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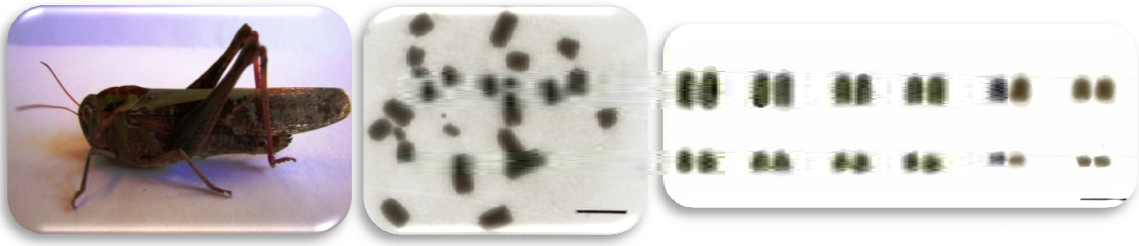
DEPARTMENT OF BIOLOGY

Chromosome Study of Some Grasshopper Species from Different Localities in Central Ethiopia



A Thesis Presented to the School of Graduate Studies, Addis Ababa University, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology (Applied Genetics)

By

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Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

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ABSTRACT

Hitherto, around 200 grasshopper species have been recorded in Ethiopia. Despite the diversity and economic importance of Ethiopian grasshoppers, the available information on their taxonomy, distribution and ecology is meager, and no report on the karyology of Ethiopian grasshoppers is available prior to this study. In this study, the karyotypes of ten taxa identified to different taxonomic levels are described. The studied grasshopper specimens were identified to belong to two families – Acrididae and Tetrigidae. Under Acrididae six genera – *Acanthacris*, *Paracinema*, *Gastrimargus*, *Acrotylus*, *Pardalophora* and *Acrida* were identified. Specimens of two additional taxa could not be identified beyond the family level. The specimens under the family Tetrigidae were found to belong to genus *Paratettix*. The grasshopper specimens were collected from eight localities in central Ethiopia. Chromosome preparations were made from tissues of the whole gut, 6 – 8 hours after the colchicine injection. The tissue was grinded in hypotonic solution (0.075M KCl). The suspension was centrifuged and the pellet was fixed in 3:1 methanol:acetic acid and followed by centrifugation. Air dry slide preparations were made from cell suspension. In some case the testis was used for the preparation of meiotic chromosomes following the same steps as in mitotic chromosome preparation. Slides were stained in Giemsa stain. Basically, all the studied species (*Acanthacris* spp., *Paracinema tricolor*, *Gastrimargus* spp., *Acrida* spp.-1, *Acrida* spp.-2, *Acrotylus* spp., *Pardalophora* spp., and the two unidentified specimens) belonging to the family Acrididae showed similarity in their karyotype, with all of them having 2n = 23 (22 autosomes + X) and 24 (22 autosomes + XX) telocentric chromosomes in male and female, respectively. *Paratettix* spp. (family Tetrigidae) had a different chromosome number, that is, 2n = 20 telocentric chromosomes. Furthermore, *Acanthacris* spp. showed some numerical instability of chromosomes in the mitotic and meiotic cells, and B chromosome like body was found in the meiotic cells of this specimen. Apart from the gross Karyotypic resemblance shown among the studied taxa of family Acrididae, slight variations in their karyotype have been noted between taxa of acridid grasshoppers. Further studies of the chromosomes of Ethiopian grasshoppers covering a wider geographic area and employing new cytological techniques were recommended.

Keywords or phrases: Karyology, Central Ethiopia, Acridid grasshopper, Karyotypic conservatism, chromosome

1. INTRODUCTION

Grasshoppers are phytophagous (herbivorous) insects, common in a variety of habitats but more frequent in arid environments like semi-deserts, open meadows and grasslands, as well as in disturbed areas such as crop fields and along road sides (Tibebu Habtewold and Landin, 1992; Capinera *et al.,* 1997; Branson *et al.,* 2006).

Grasshoppers are diversified insects which are positioned in the kingdom Animalia, and order Orthoptera together with crickets, bush-crickets, cockroaches, praying mantides and stick-insects, but, separately from these groups, they are placed under suborder Caelifera (Ragge, 1965).

The species diversity and abundance of these insects are related to numerous ecological conditions such as temperature, humidity, light intensity, precipitation and plant composition (Sirin *et al.,* 2010). In general, it has been found that their diversity and abundance would become higher at the middle altitudes and lower at high and low altitudes (Sirin *et al.,* 2010).

Among the other insect groups, grasshoppers are one of the most important insects. They have been found to impose both negative and positive effects on the environment and human beings. As pests, they cause a serious economic damage to crops and rangelands. For instance, they annually consume more than 20% of rangeland forages in the western United States at an estimated loss of $400 million (Lockwood and Lockwood, 2008). However, on the other hand, some of them are useful as a source of food (Illgner and Nel, 2000) and as a link between plants and the rest of the ecosystem (Capinera *et al.,* 1997). In addition, some grasshopper species act as a biological control for weeds (Hill and Oberholzer, 2000; Oberholzer and Hill, 2001).

As a group, grasshoppers are relatively large insects with quite distinct appearances (Pfadt, 2002; Uvarov, 1966). Grasshoppers vary widely in size. The smallest known grasshopper is the South African *Lithidium pusillum*, the apterous male of which is about 7mm long and the largest is the South American *Tropidacris latreillei*, the female being 120 mm long, with the wing-span of 230 mm (Uvarov, 1966).

Like some other insects, grasshoppers have special behavior (i.e., camouflaging) that helps them to elude from the attack of predators (Melin *et al.,* 2007). Intuitively, the commonly observed optimal camouflaging color for herbivorous insects like aphids, caterpillars and grasshoppers is green (Lev-Yadun *et al.,* 2004). According to Lev-Yadun and colleagues (2004), various color patterns in plants undermine the camouflage of herbivorous insects. This makes them to be exposed to predation and hence they obliged to remove plant organs with unsuitable coloration. In addition to camouflaging, regurgitation by grasshoppers is often considered to be a rudimentary form of defense against predators. In these phytophagous insects, regurgitate composition will vary with diet, and the type of plant secondary compounds can contribute to the effectiveness of regurgitate deterrence (Sword, 2001).

Stridulation is another typical behavior of grasshoppers. It is a species specific sound pattern produced through rhythmic rubbing of the hind leg against the forewing (Hedwig and Meyer, 1994). In many grasshopper species, this sound is useful for communication. This acoustic communication guides them in meeting of sexual partner, thereby, preventing interspecific hybridization (Krahe and Ronacher, 1993). However, acoustic communication for appropriate mate finding should be well equipped in two respects: first, individuals have to discriminate conspecific signals from numerous sounds of other species in the same biotype. Second, they have to localize and approach conspecific partner. The simplest way to achieve these tasks, as adopted by tettigoniids (bush-crickets), is unidirectional communication system, a system where the male sings and the female recognizes, localized and approaches the calling partner (Helversen and Helversen, 1997).

Finer structures of external anatomy like forms of the antennae, shape of head, shape of pronotum, carves on the ventral side of pronotum and wing venation play a prominent role in the classification and identification of grasshoppers. Body size, shape, color and pattern of stripes are also some of the other external features frequently used in this regard (Pfadt, 2002). Furthermore, internal anatomy of grasshoppers shows an important taxonomic feature in some species. For instance, the number of malpighian tubes have been found to be higher (more than 500) in two grasshopper species, namely, *Romalea microptera* and *Brachystola mogna* (Uvarov, 1966). The pattern of sound produced by these insects is taxonomically informative in their identification to the species level (Ragge, 1965).

In addition to the internal and external morphological characteristics, the data obtained from cytogenetical researches have a great taxonomical and biosystematical value. The main cytogenetic features with taxonomical relevance are: chromosome number, chromosome morphology and staining chractersitics of chromosomes (Stace, 2000).

Despite the diversity and economic importance of Ethiopian grasshoppers, little is known about their taxonomy, distribution and ecology. The lack of adequate number of studies on these insects makes their identification faint. In addition, no studies have been made regarding their chromosomes (i.e., Karyotyping) and genetic diversity using different marker systems. So, this study is aimed at generating some information on the chromosome number and morphology (karyotype) of these insects from different localities in central Ethiopia.

2. LITERATURE REVIEW

2.1. The taxonomy of grasshoppers

So long as the taxonomical position of grasshoppers is concerned, they are grouped under phylum Arthropoda, class Insecta, subclass Pterygota, division Exopterygota, order Orthoptera, suborder Caelifera, superfamily Acridoidea, family Acrididae (Meinzingen, 1993).

2.1.1. The Order Orthoptera

The Orthoptera comprises the terrestrial insects commonly known as short-horned grasshoppers, katydids, crickets, mole-cricket, grouse-locusts and related groups without common names (Richards and Davies, 1977; Rentz, 1991). This huge order, encompassing about 17000 species, formerly included groups of insects which were later classified into three orders: Grylloblattodea, Dictyoptera and Phasmida (Richards and Davies, 1977). The Orthopterans are mainly medium to large-sized, but include some of the large living insects, with bodies over 11.5 cm long and wingspans more than 22 cm. They are often abundant as individuals, forming a characteristic and striking component of the fauna in many parts of the world. The order is perhaps best known for the power of jumping possessed by nearly all species, and for their singing (Rentz, 1991). The order is divided into suborder Ensifera and suborder Caelifera (Richards and Davies, 1977).

2.1.2. The Suborder Caelifera

Caelifera is one of the two suborders of the order Orthoptera, comprising short-horned orthopterans with common names of grasshoppers and locusts, as well as some less familiar groups like the grouse-locusts (family Tetrigidae) and the pigmy mole-crickets (family Tridactylidae) (Richards and Davies, 1977). In addition to saltatorial hind limbs, the extant members under this suborder are characterized by fewer antennal segments (less than 30 segments) and the absence of auditory organs on the prothorax (Richards and Davies, 1977; New World Encyclopedia contributers, 2008). According to recent estimates, there are about 2400 valid caeliferan genera and 11,000 valid species around the world (New World Encyclopedia contributers, 2008). The 2400 genera of short-horned orthopterans are placed into 8 superfamilies and 22 families (New World Encyclopedia contributers, 2008). These superfamilies include, Acridoidea (grasshoppers and locusts), Pyrgomorphoidea (lubber and bush grasshoppers), Trigonopterygoidea, Tanaoceroidea (desert grasshoppers), Eumastacoidea (monkey grasshoppers), Pneumoroidea (bladder grasshoppers), Tettigonoidea (grouse- or pygmy- grasshoppers), and Tridactyloidea (pygmy mole-crickets and sandgropers) (New World Encyclopedia contributers, 2008). Following, focus will be made on the acridids of superfamily Acridoidea and family Acrididae.

2.1.3. The Superfamily Acridoidea

The Acridoidea is the largest superfamily of Orthoptera with 14 families, more than 1500 genera (Rentz, 1991) and over 10,000 species (Richards and Davies, 1977). Some of the general characters of this superfamily are: hind-, mid- and fore-tarsi with three segments, pretarsus usually with arolium (Richards and Davies, 1977; Rentz, 1991), non-moniliform short antennae, absence of antennal organs, absence of dorsal spines and tubercles at the basal segments of hind tarsi and presence of variable wings, i.e., from fully developed to apterous (Rentz, 1991).

2.1.3.1. The Family Acrididae

Of the 14 families of the superfamily Acridoidea, Acrididae is the largest and the most recognizable grasshopper family (Richards and Davies, 1977; Picker *et al.,* 2004). This family comprises about 9000 species (Richards and Davies, 1977). They are characterized by stout antennae, presence of pegs on inner side of the hind legs and hearing organ (tympanum) on each

side of the abdomen (Picker *et al.,* 2004). Of the 17 subfamilies included under Acrididae, subfamily Acridinae is the largest group with world-wide distribution (Rentz, 1991).

2.1.4. Taxonomic composition of grasshoppers in Ethiopia

Hitherto, little is known about the taxonomic composition and assemblage of grasshoppers in Ethiopia. However, the pioneer work of Jago (1977) cited in Tibebu Habtewold and Landin (1992) indicated that there are at least 200 species of grasshoppers in the country. A few of them constitute high risk pests to economic crops in different parts of the country. In addition, Tibebu Habtewold and Landin (1992) have recognized about 29 species of short- and long-horned grasshoppers from central Ethiopia, and these are grouped into 2 superfamilies, 3 families and 9 subfamilies (Appendix 1).

2.2. Feeding and distribution

As a group, grasshoppers are herbivorous insects that range from monophagous to polyphagous feeders although most species fall in the oligophagous to polyphagous group (Joern, 1979). Depending on the feeding habit, grasshoppers can be divided into two. These are: specialists and generalists and it has been found that specialists are less efficient on their host plant than are generalists on convenient host plants, but some specialists fed on plants which are not convenient for generalists. For example, the feeding preference of grasshopper species *Anthermus granosus* and *Eucoptacra spathulacauda* to *Lippia muliflora* is not convenient for a generalist like *Eucoptera anguliflava* (Philipe, 1991).

In connection with the type of food grasshoppers eat, three different types of feeder are described: grass-feeders, mixed-feeders and forb-feeders (Philipe, 1991). For example, *Eyprepocnemis plorans* and *Catantops sylvestris* are grasshopper species known to feed on mixed-food types and forbs, respectively (Philipe, 1991). These feeders have distinct mouthpart (mandible) constructions in accordance to the general structure and characteristics of the diet they consume. These are: graminivorous (grass-feeding type) with grinding molars and incisors typically fused into a scythe-like cutting edge, forbivorous (forb or broad leaf-feeding type) which has a molar region with a depression surrounded by raised teeth and sharp interlocking incisor teeth and herbivorous (mixed feeding type) with mandibular characteristics of both of the aforementioned groups (Smith and Capinera, 2005).

Feeding behavior in grasshoppers has a direct influence on their distribution. There is an intimate association between the distribution of grasshoppers and the type of environmental vegetation. As a result of this, they have non-random distribution in different enivironments (Almeida and Camara, 2008). For instance, an endangered grasshopper species in New Zealand, *Sigaus minutus*, is restricted to the area dominated by plantation of *Raoulia australis* (Jamieson, 1996). In other words, vegetation structures, plant species diversity, microclimate and availability of suitable host plant are factors that can potentially control grasshopper distribution (Heidorn and Joern, 1987).

Few studies made indicated that grasshoppers are more or less ubiquitous in their distribution. They occur from lowland habitats to highlands (Adis *et al.,* 2007), except the coldest part of the earth's surface (Rentz, 1991). In Ethiopia, grasshoppers are distributed in lowlands, mid-highlands and highland regions (Stretch-Lilija, 1977). More specifically, different species of grasshoppers and locusts have been recorded from Ilubabor, Gojam, Gonder, Welega, Shewa, Harerghe, Bale, Sidamo, Tigray, Kefa, Gemu Gofa and Welo regions (Stretch-Lilija, 1977; Tibebu Habtewold and Landin, 1992).

2.3. Flight and migration

Flight is a prominent feature of many grasshopper species. They start to fly by jumping into the air. The jump provokes flight by consequence of the loss of tarsal contact to the ground and by setting up an air current to wind sensitive hairs on their forehead. Flight initiation, especially in locusts, is phase dependent; for any given wind stimulus the chance that a gregarious locust would initiate flight is significantly higher than that of an isolated one (Ayali *et al.,* 2004). Flight has a direct relationship with reproduction. The juvenile hormone concentration has been found to be increased by performance of long-duration flight. The increment in the concentration of this hormone enhances reproduction (Min *et al.,* 2004).

In grasshoppers, flight is a major mechanism that facilitates migration. Migration helps grasshoppers to escape from detrimental environmental condition, such as cold or drought, to find a suitable habitat for reproduction and to search for a new resource (Lorenz, 2009).The frequency of migration is a major determinant of the rate of gene flow between populations, and dispersal can stabilize populations in heterogeneous environments (Roff, 1974 cited in, Mcanelly

& Rankin, 1986). During migration, the speed and direction of these insects is assisted and determined by wind. For example, desert locust (*Schistocerca gregaria*) crossed the Atlantic from West Africa to the Caribbean in 1988, a non-stop flight of about 5000km (Lorenz, 2009).

2.4. Life history

The life cycle of grasshoppers comprises three stages, namely, egg stage, hopper stage and adult stage (Meinzingen, 1993). The first stage is the stage where female grasshoppers lay two or more egg pods in the soil; each egg pod encapsulates 20 – 100 eggs depending on the species (Meinzingen, 1993). The eggs are usually laid in areas of bare sandy moist soil at about 5 – 10 cm below the surface (Symmons and Cressman, 2001). During this stage, the eggs need warm temperature and adequate moisture. However, if environmental condition is unfavorable, the eggs will undergo diapause (Meinzingen, 1993).

During hatching, the emerging hoppers make their way out through the froth plug to the surface and they immediately moult to the first instar. The hoppers then pass through 5 – 6 instars by shedding an exoskeleton between each instar stage (Meinzingen, 1993; Symmons and Cressman, 2001).

Finally, the adult stage begins with the completion of the last moult. The newly emerged adult (fledging) has delicate wings and weak integument color (Meinzingen, 1993). In the majority of grasshopper species, it will take nearly 10 days for the fledging to become a fully grown adult by hardening its wing so that it is capable of sustained flight. The adults then remain sexually immature until they encounter favorable conditions that stimulate maturation (Symmons and Cressman, 2001). Food availability in response to good rain is a favorable ecological condition that triggers the beginning of rapid reproductive maturation (vitellogenesis) (Franc and Luong-Skovmand, 2009).

2.5. Importance of grasshoppers

2.5.1. Positive impact

Like the other major insect orders, including Lepidoptera (moths and butterflies), Hymenoptera (bees and ants), Isoptera (termites), Coleoptera (beetles) and Hemiptera (true bugs), Orthoptera (locusts and grasshoppers) also serve as a supplementary food source for human beings (Table 1), predominantly in Africa and Australia (Illgner and Nel, 2000). Additionally, they are critical elements in the food supply of many birds and mammals. They are also central to the conversion of plant matter into animal matter and in nutrient re-cycling (Capinera *et al.,* 1997).

Moreover, grasshoppers are important in the biological control of invasive weed species. The grasshopper *Cornops aquaticum* is found to be a natural enemy for water hyacinth (aquatic weed) in South Africa, both the adults and the nymphs are very damaging to water hyacinth plants (Hill and Oberholzer, 2000; Oberholzer and Hill, 2001).

Table 1. Lists of some edible grasshopper species, modified from Illgner and Nel (2000).

Order	Species	Edible stage	Protein content %
Orthoptera	*Locusta migratoria*	adult	—
Orthoptera	*Locustana pardalina*	adult	55.5
Orthoptera	*Schistocerca gregaria*	adult	—
Orthoptera	*Zonocerus elegans*	hoppers, fliers	29.2(hoppers)

2.5.2. Negative impact

Acridid pests, locusts and grasshoppers, create massive threats to rural communities in developing countries, including sub-Saharan Africa, where human and material resources for controlling these insects are meager to none (Yeneneh Taye, 2005). The desert locust *Schistocerca gregaria*, one of the 20 locust species present in tropical and subtropical areas, is

probably one of the world's most serious agricultural pest, mainly due to its vast area of distribution (Wiktelius *et al.,* 2003). The amount of damage it can cause is estimated from the fact that a locust eats approximately its own weight, 1.5-3.0 g, of fresh vegetation each day. As there are often some 50,000,000 individual in each km^2 of a medium density swarm, a small swarm of 1 km^2 can eat 100t of food every day. A very large swarm covering 1000 km^2 requires about 100,000t of food each day, enough to feed 500,000 people for a year (Meinzingen, 1993).

Apart from the damage locusts cause by eating leaves, flowers, seeds and growing tips, they sometimes settle so densely that they weigh down and break the branches of trees and plantation crops such as coffee, which they do not eat (Meinzingen, 1993).

2.5.2.1. Control

Different grasshopper and locust controlling techniques have been developed and employed. Some of them are:

• **Biological control:** It is the use of environmentally benign entomopathogenic fungi. In this regard, the application of *Metarhizium flavoviride/ anisopliae* (Green Muscle) as a biological control agent provides a magnificent result in minimizing/ effectively suppressing the growth of grasshopper population (Thomas *et al.,* 1995; Kooyman *et al.,* 1997; Lomer *et al.,* 2001; Emiru Seyoum and Merid Negash, 2007). In addition to this, Tibebu Habtewold *et al.,* (1995) obtained high mortality rate of grasshopper population using a protozoan parasite, *Nosema locustae* (Nosematidae), as a control agent.

• **Cultural control:** It involves the use of one or a combination of different techniques (like crop rotation, tillage, burning of crop stubble and intercropping or polyculture) to reduce insect population in crops (Gullan and Cranston, 2010). Furthermore, careful timing or placement of plantings to avoid synchrony with pests might have a significant role in avoiding the damage of crops by the pest infestation (Tibebu Habtewold, 1993; Gullan and Cranston, 2010).

• **Chemical control:** The uses of various chemical insecticides to control infestation have replaced traditional methods such as scaring, burning and trenching. Their intensity differs from species to species and among different stages of insect development. For example, insect growth regulators (IGR) are promising for the effective control of locust hoppers. However, most of the

pesticides available for locust control are not environmentally rational; they are contact poisons (Meinzingen, 1993).

• **Physical control:** It is the use of non-chemical and non-biological methods to destroy the pest or to make the environment unsuitable for the entry or survival of the pest. Most of these control methods may be classified as passive such as fences, trenches, traps, inert dusts, and oils or active (e.g. mechanical and thermal treatments) (Gullan and Cranston, 2010).

2.6. Cytogenetics in systematics

Cytogenetics is a branch of genetics that is concerned with the study of the structure and function of the chromosomes. Chromosomal data are important in phylogenetic studies because of the obvious potential that cytogenetic studies have to reveal both structural and functional homologies among taxa (Dobigny *et al.,* 2004). Cytogenetical data also have a significant role in taxonomic decision-making and biosystematic investigation (Stace, 2000). More specifically, chromosome cytology has contributed to insect systematics in several different ways that are not always distinguished conspicuously through conventional taxonomy (White, 1957). It is a powerful means for discriminating or characterizing different species, and sometimes sibling or cryptic species (White, 1957).

Chromosomal data including chromosome number and morphology, collectively known as karyotype, are often variable among different species. Close examination of karyotypes and different types of chromosomes other than A-chromosomes (i.e., B-chromosomes) would give an inference in the study of evolutionary relationship of organisms.

2.6.1. Important features by which Karytypes of species differ

Karyotype can be defined as the phenotypic appearance of the chromosomes at mitotic metaphase stage (Lewitsky, 1931 cited in, Jackson, 1971; Stace, 2000). It is usually depicted by a histogram-type, ideogram or karyogram (Stace, 2000). Karyotypes may differ from one species to another in a number of ways. Some of the features by which the karyotype of one species may differ from that of another species include: chromosome number, kind of centric activity, size and number of localized centromeres, arm ratio, number, size and position of secondary constrictions and satellites, absolute size of the chromosome, chromosome size difference within

the complement and position, number, size and distribution of differentially staining heterochromatic segments (Jackson, 1971).

2.6.1.1. Chromosome number and size

Chromosome number is a product of two variables, base number (x) and ploidy level (Stace, 2000). Chromosome number varies to a great extent among species. For instance, among animal species the diploid chromosome number is known to vary from the smallest (2n = 2) in the ant species *Myrmecia pilosula* (Imai *et al.,* 1988) to the highest (2n > 1200) in the nematode *Parascaris univalens* (Sumner, 2003).

Regarding chromosome size, a variation might exist as much as 20-fold between genera of the same family having the same or similar basic chromosome numbers. For instance, in plants, variation in absolute chromosome size is evident between advanced and primitive plants. Advanced vascular plant families like Cruciferae (*Arabidopsis* spp.) and Gramineae (*Panicum* spp.) have smaller chromosomes and lower DNA content than primitive vascular plants such as *Psilotum* and *Tmesipteris* (Stebbins, 1971).

2.6.1.2. Chromosome morphology

Another crucial feature by which the karyotypes of different species differ from each other is by the morphology of their chromosomes. This aspect is one of the relevant characteristics of chromosomes in systematics (Stace, 2000). Primarily, chromosome morphology is determined by the position of the centromere (primary constriction) and to some extent by the nucleolus organizer region (secondary constriction). Telomeres, repetitive nucleotide sequences at the ends of chromosomes, are also essential parts that keep the integrity of chromosome morphology (Sumner, 2003). These three features of chromosome are discussed, in some details, below.

2.6.1.2.1. Centromeres and chromosome nomenclature

The centromere is a constricted region seen on somatic metaphase chromosomes. Functionally, it is a region of the chromosome where the sister chromatids are held together until anaphase separation, and which interacts and attaches to the spindle fiber through specific structure called kinetochore, housed within the constriction (Sumner, 2003). In short, centromere is critically important for stability, integrity and proper segregation of chromsomes (Ketel *et al.,* 2009).

Because of the fact that the position of the centromere is normally constant for a given homologue but may vary among different homologues, the location of the centromere is the most valuable landmark in the morphologic identification and characterization of chromosomes or karyotypes (Levan *et al.,* 1964). On the basis of centromeric positions, Stebbins (1971) classified chromosomes into four types as metacentric, submetacentric, acrocentric and telocentric. However, it seems less precise as compared with the one suggested by Levan *et al.* (1964). These authors categorized chromosomes into six groups based on their arm ratio ($\text{i.e., arm ratio (r)} = \text{long arm}/\text{short arm}$), and desiginated those chromosomes with an arm ratio of 1.0 as M; 1.0 – 1.7 as m; 1.7 – 3.0 as sm; 3.0 – 7.0 as st; 7.0 – ∞ as t; and at terminal point (∞) as T. This system of nomenclature to define centromeric position based on the numerical value of the arm ratio has widely been used in the literatures.

Various modifications to the classification system proposed by Levan *et al.* (1964) have been made by different people. Adhikary (1974) have classified chromosomes into eight groups. Later, Abraham and Prasad (1983) have suggested a system of nomenclature of centromeric position in which they recognized 10 positions on a chromosome.

2.6.1.2.2. Nucleolar organizing regions (NORs) and satellites

The nucleolar organizing region (NOR) is another constricted region found on some chromosomes of a species and it is frequently referred to as a secondary constriction in order to differentiate it from the centromere (Jackson, 1971; Heitz, 1931 cited in, Esponda and Gimenez-Martin, 1974). NOR is located either close to a terminal or in the interstitial regions between the centromere and the end of a chromosome arm (Schubert, 2007). Usually, only a few chromosomes of the complement possess secondary constriction and satellites. There is at least one chromosome in each haploid complement of monocentric chromosomes that possesses a secondary constricion and consequently a satellite (Jackson, 1971).

Secondary constrictions and satellites are useful morphological landmarks to characterize chromosomes and karyotypes. A chromsome with a secondary constriction can easly be distinguished from the rest of the chromosomes and if two or more pairs of such chromosomes are present in the complement, they may be distinguished from each other by the position and size of the secondary constrictions and satellites. In the same way, karyotypes of different

species may be distinguished by the number, size, position and distribution of the secondary constrictions and satellites they possess (Rocha *et al.,* 2004), in addition to the other morphological landmarks.

Functionally, the NOR contains a multiple-copy cluster of 5.8, 18 and 28 S ribosomal RNA (rRNA) genes (Cross *et al.,* 2003). These rRNA genes are coded by 45 S ribosomal DNA (rDNA) gene (Ferro *et al.,* 2001). In general, the NOR in the transcriptionally active state contains DNA, RNA and proteins (Cross *et al.,* 2003). Along the length of NOR, the 45 S DNA can easly be identified in the chromosome by means of silver nitrate staining (Ag-NOR staining) (Ferro *et al.,* 2001).

2.6.1.2.3. Telomeres

Telomeres are structures that constitute the ends of eukaryotic chromosomes (Shachar *et al.,* 2008). The term telomere was first coined by Hermann J. Muller (1938) from two Greek words, "telos" (end) and "meros" (part) to designate the terminal part of the chromosome (Chuaire, 2006). Telomeres are essential regions of the chromosome in maintaining its stability and integrity (McClintock, 1941; Shachar *et al.,* 2008). The establishment of chromosome stability as a result of telomeres is conferred by a protein-DNA structural cup that protects the chromosomal ends, and the telomeric sequence of nucleotides is conserved from yeast to human cells (Shachar *et al.,* 2008). Apart from this, telomeres do not have much to offer by way of cytologically detectable morphological landmark for chromosome characterization, although it is known that telomeres may differ, in the number of telomeric repeat units of nucleotides they possess, within a complement of a species or between complements of different species.

2.6.2. Chromosome mutation and karyotypic evolution

Though species may differ from one another in their karyotypic features, to a lesser or larger extent, it is a common observation that closely related species have more similar karyotypes than do distantly related species (Sumner, 2003). The similarity is ascribable to the sharing of many of the features of the karyotype of the common ancestral species while the difference could be due to the evolution of the karyotypes, since their separation from the common ancestor, that fixes different chromosomal mutants in different lineages. This means that karyotypes are liable

to evolution; chromosomal mutations produce the raw material or chromosome variants which evolutionary forces shape into a new karyotype.

Eukaryotic chromosomes may vary in size, shape and composition of DNA, proteins and RNA, as well as in their number and redundancy. All these features are subject to evolutionary changes, ultimately resulting in karyotypic evolution. As a result, karyotypic variation exists between species and sometimes it might even exist within species as polymorphism (Schubert, 2007). According to Jackson (1971), in order for chromosome structural rearrangements to take place, survive and get fixed, the following five conditions have to be met. A cellular environment must exist that is conducive for chromosome breakage, breakage points must occur in essentially nonfunctional portions or else there should a duplicate cistron be present, chromosomal rearrangement or addition must resist the rigors of mitosis and meiosis, rearrangements or additions must carry with them adaptive gene complexes and the breeding system must be such that a reasonable probability of survival exists.

There are two major types of mutations recognized. Structural chromosome mutations that include deletions, translocations, inversions and duplications etc, and numerical chromosome mutation such as aneuploidy and polyploidy. Following, the main types of chromosomal mutations and their effects on the chromosome morphology and number will be presented in some details.

2.6.2.1. Morphological chromosome aberration

In eukaryotes, chromosome morphological alterations are frequent and range from part of a gene to hundreds of genes, and there is also a strong correlation between the number of rearrangement breakpoints and that of repeat sequences (Coghlan *et al.,* 2005).

Speciation is often linked with chromosome structural changes (Dobzhansky, 1937 cited in, Sax, 1940) although, gross structural changes in chromosome are not always the requirement for speciation as can be seen from the remarkable karyotypic similarity found between a number of species. For instance, birds are generally very conservative karyotypically. Even, in cases where whole karyotypes have not been maintained unaltered, it may be possible to recognize individual chromosomes that appear to have remained unchanged during the divergence of species, even

between different orders. For example, several human chromosomes have been identified unchanged in cats (Sumner, 2003).

The morphology of chromosomes can be altered by pericentric and paracentric inversions. The former can result in centromeric shift if breakpoints occur at different distances on either side of the centromere (Stebbins, 1971; Schubert, 2007), or else centromeric shift may not be detected in the case of isomorphic pericentric inversion (Jackson, 1971). Contrariwise, paracentric inversion does not result in centromeric shift. However, it may bring about a different type of morphological change such as when the breakpoints within the same arm occur at different distances on either side of a detectable landmark in that arm such as a secondary constriction or heterochromatin block (Jackson, 1971; Schubert, 2007).

The shape and size of chromosomes can also be altered by translocation (unequal-reciprocal or non-reciprocal), insertion (for example, via transposition), deletion of dispensable parts, or by sequence amplification (Schubert, 2007). Reciprocal translocations may occur when two non-homologous chromosomes break simultaneously and exchange segments (Jackson, 1971; Stebbins, 1971; Lorite and Palomeque, 2010). They may also occur between homologous chromosomes (Jackson, 1971). If the exchanged segments are unequal in size, there will be an alteration of chromosomal shape and size. However, translocations involving interchange of segments with essentially equal length do not alter the structure and length of chromosomes involved, and hence are not detectable by the usual karyotypic methods (Jackson, 1971). In non-reciprocal translocations, in addition to the morphological change that occurs in both the donor and recipient chromosomes, there will be size increment in the recipient chromosome and decrement in the donor.

Deletion is another means by which chromosomes are altered morphologically. It takes place when part of a chromosome segment is lost (Lorite and Palomeque, 2010). A deletion can occur on any chromosome, at any place on the chromsome, and it may be a large or a small deletion. It ultimately leads to the loss of genes. In some special cases, the whole chromosome could also be deleted resulting in chromosome number reduction (White, 1957).

Even if the new chromosomes produced by rearrangement are capable of persisting through a number of cell generations and do not lead to loss of essential genes they are still liable to be

eliminated from the population as a result of natural selection unless individuals possessing them in the heterozygous condition have an adaptive superiority over those lacking them (White, 1957).

2.6.2.1.1. A special type of translocation

Robertsonian translocation (i.e., centric fusion and fission) is among the important means of karyotypic evolution since it results in concomitant changes in chromosome morphology and chromosome number (Jones, 1998; Perry *et al.,* 2004). Robertsonian fusion can result when breakage occurs in the region proximal to or within the centromere of two, usually non-homologous, acro- or telocentric chromosomes, followed by fusion of the broken segments/ chromosome arms (Jones, 1998). This evolutionary process ultimately results in the formation of meta- or submetacentric chromosome (Sumner, 2003). This would decrease the chromosome number while increasing the karyotype symmetry, without a change in the number of major chromosome arms or fundamental number (FN) (Jones, 1998).

In contrast, Robertsonian fission results from transverse breakage of a chromosome horizontally through the centromere (Jones, 1998; Perry *et al.,* 2004; Coghlan *et al.,* 2005). This would produce either two telocentric chromosomes (if fission occurs befor chromosome replication) or two isochromosomes (i.e., if fission occurs after replication) (Perry *et al.,* 2004). Either way, fission increases chromosome number, but the FN remains unchanged (Jones, 1998). However, the karyotype symmetry decreases or conversely the asymmetry increases (Stebbins, 1971).

In general, Robertsonian translocation is more prevalent in animals, like molluscs, insects, reptiles and mammals, than in plants (Jones, 1998).

2.6.2.2. Numerical chromosome aberration

The karyotypes of species may differ from each other not only in the morphology of their chromosomes, as described in the preceding sections, they may also differ in the number of chromosomes that constitute the karyotypes. This, in other words, indicate that karyotypes evolve not only through changes in chromosome morphology but also through numerical chromosome changes.

Any deviation from the normal chromosome number of an organism at any stage in its life cycle is a result of either aneuploidy or polyploidy. Aneuploidy involves changes in chromosome number by additions or deletions of individual chromosomes. The number being less than a whole set, whereas polyploidy involves changes (gain or loss) in the whole set of chromosomes. Each of the major types of chromosomal numerical mutations are discussed, in some depth, below.

2.6.2.2.1. Aneuploidy

Aneuploidy is one of the major category of chromosomal aberrations which corresponds to a change in the number of chromosomes in a cell, resulting from the gain or loss of one or more but less than the basic chromosome number of the species (Tamarin, 2001). Non-disjunction and chromosome loss through anaphase lagging are the two classical processes that lead to aneuploidy. In addition to these, non-conjunction (failure of homologous chromosomes to synapse), defective centromeric division (as in precocious centromeric division that results in the separation of sister chromatids at anaphase I) and extra-replication of chromosome could cause aneuploidy (Kirsch-Volders *et al.,* 2002). Once an aneuploid gamete is formed, its union in fertilization with a normal or another aneploid gamete will produce chromosomally abnormal (aneuploid) zygote (Tamarin, 2001). The proper development and viability of the aneuploid zygote would depend on a number of factors such as the number and type of the chromosome involved (Tamarin, 2001), and sometimes the ploidy level of the species (John, 1976).

2.6.2.2.2. Polyploidy

Polyploidy is the phenomenon of possessing more than two complete sets of chromosomes (Jackson, 1971; Stebbins, 1971). It involves an increase in chromosome number by an exact multiple of the basic chromosome number and it is a typical feature of plant species, albeit it is also found in almost all organisms from protists to certain cells of humans (Baatout, 1999). The sets of chromosome possessed by a polyploid cell either belong to the same genome or derived from two or more different genomes contributed by completely or partially differentiated species and are respectively known as autopolyploidy and allopolyploidy (Stebbin, 1971; Chenuil *et al.,* 1999; Parisod *et al.,* 2010). Allopolyploids are referred to classical or genomic allopolyploids when the chromosomes of the parental species are completely differentiated from one another

(Stebbin, 1971; Chenuil *et al.,* 1999; Parisod *et al.,* 2010) whereas they are called segmental allopolyploids when the parental genomes are only partially differentiated (Stebbins, 1971).

Polyploidization is one mechanism that can produce instantaneous speciation because it imposes immediate reproductive isolation with the parental species. Speciation by polyploidy has a major role in diversification of lineages (Holloway *et al.,* 2006). Apart from reproductive isolation, the evolutionary role of polyploidy is to endow extra-copies of genes, whose subsequent alteration leads to new functions, increased biological complexity, and, at the end of the day, speciation (Becak and Kobashi, 2004). Polyploidy confers permanent heterozygosity and heterosis which increases the fitness of the polyploid (Comber and Smith, 2004; Comai, 2005).

In comparison with plants, polyploidy is too rare in animals (Muller, 1925; Mable, 2004; Chen and Ni, 2006), and it has low significance in animal evolution (Mable, 2004). The paucity of polyploidy in animals is usually associated with either the direct disruption of sex determination machanism or indirect effects like the altered dosage of sex linked genes in polyploids (Muller, 1925; Orr, 1990), and animal development, which is disrupted by polyploidization (Orr, 1990; Mable, 2004; Chen and Ni, 2006). In addition, as it is inferred by Tamarin (2001), the ability of plants to avoid meiotic problems of polyploidy as compared with animals makes the incidence of polyploidy high in plants because some plants exist vegetatively, allowing more time for the rare somatic doubling event to occur that will produce an amphidiploid whereas the precisely defined life span in animals permit less time for a somatic doubling. However, in animals like the earthworms and fresh-water snails, which are normally hermaphroditic, tetraploidy or even higher forms of polyploidy could occur as readily as amongst most plants (Muller, 1925) because in such group of animals sex chromosome mechanisms are absent (Takenouchi *et al.,* 1983).

2.6.2.2.2.1. Incidence and effect of polyploidy in insects

Despite the diversification of insects as compared to any multicellular life forms on earth, they have experienced low incidence of polyploidy. However, some cases of polyploidy with 3x, 4x, and rarely 5x, 6x or more have been reported (Table 2) (Gregory and Mable, 2005). For instance, polyploidy is extremely rare in aphids (order Hemiptera) eventhough they are reproduced by cyclic parthenogenesis/ thyletoky (Takada *et al.,* 1978). Likewise, although many butterflies

(order Lepidoptera) show extremely high chromosome numbers, these are the result of the fragmentation of existing chromosomes and not of polyploidy (Gregory and Mable, 2005).

In general, polyploid insects are found to be flightless and to have slow life cycles lasting two or more years. There is also a positive correlation between body size and level of polyploidy in weevils (order coleoptera, family Curculionidae) (Gregory and Mable, 2005).

Table 2. Incidence of natural polyploidy in some insect species (Gregory and Mable, 2005).

Taxonomic Groups			Ploidy		Mode of Reproduction
Order	Family	Species	x	Ploidy level	
Coleoptera	Curculionidae	*Catapionus gracilicornis*	11	2x, 3x, 4x, 5x, 6x, 10x	T
Hemiptera	Coccidae	*Physokermes hemicryphus*	9	2x, 3x	T (3x)
	Delphacidae	*Muellerianella fairmairei*	14	2x, 3x	G (3x)
Lepidoptera	Psychidae	*Solenobia fennicella*	__	4x	T
		Solenobia lichenella	__	2x, 4x	T (4x)
		Solenobia seileri	__	4x	T
		Solenobia triquetrella	__	2x, 4x	B or T (2x), T (4x)
Orthoptera	Tettigoniidae	*Saga pedo*	14	5x	T

Note: The level of polyploidy is indicated as a multiple of basic chromosome number 2x = diploid, 3x = triploid, etc. and regarding mode of reproduction: bisexual = B, thyletokous = T and gynogenetic = G.

2.6.3. Karyotypic conservatism

Karyotype of different species often show certain degree of variation. However, in some sporadic cases different species within a genus or rarely within a family demonstrate uniformity in their karyotypes (Stebbins, 1971). In other words, this is to say that species may have a conservative nature on chromosomal evolution (Baker and Bickham, 1980). In plants, for instance, genus *Pinus* and other conifers show high degree of karyotypic conservatism (Stebbins, 1971).

Climatic stability and similarity of habitats in the distribution of species of the same genus or family may have an important role in creating and maintaining karyotype conservatism (Vosa, 2005). In addition, selection seems to play a role in karyotypic uniformity (Vij *et al.,* 1980). This case is observed in the majority of the species belonging to the genera *Aloe*, *Haworthia*, *Gasteria* and *Poellnitzia*, (family Asphodelaceae, tribe Aloineae). The karyotype of all the species belonging to these genera, apart from few species of *Aloe* and *Haworthia*, are generally bimodal composed of eight large and six small chromosomes (Vosa, 2005). In these species, cells with numerical deviations from the normal diploid number of the complement will be weeded out, as they enter in the germ line, through a regrous selection (Vij *et al.,* 1980).

It has also been indicated that there is high degree of karyotypic conservatism in grasshoppers especially in the family Acrididae (Mesa and Fontanetti, 1983; Rocha *et al.,* 2004; Souza and Melo, 2007). In addition to what have been indicated above as responsible for conservatism, the conservatism in grasshoppers is attributed to the lack of heterochromatin/ or repetitive DNA sequence (Jackson, 1971).

The maintenance of chromosomal linkage groups between species could also be regarded as a major strategy in karyotype conservatism (Patton and Baker, 1978). Moreover, active telomeres play a prominent role in conferring karyotypic conservatism (Cui *et al.,* 2002).

2.7. B-chromosomes

In addition to the normal chromosome complements possessed by all members of the species, a number of plant and animal species possess additional one or more chromosomes, designated as B-chromosomes, which would bring about chromosome numerical differences among members of a species as well as between species.

B-chromosomes can be defined as extra/ supernumerary chromosomes, (Cabrero *et al.,* 1999; Camacho *et al.,* 2000; Camacho, 2005), that display noticeable heterogeneity in their nature, behavior and evolutionary dynamics and they are also considered as an additional nonessential chromosomes that are present in some individuals in some populations of some species (Beukeboom *et al.,* 1996; Camacho, 2005).

B-chromosomes have been described in more than 1300 species of plants and 500 species of animals (Jones and Rees, 1982 cited in, Camacho *et al.,* 2000; Camacho, 2005). They have also been reported in 10 species of fungi (Camacho, 2005).

The presence of B-chromosomes is usually explained by their violation of Mendelian rules, based on mitotic and meiotic instability, leading to their accumulation in the germ line (Araujo *et al.,* 2001). In other words, B-chromosomes lack regular meiotic behavior, i.e., they do not often pair up and segregate during meiosis, and this impedes their stable integration into the A genome. The fact that they undergo directed non-disjunction into gametes or cells destined to form gametes facilitates their accumulation in the germ line (Camacho, 2005).

B-chromosomes can originate from A-chromosomes (autosomes and sex-chromosomes) in various ways, which include origin from polysomic A chromosomes, centric fragments resulting due to A-chromosome fusion or from amplification of the paracentromeric region of the fragmented A-chromosome (Camacho *et al.,* 2000).

Camacho and his colleagues (2000) have suggested four factors that determine the frequency of B-chromosomes: (i) their average transmission ratio, (ii) harmful effects exerted by B-chromosomes on host fitness (iii) random changes if the Bs are in the near-neutral stage and (iv) time from B invasion.

Generally, B-chromosomes, in most cases, are heterochromatic in nature owing to the high content of repetitive DNA of various types, especially satellite, ribosomal, and mobile element DNA. In spite of this fact, they are significant factors in genome evolution because their presence increases the level of A-chromosome recombination (Muntzing, 1974; Camacho, 2005).

2.8. Chromosome study reports on some grasshopper species

2.8.1. Chromosome number and morphology

Different studies have revealed variations and similarities in chromosome number and chromosome morphology (i.e., in general, karyotypes) among different grasshopper species. Most species of *Dichroplus* and related *Melanoplines* (family Acrididae and subfamily Melanoplinae) share the ancestral all-telocentric, 2n = 22 + X (male) or 22 + XX (female),

karyotype of the ancestral Cryptosacci Acridoids. However, exceptionally, the karyotype of *D. pratensis* comprises nine pairs of telocentric autosomes and a telocentric X chromosome (2n = 18 + X/ 18 + XX), which could be derived through two tandem fusions from the Cryptosacci complement (Bidau and Marti, 1995).

Likewise, in the genus *Caledia* (family Acrididae, subfamily Acridinae), *C. species nova I* and *C. captiva* possess karyotypes with 2n = 22 autosomes + XX (female)/ X (male) sex chromosomes (Shaw *et al.,* 1995). Regarding chromosome morphology, the *C. species nova I* exclusively contains telocentric chromosomes with little or no centric heterochromatin. In contrast, all subspecies within *C. captiva* show distinctive karyotypes based up on variation in centromeric location, i.e., chromosome of the first subspecies contains both acrocentric and telocentric chromosomes, with centromeric heterochromatin restricted to 3 pairs of autosomes, whereas the second subspecies shows extreme levels of variation in the location of the centromere (i.e., sub-median to terminal) on all of its 12 chromosomes (Shaw *et al.,* 1995). According to Mesa *et al.* (1990), in *Spathalium helios* the diploid chromosome number was found to be 22 in both male and female individuals. Furthermore, this species was found to possess a neo XY (male)/ XX (female) sex determining mechanism, with metacentric X chromosome and acrocentric Y chromosome. Additionally, few chromosomal studies on the longhorned grasshoppers indicate a wide range of chromosome numbers although, they show certain degree of karyotypic conservatism. For instance, the somatic chromosome number of family Tettigoniidae ranges from 20 to 35 (Turkoglu and Koca, 2002). Conversely, Dutrillaux *et al.* (2009) have reported a polyploidy case in one species of family Tettigoniidae. In this parthenogenetic species, *Saga pedo*, the somatic chromosome number was found to be 70. In contrary to the previous studies that proposed the species as if it is tetraploid, the DNA content analysis, currently, revealed that *Saga pedo* is a pentaploid species.

In contrast to family Tettigoniidae, acridid grasshopper illustrates a very stable karyotype over a wide range of species. Surprisingly, however, *Gastrimargus* spp. (family Acrididae, subfamily Oedipodinae) demonstrates a facet of "karyotypic mosaicism" in the male germ line (Channaveerappa and Ranganath, 1997). From the study conducted by these people on the genus *Gastrimargus,* it was found that the somatic and germ line cells of all the females and 82 of the 94 male individuals studied possessed 2n = 24 (22AA + XX) and 2n = 23 (22AA + X),

respectively. On the other hand, the germinal cells of the rest 12 male individuals showed five types of cells, i.e., 2n = 19, 21, 23, 25 and 27. This chromosome numerical inconsistency in some of the male germ line cells is attributed to aneuploidy.

Additionally, Turkoglu and Koca (2002) have examined chromosomes of two grasshopper species belonging to the subfamily Oedipodinae, i.e*., Oedipoda schochi schochi* and *Acrotylus insbricus*. The karyotype of male *O. schochi schochi* with diploid chromosome number of 25 (24AA + X and FN = 48) contains eight pairs of metacentric, two pairs of submetacentric, one pair of acrocentric, one pair of subacrocentric autosomes and a metacentric X chromosome, whereas male *A. insbricus* with 2n = 23 (22 + X and FN = 44) possesses metacentric autosomes and X chromosome. The grasshopper species of the family Acrididae are known to have a diploid chromosome number 2n = 23 (males) and 24 (females), with acrocentric chromosomes. Hence, the cause of the deviation either in chromosome number or chromosome morphology in *O. schochi schochi* is ascribed to centric fission and pericentric inversion, whereas in *Acrotylus insbricus* the variation in chromosome morphology is attributed to only pericentric inversion.

2.8.2. B-chromosomes in grasshoppers

Several investigations confirmed that B-chromosomes are frequently found in Orthoptera, where they sometimes constitute apparently stable polymorphisms (Henriques-Gil and Arana, 1990; Bakkali *et al.,* 1999). The grasshopper species, *Eyprepocnemis plorans,* was found to possess an extremely widespread polymorphism for B chromosomes (Bakkali *et al.,* 1999; Camacho *et al.,* 2003; Bakkali and Camacho, 2004). The B-chromosomes of this species have evolved through several stages of parasitic and near-neutral nature, most probably because of competition between the standard A and B-chromosomes. The possible ways to circumvent this intragenomic conflict can either be extinction of neutralized B-chromosome or its replacment by a mutant version of B-chromosome, i.e., being parasitic again (Camacho *et al.,* 2003).

2.8.2.1. Origin of B-chromosomes in grasshoppers

The origin of B-chromosomes in grasshoppers has been investigated by several people. Loreto and his colleagues (2008a) have pointed out the possible origin of acrocentric macro-B-chromosomes in two grasshopper species. According to their finding, the acrocentric macro B-chromosomes found in *Rhammatocerus brasiliensis* (Acrididae, Gomphocerinae) and *Xyleus*

discoideus angulatus (Romaleidae, Romaleinae) are highly similar to their X chromosome in terms of morphology, size and pycnosis. In spite of this fact, the results of FISH experiments using 45S and 5S rRNA probes revealed that, in both species, the most likely origin of the B-chromosome is autosomal chromosome. In addition, Cabrero *et al.* (2003) tried to scrutinize the multiregional origin of B-chromosomes in *Eyprepocnemis plorans* using three molecular markers, 18S-5.8S-28S rDNA, 5S rDNA and a 180bp sat DNA. From the study they found that the B-chromosomes in this species originated independently in Eastern (Caucasus) and Western (Spain and Morocco) populations. The Eastern B chromosomes are derived from the smallest autosomes, which is the only A chromosome that possess all the three markers whereas, the B chromosomes from Western populations lack 5S rDNA and were most likely derived from the X chromosome.

2.8.2.2. Effect of B-chromosomes

Despite the fact that B-chromosomes seem to appear neutral with no visible negative effect in the individuals where they exist, different people tried to investigate the possible effect of this extra chromosomes. Hewitt and East (1978) have tried to determine the overall influence of B-chromosomes in grasshopper species, *Myrmeleotettix maculatus*. They have found that B-chromosomes produce a variety of chromosomally mutant embryos, many of which were arrested prior to diapause and only a few developed to hatching stage.

Several other studies have also been done to investigate the possible effect of B-chromosomes in another grasshopper species, *Eyprepocnemis plorans*. These were found to decrease egg fertility, i.e., reduce the proportion of eggs containing embryo (Munoz *et al.,* 1998). In addition, the presence of B-chromosome in this species was found to cause absence of sexual selection and random mating. These effects of B-chromosomes are believed to occur only due to their physical presence rather than their genetic activity, since they are devoid of active genes because of their heterochromatic nature (Lopez-Leon *et al.,* 1992). Furthermore, the presence of B-chromosomes is greatly linked with an increase in chiasma frequency, (Camacho *et al.,* 2002; Riera *et al.,* 2004). This is believed to be favored by natural selection because it increases the proportion of recombinant progeny which are resistant to both B-chromosome effects and B-accumulation in the germ line (Camacho *et al.,* 2002).

2.8.3. Nucleolar organizer regions (NORs) in grasshopper chromosomes

NORs have been identified in different grasshopper species. In grasshopper (*Chorthippus parallelus*), an active NOR is found at the distal end of the X-chromosome (Flanagan *et al.,* 1999). However, in the case of some Neotropical gomphocerine grasshopper species, the NOR (rDNA) is restricted to autosomal pairs (Loreto *et al.,* 2008b). In *Eyprepocnemis plorans*, NOR has been found to exist distally on the B-chromosome (Lopez-Leon *et al.,* 1995). And hence, it is a useful chromosome marker for interspecific comparisons, and has been frequently used in insects (Loreto *et al.,* 2008b).

3. OBJECTIVE OF THE STUDY

3.1. General Objective:

To study the chromosomes of some grasshopper species, Orthopteran species, found in some parts of central Ethiopia and generate cytogenetic data that would be helpful for cytotaxonomy and evolutionary study of grasshoppers.

3.2. Specific Objectives:

► To determine the chromosome number of the ten grasshopper species

► To describe chromosome morphology of the ten grasshopper species

► To compare the karyotypic relationships between the studied grasshopper species

4. MATERIALS AND METHODS

4.1. Specimen collection sites and methods of collection

The grasshopper specimens were collected from eight localities in central Ethiopia (Fig. 1). The geographical locations (i.e., latitudes, longitudes and altitudes) of each locality and the number of specimens collected from each locality are presented in Table 3.

The specimens were captured from their natural habitat specifically, fallow farmlands, wheat farmland, grasslands and forests, using insect net. Hand collection was also adopted in the situation where using insect net became difficult.

Table 3. Name of localities, coordinates and altitudes of collection sites and the number of male and female grasshopper specimens collected from each locality.

Locality	Coordinate (latitude, longitude)	Altitude (masl)	Number of grasshoppers		
			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	18	21
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	58

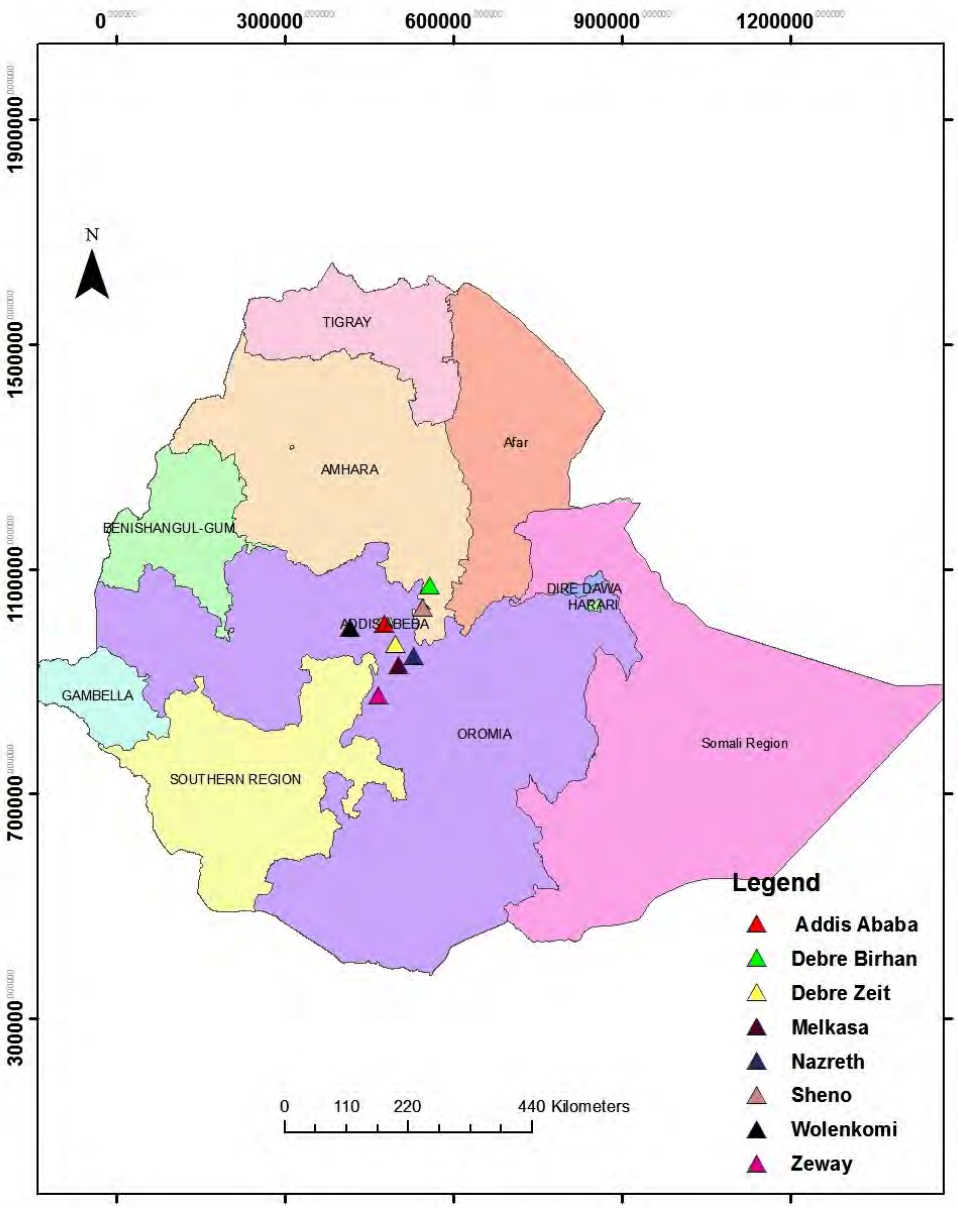


Fig. 1. Map of Ethiopia showing the locations of specimens collection sites (Source: Ethio-GIS database)

4.2. Preservation of the specimens

Each grasshopper specimen was pinned using an ordinary pin that is placed vertically through the thorax, slightly to the right of the center. The studied specimens were properly labelled with specimen code, name of collection locality, date of collection and sex of the individual specimen. Pinned and labelled specimens were stored in a sealed box and kept in the Entomology Laboratory, College of Natural sciences, of Addis Ababa University, for further reference. The

preservation was done after the internal organs were removed carefully for chromosome preparation by opening the animal ventrally following colchicine treatement.

4.3. Taxonomic identification of grasshopper specimens

For the taxonomic identification, information from different literatures including books by Stretch-Lilija (1977), Rentz (1991) and Picker *et al.* (2004), and a web page known as bugguide (http://bugguide.net) were consulted.

4.4. Preparation of somatic chromosomes

4.4.1. Metaphase chromosome slide preparation

Using fine needle (insulin needle) the animal was injected 0.05 – 0.2 ml of 0.1% colchicine in the thorax and abdominal regions. After 6 – 8 hours following colchicine injection, the specimen was over anesthetized by placing it in a glass jar containing a piece of cotton soaked in ethyl-ether. The whole gut (front-gut, mid-gut and hind gut) was dissected out and meshed in about 1 – 2 ml of hypotonic solution (0.075 M KCl) by grinding the tissue with a glass rod in Cyracus dish. 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 it 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 it was centrifuged again for about 3 minutes. This particular step of removing the supernatant, addition of fixative and centrifugation, was repeated twice or more times 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.

Finally, some amount of the cell suspension was drawn with a Pasteur pipette and several drops were splashed on the microscope glass slides, inclined at about 45 degree, from a height of about half-a-meter. The slides were allowed to air-dry at room temperature, until needed for staining.

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

4.4.2. Staining and mounting of the slides

Air-dried slides were stained with Giemsa stain in Sorenson's phosphate buffer solution (pH=6.8) for about 30 minutes. The buffer was prepared by dissolving a phosphate buffer tablet in distilled water. The stained slides were rinsed in distilled water and allowed to air-dry. Slides with good chromosome spreads were screened through microscopic observation and mounted under a 22mm by 50mm cover slip in Depex mounting medium.

4.4.3. Analysis of Karyotype

Cells containing complete and well spread chromosome complement were photographed with a total magnification of 1000× using a camera-fitted microscope. From two to six mitotic metaphase cells per individual were analyzed for the karyotypic description. Karyotypes were constructed from photographic prints (photomicrographs) of somatic metaphase plate with good chromosome spread. The photomicrographs of mitotic metaphase chromosome spreads were edited using Image J software which was downloaded freely from http://rsb.info.nih.gov/ij/.

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

5. RESULT

5.1. Taxonomic identification

The identification of specimens to the species level was difficult due to unavailability of sufficient previous studies on the taxonomy and species composition of Ethiopian grasshoppers. As a result of these difficulties, identification was possible only to the genus level for most of the specimens, to the family level for two specimens and to the species level only for one specimen. Generally, all the collected specimens were identified to belong to ten different taxa which are further identified to belong to two families – Acrididae and Tetrigidae (Table 4). The photographs of the representatives of the identified grasshopper specimens are shown in Fig. 2.

Table 4. Results of taxonomic identification of the grasshopper specimens used in the present study.

Family	Subfamily	Genus	Species	Collection site	Number of grasshoppers	
					Male	Female
Acrididae	Cyrtancanthacridinae	*Acanthacris*	not identified	Wolenkomi	6	__
	Oedipodinae	*Paracinema*	*Paracinema tricolor*	Debre Birhan	__	5
				Addis Ababa	__	3
		Gastrimargus	not identified	Zeway	__	1
				Melkasa	__	1
				Debre Birhan	4	7
		Acrotylus	not identified	Nazreth	__	5
		Pardalophora	not identified	Nazreth	3	2
	Acridinae	*Acrida*	*Acrida* spp.-1 (not identified)	Debre Zeit	1	__
				Sheno	__	1
				Melkasa	__	3
			Acrida spp.-2 (not identified)	Melkasa	__	1
	not identified	__	Designated as Acrididae MU	Melkasa	__	1
	not identified	__	Designated as Acrididae WDZU	Wolenkomi	__	2
				Debre Zeit	__	1
Tetrigidae	Tetriginae	*Paratettix*	not identified	Nazreth	__	11

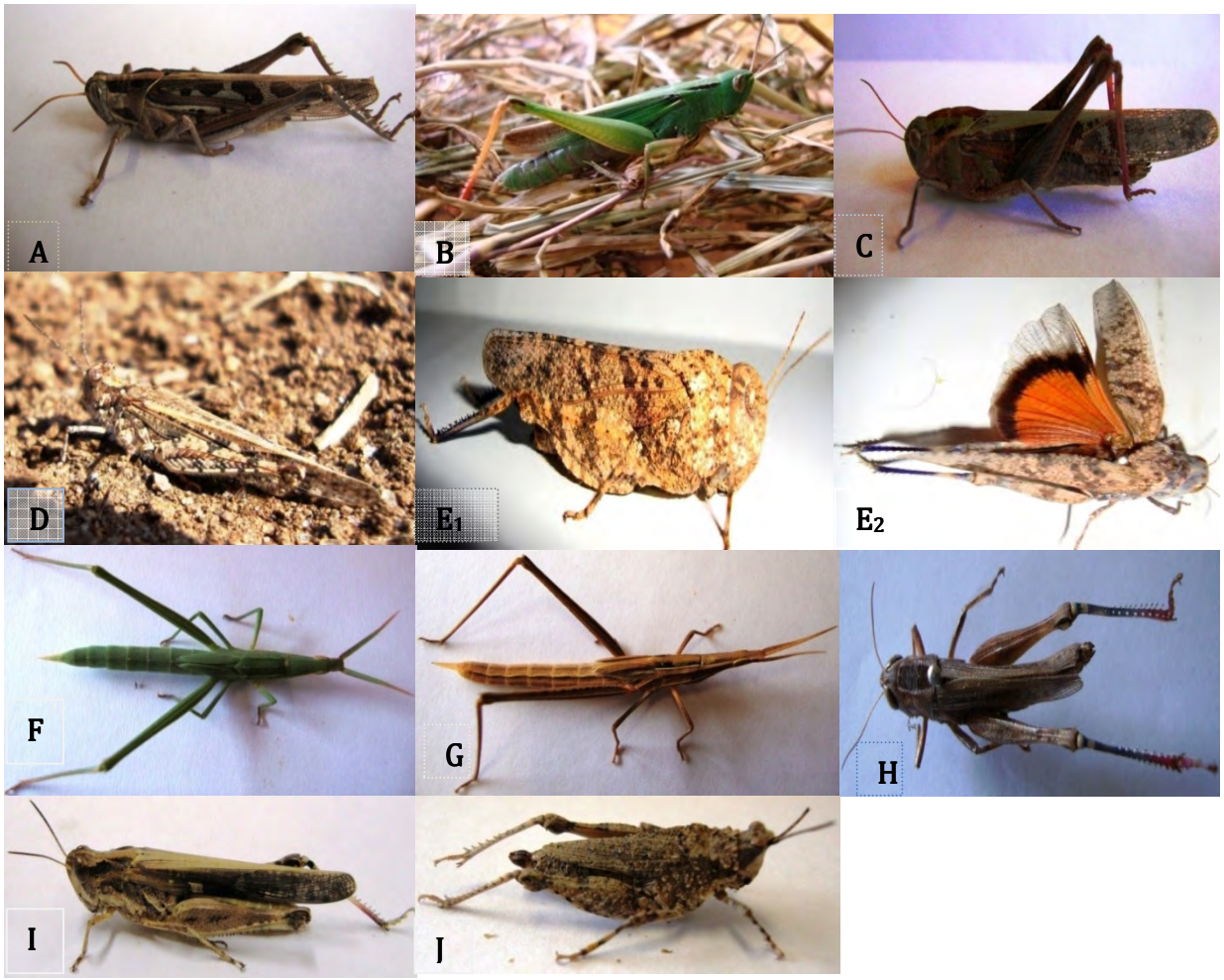


Fig. 2. Pictures of grasshopper specimens belonging to different species collected for the study from different localities of Ethiopia: (A) *Acanthacris* spp., (B) *Paracinema tricolor*, (C) *Gastrimargus* spp., (D) *Acrotylus* spp., (E_1 and E_2) *Pardalophora* spp., (F) *Acrida* spp.-1, (G) *Acrida* spp.-2, (H) Acrididae MU, (I) Acrididae WDZU, (J) *Paratettix* spp.

Note: In figure E_2, the color of the hind wing, the reddish color with thick dark or brown band, is the characterstic feature of this species (http://bugguide.net).

5.2. Karyotypic description

5.2.1. Family Acrididae

5.2.1.1. Subfamily Cyrtancanthacridinae

5.2.1.1.1. Karyotype description of *Acanthacris* spp.

The representative mitotic metaphase chromosome spreads (Fig. 3), karyotype (Fig. 4) and meiotic bivalent spreads (Fig. 5) of six male individuals of *Acanthacris* spp. that were collected from Wolenkomi are presented. Chromosome numerical instability has been noticed in the gonadal tissue of these specimens. Diploid cells with the normal chromosome number were found to possess 23 telocentric chromosomes (22 autosomes + X), and the autosomal fundamental number (FNa) was found to be 22. When chromosomes, in the normal complement, are arranged according to their decreasing size, they showed a gradual decrement. In comparison, the 11^{th} and 12^{th} chromosomal pairs were found to be significantly smaller than the rest and the average lengths of the homologues of these pairs were 2.11 µm and 1.84 µm, respectively (Appendix 2). Furthermore, the 4^{th} chromosome in the karyotype is with small second arm and has no a homologue. Hence, it is assumed to be an X chromosome. The rest of the chromosomes also show tiny second arms (Fig. 3A). Regarding chromosome numerical instability, cells with chromosome complements of 2n = 22, 24 and 27 have been found (Fig. 3B – D). As shown in the karyotypes (Fig. 4B and C), in cells with 2n = 22 chromosomes, one homologue from the third chromosomal pair is missing whereas in those with 2n = 24 the homologues of the second chromosomal pair are occurring in three copies.

Meiotic chromosomes are presented in Fig. 5. Like as in the somatic cells, variation in the chromosome number has been observed in meiocytes (Fig5A – E). Meiocytes with 10II + X+1 (total = 22 chromosomes), 11II + X + 1 (total = 24 chromosomes), 12II + X + 1 (total = 26 chromosomes) were observed. One of the chromosomes designated above as +1 appeared to be unique. As could be seen from the condensation patterns, this chromosome appears to consist almost wholly of heterochromatin (Fig.5A – E). It is of about same size as the X chromosome, and like the X chromosome it condenses ahead of the rest of the chromosomes (positively heteropycnotic). Sometimes, it bends on itself (Fig. 5D) other times it is straight (Fig. 5B, C, E). In some meiotic cells this extra unpaired chromosome shows some sort of association with the X

chromosome (Fig. 5B, C) while in the others it exists independently (Fig. 5A, D, E). This extra univalent chromosome is presumed to be a B-chromosome.

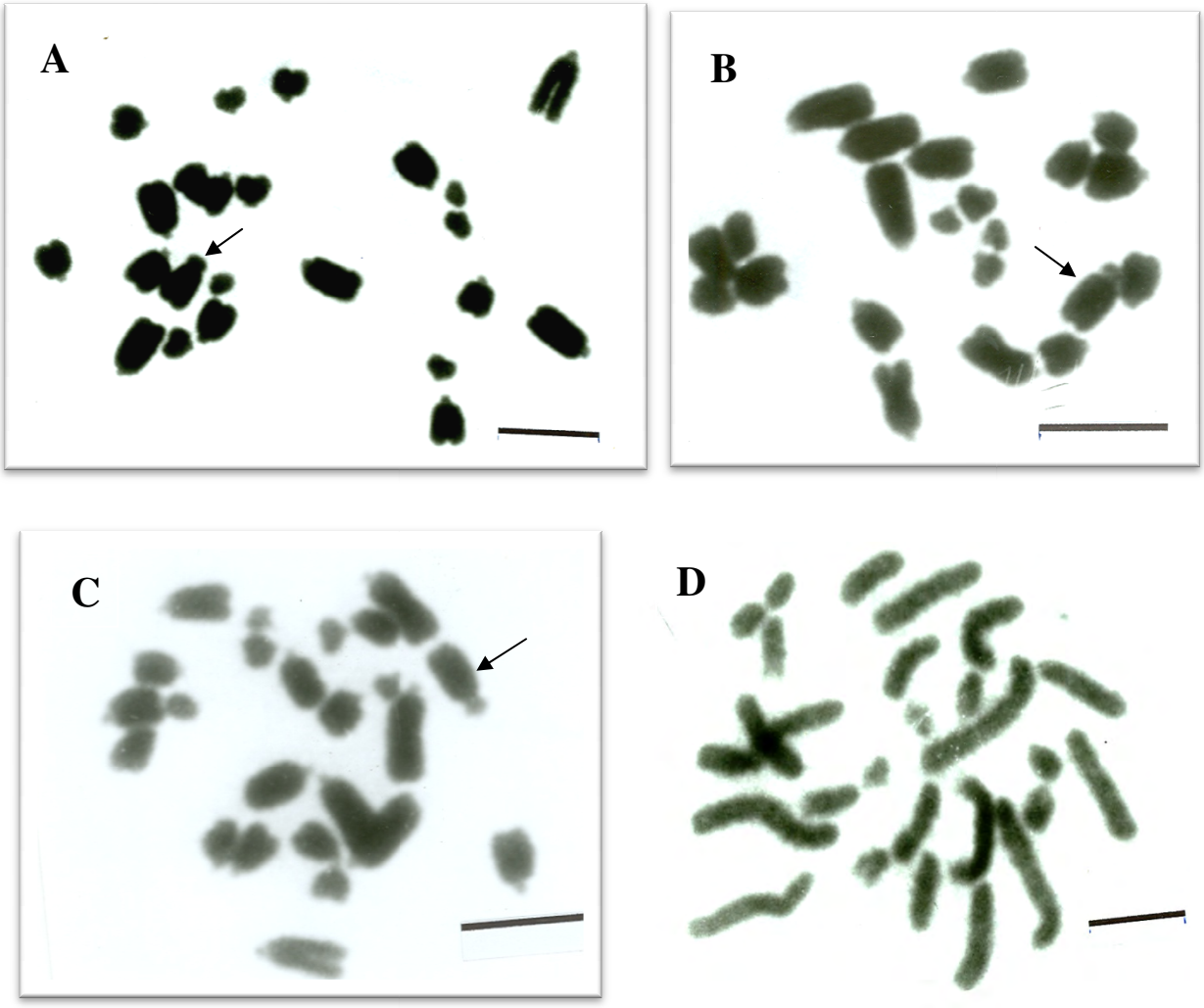


Fig. 3. Somatic metaphase chromosomes of *Acanthacris* spp. showing variable number of chromosomes in different cells. (A) A cell with 2n = 23, (B) A cell with 2n = 22, and (C) A cell with 2n = 24, (D) A cell with 2n = 27 chromosomes. Arrows point to the biarmed chromosome, which is presumed to be the X chromosome. Bar = 10 µm

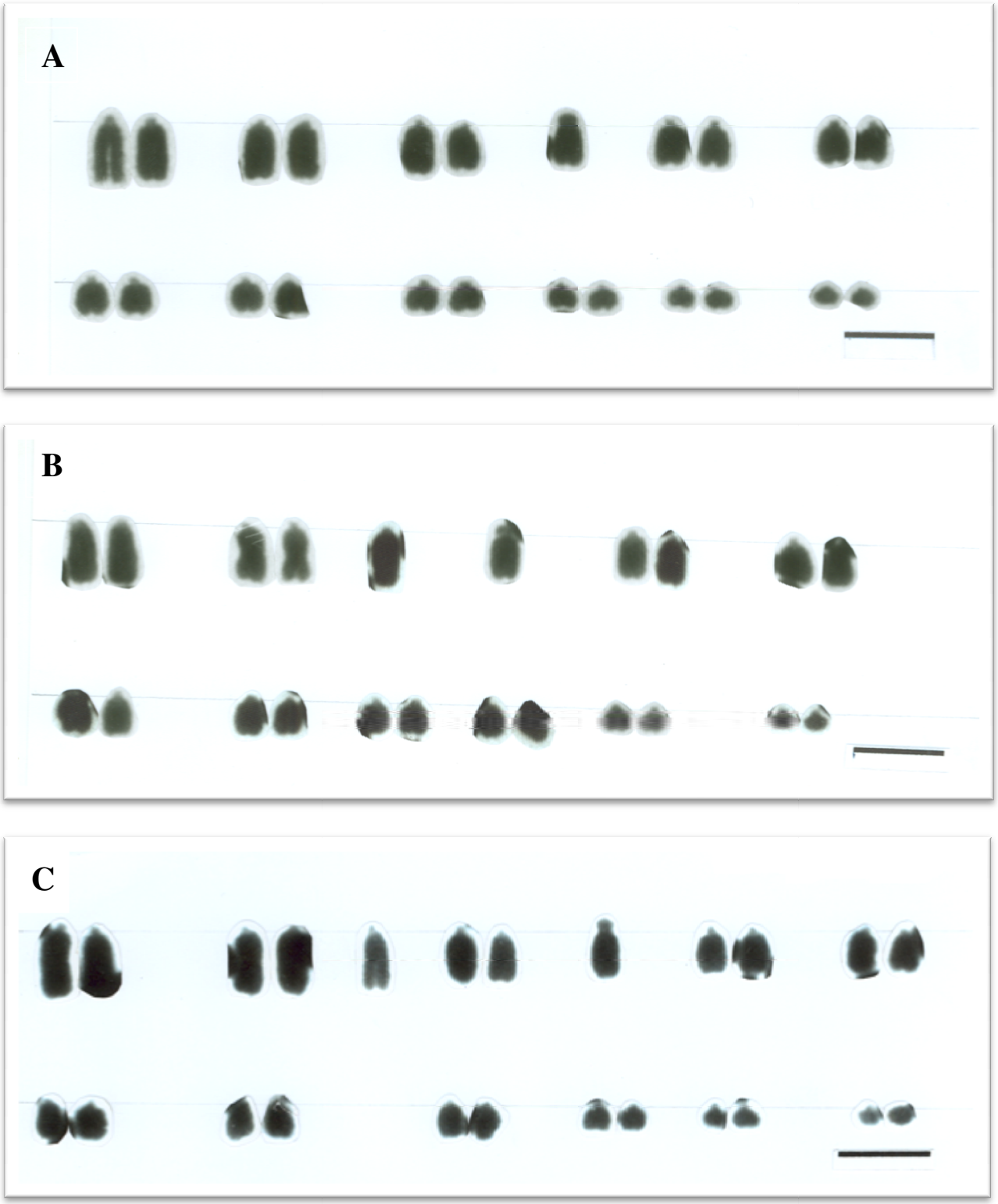


Fig. 4. Karyotype of *Acanthacris* spp. showing cells with different chromosome numbers. (A) from a cell with 2n = 23, (B) from a cell with 2n = 22 and (C) from a cell with 2n = 24. Bar = 10 µm

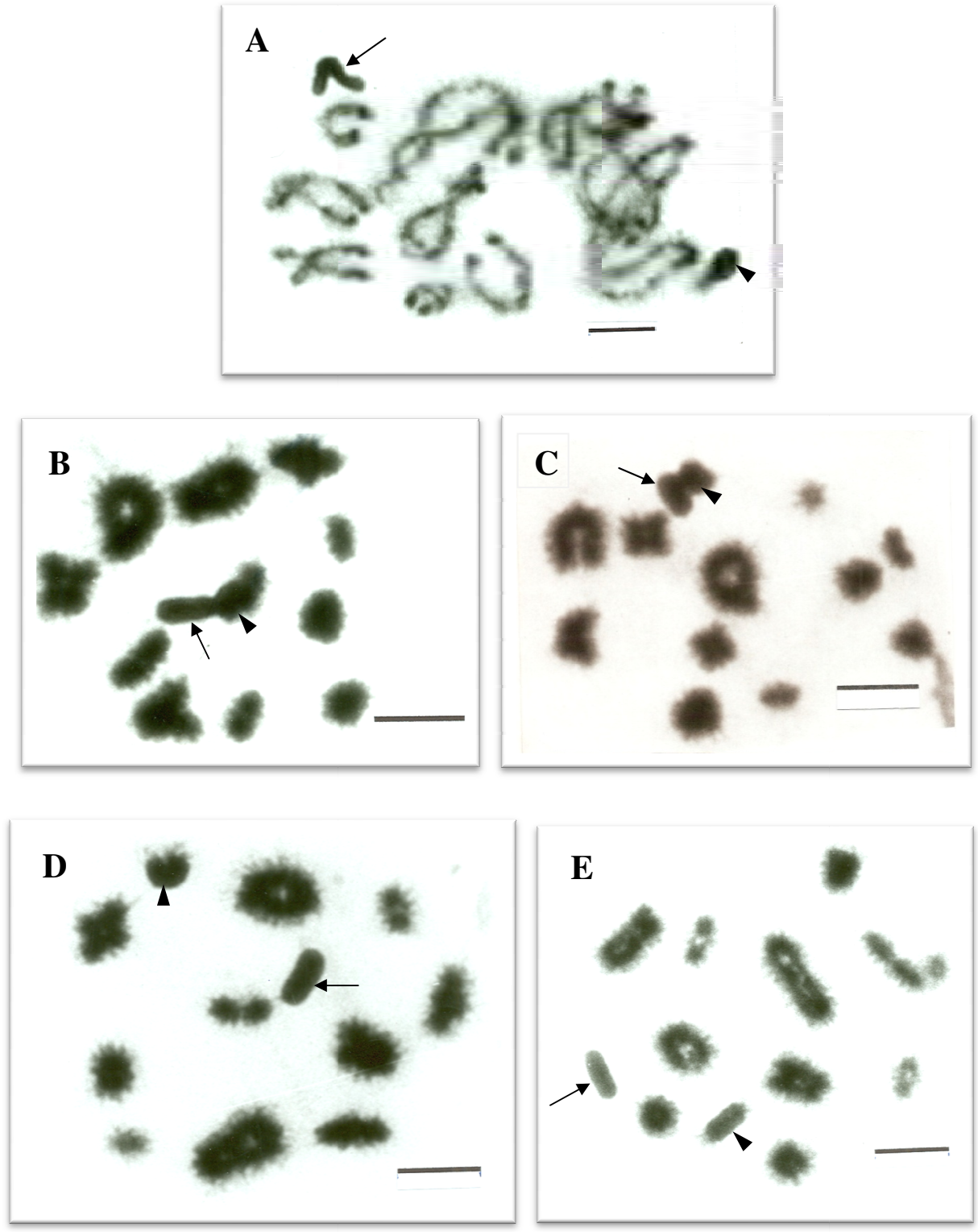
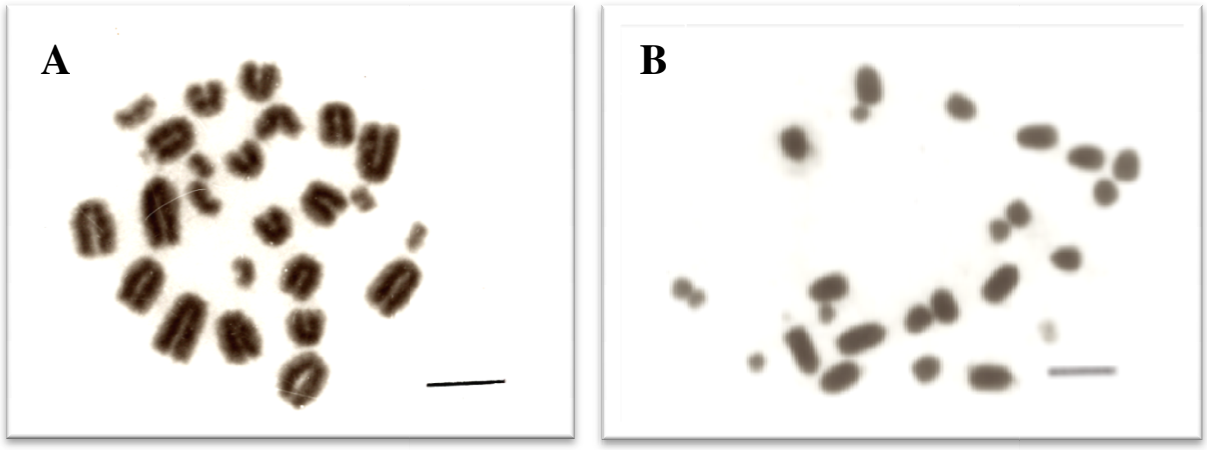


Fig. 5. Meiotic chromosomes of *Acanthacris* spp. (A) A cell in diplotene stage with heavily staining extra chromosome (arrow head) and X chromosome (arrow) (10II + X + 1, 2n = 22), (B) A cell with 11II + X + 1 (2n = 24). The extra chromosome is associated with X chromosome end

5.2.1.2. Subfamily Oedipodinae

5.2.1.2.1. Karyotypic description of *Paracinema tricolor*

Karyotypic analysis was made for eight female specimens of this species collected from Addis Ababa and Debre Birhan. A representative somatic metaphase chromosome spread and a karyotype are depicted in Fig. 6. The karyotype of these specimens consisted of 12 pairs of telocentric chromosomes (2n = 24). Since all the chromosomes are uni-armed, the autosomal fundamental number is equal to the number of autosomal chromosomes (FNa = 22). When the chromosomes are arranged according to their decreasing size order, a gradual length differences observed from chromosome pair 1 to 9. However, a sharp size difference exists between the 9th chromosomal pair and the last three pairs (pair 10, 11, 12).



to end. (C) A cell with 12II + X + 1 (2n =26). The X and the extra chromosome are associated laterally. (D) A cell with 10II + X + 1 (2n = 22), the extra chromosome is bent on itself. (E) A cell containing 11II + X + 1 (2n = 24), the extra chromosome remains straight and indipendently of the X chromosome. Arrows indicate X chromosome and arrow heads indicate the extra chromosome. Bar = 10 µm

Fig. 6. Somatic metaphase plates and karyotype of *Paracinema tricolor* specimens. (A) Metaphase plate of a specimen from Addis Ababa; (B) Metaphase plate of a specimen from Debre Birhan; (C) Karyotype of a specimen from Addis Ababa. Bar = 10 µm

5.2.1.2.2. Karyotypic description of *Gastrimargus* spp.

The chromosomes of 4 male and 9 female specimens of *Gastrimargus* spp. captured from Melkasa, Zeway and Debre Birhan were studied, and representative metaphase cells with good chromosome spread (Fig. 7) and karyotypes (Fig. 8) are presented below.

All the analyzed male and female individuals of the genus *Gastrimargus* from the three sites were found to have chromosome number of 2n = 23 and 2n = 24, respectively, with all being telocentric in morphology (Fig. 7A, B, C, D). The size of the chromosomes is decreasing in a gradual manner from pair 1 to 10, while pair 11 and 12 are distinctly smaller than the rest of the pairs but the two pairs are more or less of equal size to each other (Fig. 8A, B, C). Surprisingly, in some male individuals from Debre Birhan, gametic cells from the testis at anaphase-I contained dyads in which the sister chromatids have completely opened apart (180°) and show apparent resemblance to metacentric chromosomes, with one sister chromatid forming one arm and the other sister chromatid forming the other arm held together at the center by a centromeric region (Fig. 9A, B, C). In such cells, the pole in which the X chromosome is included contains 12 such chromosomes, whereas the opposite pole contains 11 such chromosomes.

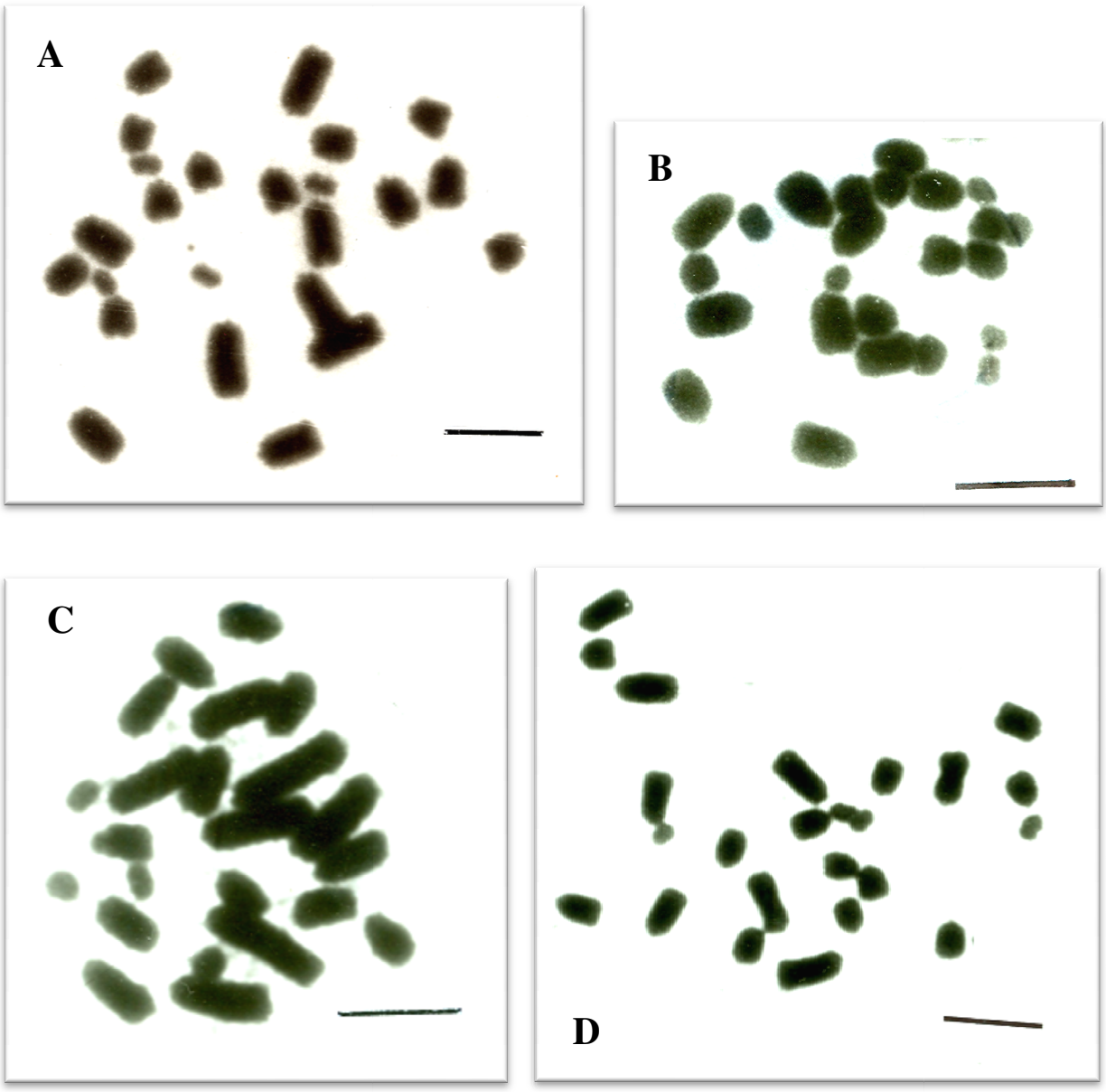


Fig. 7. Somatic metaphase chromosomes spreads of *Gastrimargus* spp. (A) Female specimen from Melkasa; (B) Female specimen from Zeway; (C) Male specimen from Debre Birhan and (D) Female specimen from Debre Birhan. Bar = 10 µm

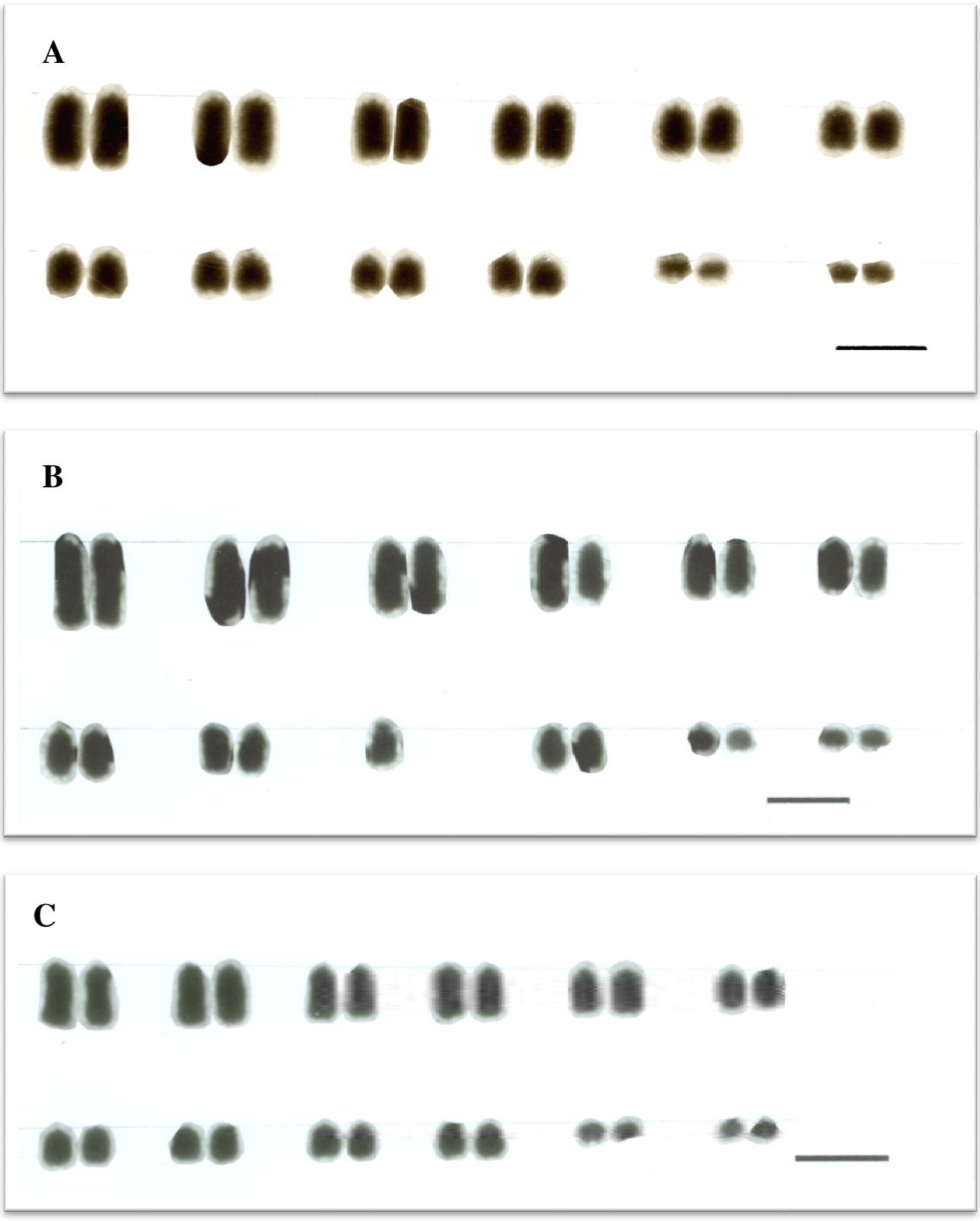


Fig. 8. Karyotype of *Gastrimargus* spp. (A) Female specimen from Melkasa; (B) Male specimen from Debre Birhan (the unpaired chromosome is assumed to be the X chromosome); (C) Female specimen from Debre Birhan. Bar = 10 µm

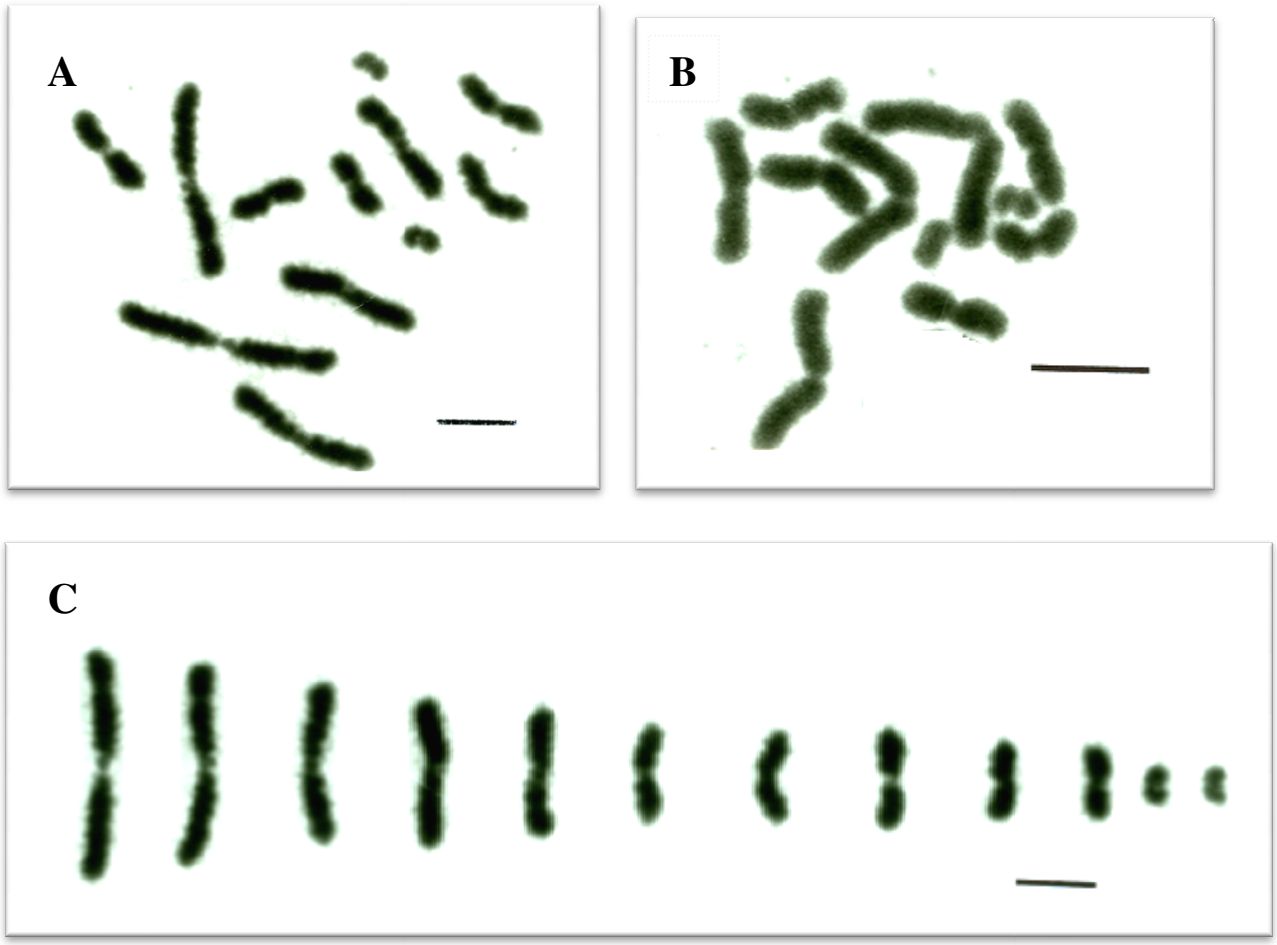


Fig. 9. Dyads at anaphase-I stage of a male specimen of *Gastrimargus* spp. from Debre Birhan with the dyads apparently looking like metacentric chromosomes due to the opening apart of the sister chromatids. (A) Group of 12 dyads including the X chromosome; (B) Goup of 11 dyads consisting of only autosomes; (C) Dyads arranged in decreasing size order in the manner of a karyotype. The two halves of each dyad are sister chromatids. It is evident that the last two chromosomes are distinctly smaller than the rest of the chromosomes. Bar = 10 µm

5.2.1.2.3. Karyotypic description of *Acrotylus* spp.

Five female specimens of this species were collected from Nazreth. The somatic metaphase spread and partial karyotype is presented in Fig. 10. The specimens possess a karyotype with 2n = 24 telocentric chromosomes and FNa = 22. The chromosome complement is composed of large, medium and small chromosomes. Only a partial karyotype of this species was constructed because a good mitotic metaphase chromosome spread was not obtained.

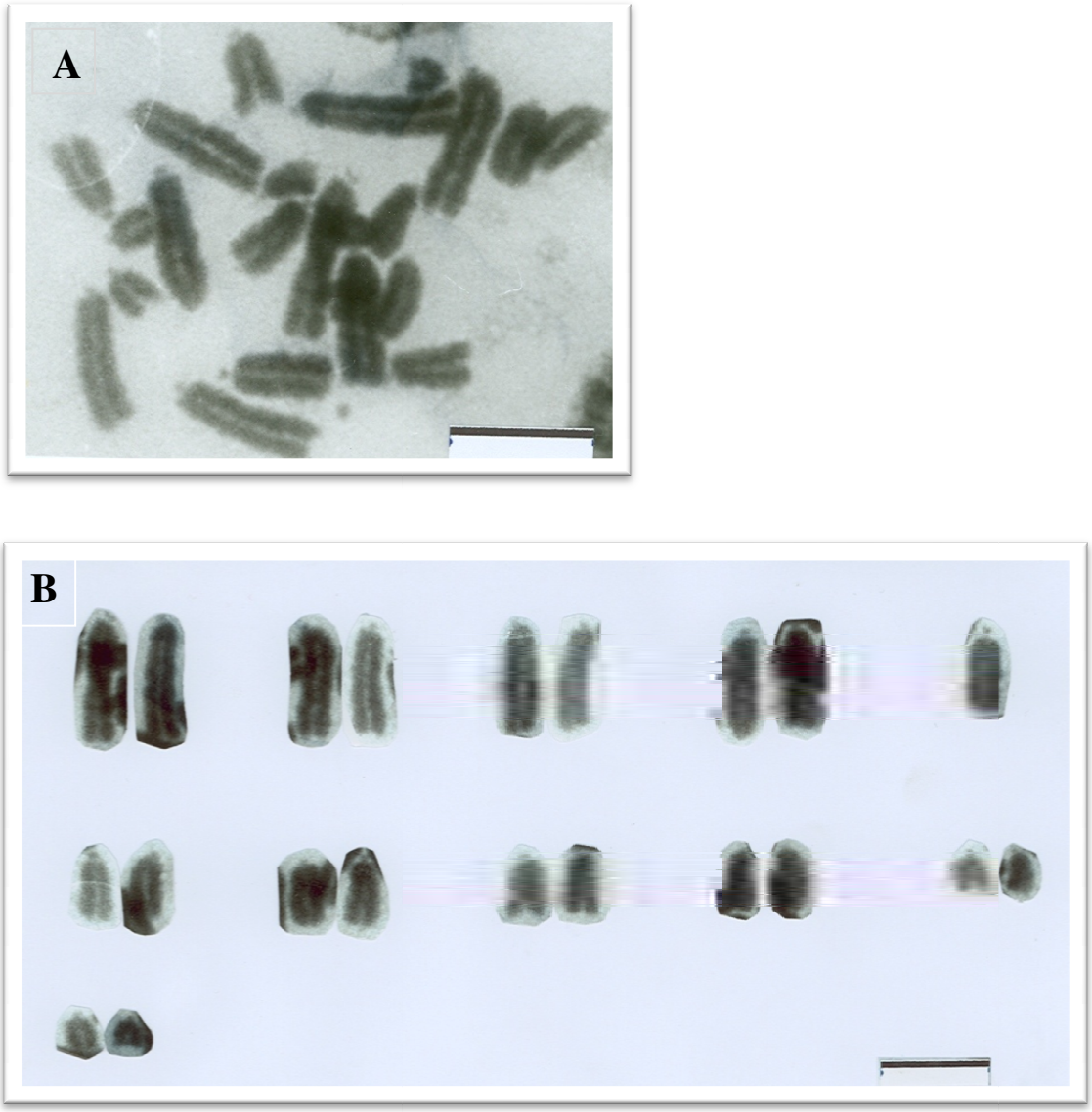


Fig. 10. Partial somatic metaphase chromosome spread (A) and partial karyotype (B) of a female specimen of *Acrotylus* spp. Bar = 10 µm

5.2.1.2.4. Karyotypic description of *Pardalophora* spp.

Cytological study has been carried out on a total of five specimens (3 males and 2 females) of *Pardalophora* spp. The count of chromosomes in mitotic metaphase spreads (Fig. 11A, B) showed the diploid number to be 23 in males and 24 in females. The autosomal fundamental number is 22 (FNa = 22). All the chromosomes of this species are telocentric. A sharp size reduction is observed in pair 10 and 11 relative to the rest of the pairs. Thus, there are two pairs (pair 11 and 12) of smaller chromosomes in the karyotype. When the chromosomes are arranged

in decreasing order (Fig. 11C), the one without a homologous pair is assumed to be an X chromosome.

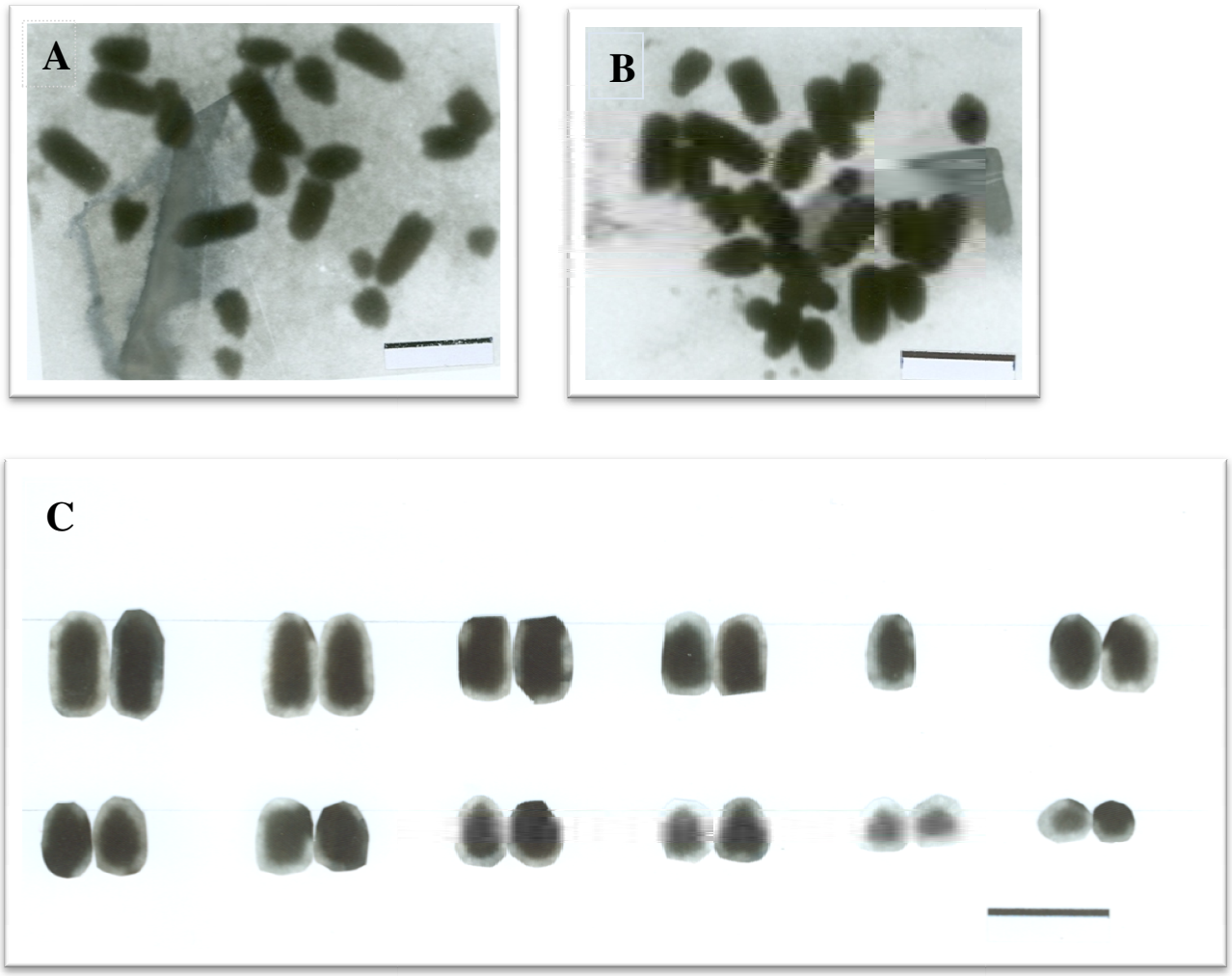


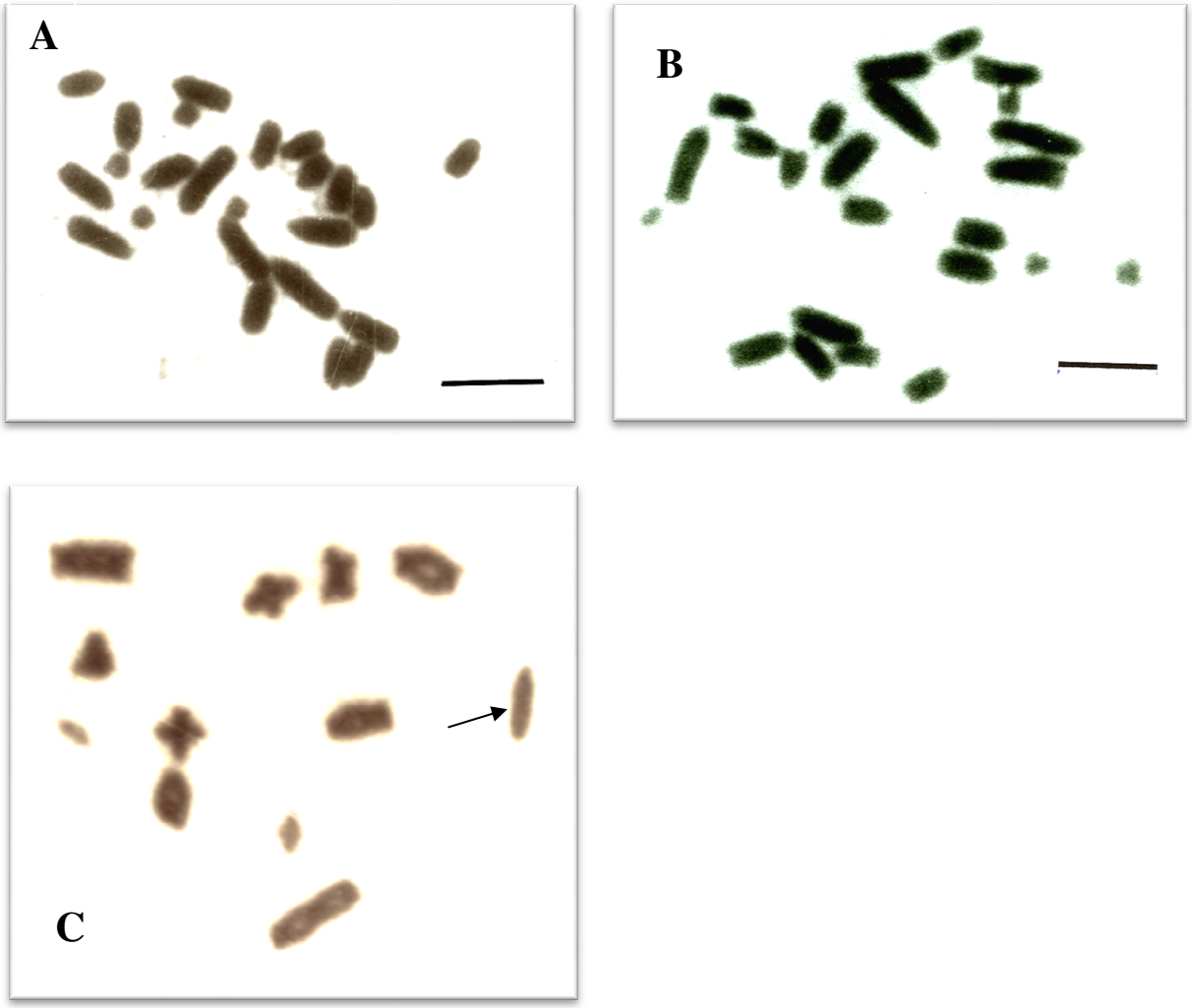
Fig. 11. Mitotic metaphase chromosome spreads of male (A) and female (B) *Pardalophora* spp. from Nazreth, and karyotype (C) of a male specimen. Bar = 10 µm

5.2.1.3. Subfamily Acridinae

5.2.1.3.1. Karyotypic description of *Acrida* spp.-1

Four females and one male specimens of *Acrida* spp.1, captured from Sheno, Melkasa and Debre Zeit, were analysed. The mitotic metaphase chromosome spreads from female specimens (Fig. 12A, B), meiotic bivalent spread from testis (Fig. 12C) and a representative karyotype (Fig. 12D) are presented below. The karyotype of the examined female specimens of *Acrida* spp.-1 is consisting of 2n = 24 telocentric chromosomes and hence, the autosomal fundamental number is

equivalent to the number of autosomes (FNa = 22). When the chromosomes are arranged in decreasing order they show gradual decrement up to the 10th chromosomal pair. However, the last two pairs of chromosomes (11th and 12th pairs) are significantly smaller, and hence they are so easily distinguishable from the rest of the chromosomes. Due to the lack good mitotic metaphase chromosome spreads in the male, only the meiotic bivalent spread is presented to show the diploid chromosome number (11 bivalents + X chromosome) which is 2n = 23. Here again, the two small pairs of chromosomes are recognized by their formation of two distinctly small bivalents (Fig. 12C).



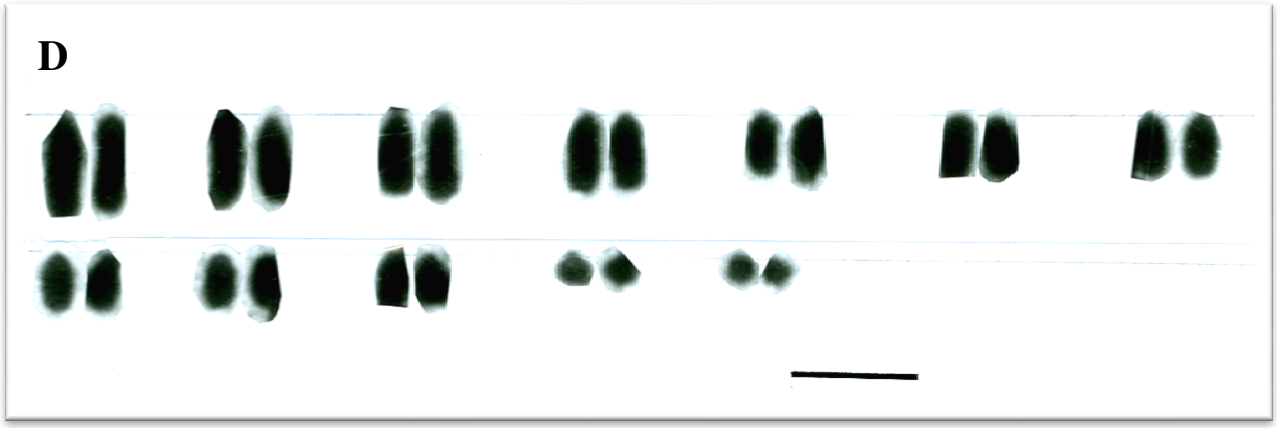
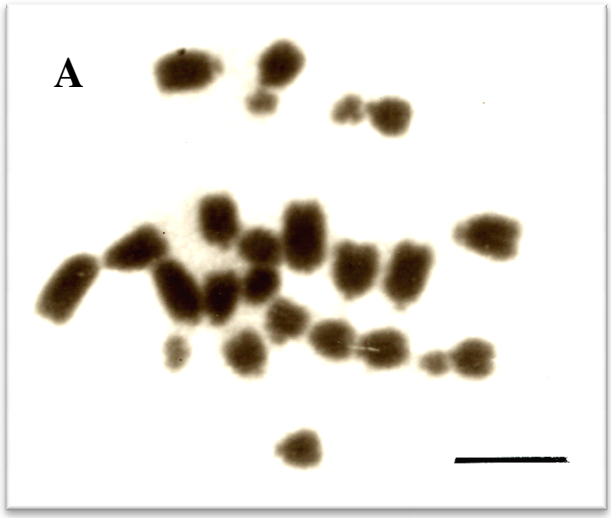


Fig. 12. Chromosomes of *Acrida* spp.-1. (A) Mitotic metaphase chromosome spreads of female individuals from Melkasa and (B) From Sheno; (C) Meiotic bivalents of a male specimen from Debre Zeit (arrow indicates X chromosome); (D) Karyotype of a female specimen from Melkasa. Bar = 10 µm

5.2.1.3. 2. Karyotypic description of *Acrida* spp.-2

Cytological examination of the female material captured from Melkasa showed 2n = 24 telocentric chromosomes (Fig. 13) and FNa = 22. When the chromosomes in the complement are arranged into putative homologous pairs of decreasing order, they show gradual size differences up to the 10^{th} pair whereas pair 11 and 12 are distinctly smaller. The latter two have average length of 1.84 and 1.58 µm, respectively (Appendix 2). Furthermore, the chromosomes show miniature arms.



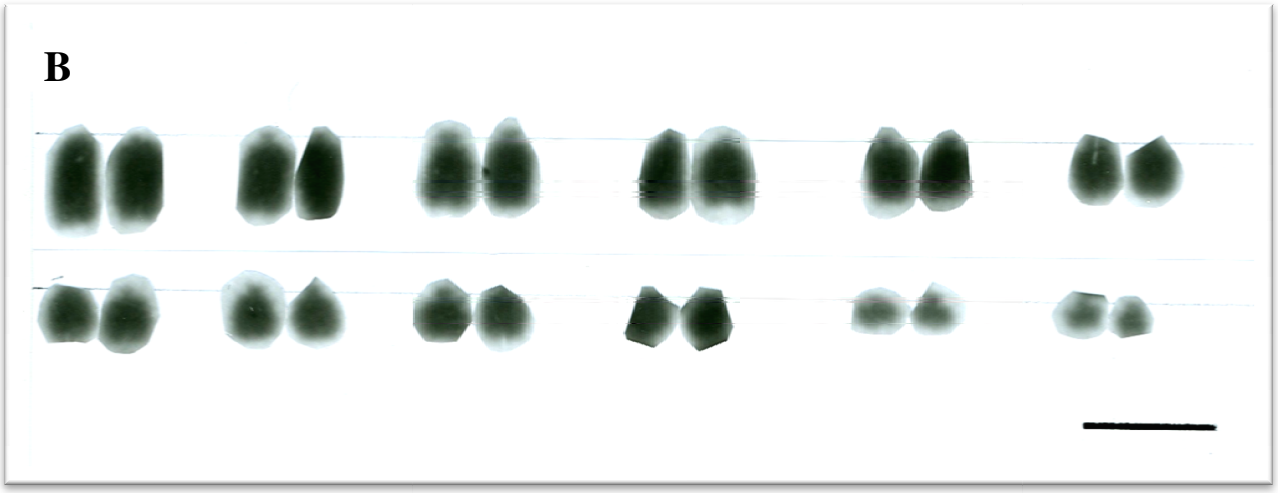
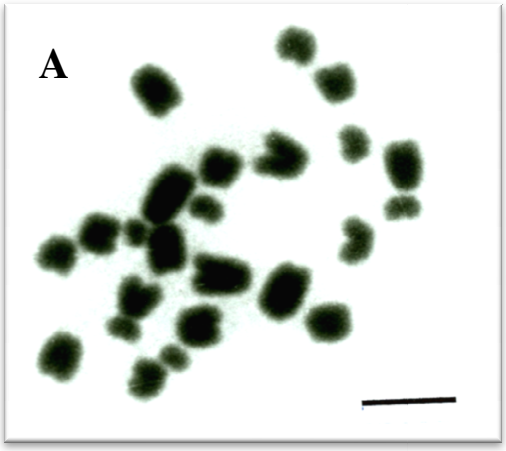


Fig. 13. Somatic chromosome spread (A) and karyotype (B) of a female specimen of *Acrida* spp.-2. Bar = 10 µm

5.2.1.4. Karyotypic description of specimen from Melkasa (Acrididae MU)

Due to the ambiguity or vagueness of taxonomically important characters, the identification of this female specimen to the genus level became difficult. As a result, it has only been identified to the family level (family Acrididae). Thus it is designated as Acrididae MU to mean unidentified specimen of the family Acrididae collected from Melkasa site, where M stands for Melkasa and U = unidentified. As it can be observed from mitotic metaphase chromosome presented in Fig. 14A, this taxa contains 2n = 24 telocentric chromosomes. The 12 pairs of chromosomes in the karyotype (Fig. 14B) are decreasing in a gradual manner, and unlike karyotypes of other specimens considered earlier, the last three or two pairs are not distinctly smaller than the rest of the pairs.



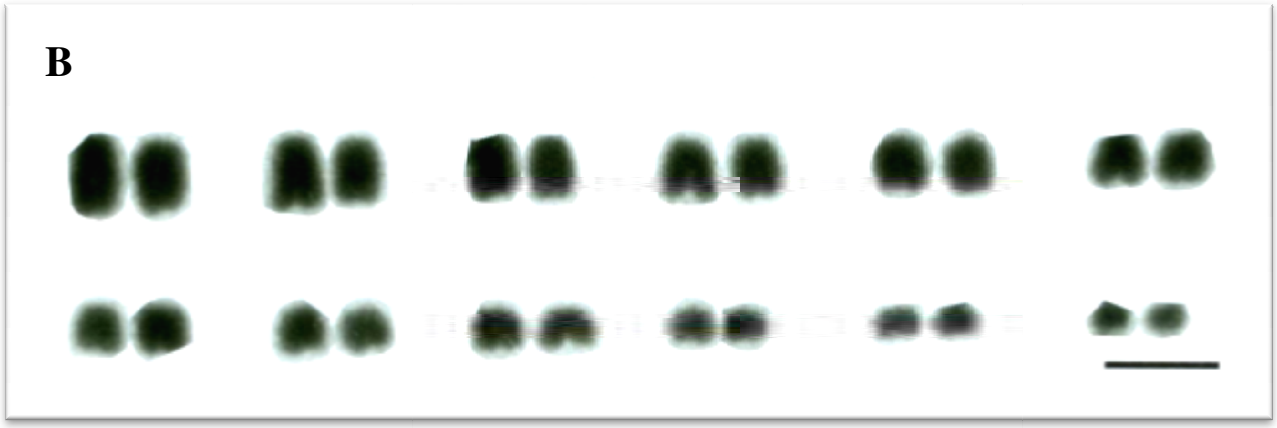


Fig. 14. Mitotic metaphase chromosome spread (A) and karyotype (B) of a female Acrididae - MU specimen from Melkasa. Bar = 10 µm

5.2.1.5. Karyotypic description of Acrididae WDZU specimens from Wolenkomi and Debre Zeit

Three female specimens were collected from Wolenkomi and DebreZeit. Like Acrididae MU (above), the taxonomic identification of these materials was also intricate. Hence, they have been identified only to the family level, and were designated as Acrididae WDZU by affixing WDZU to the family name Acrididae, where W stands for Wolenkomi, DZ for Debre Zeit and U for unidentified. This means that taxonomically unidentified specimens of the family Acrididae collected from Wolenkomi and Debre Zeit.

These specimens were found to have a chromosome number of 2n = 24, and FNa = 22 (Fig. 15A – C). The karyotype (Fig. 15C) contains three small (10^{th}, 11^{th} and 12^{th} pairs) telocentric chromosome pairs. The three pairs of smaller chromosomes are more or less of equal size, while the rest of the pairs decrease in size in a gradual manner.

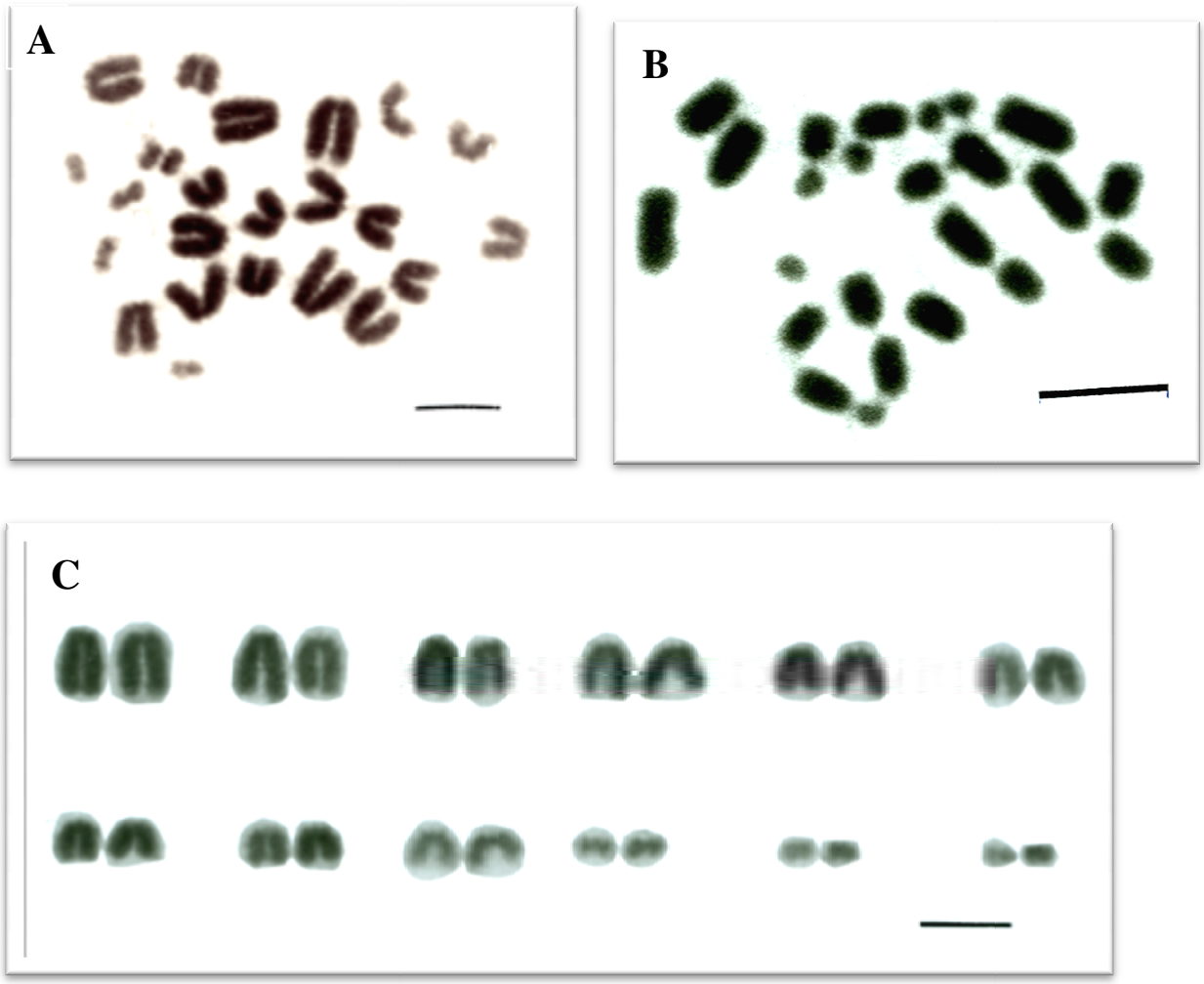


Fig. 15. Mitotic metaphase chromosome spreads of female Acrididae WDZU specimens from Wolenkomi (A) and Debre Zeit (B), and karyotype of the specimen from Wolenkomi (C). Three pairs are distinctly smaller than the rest pairs of chromosomes. Bar = 10 µm

5.2.2. Family Tetrigidae

5.2.2.1. Karyotypic description of *Paratettix* spp.

Karyotypic description was made from the specimens of this species collected from Nazreth. The chromosome complement (Fig. 16) of this species is composed of 2n = 20 telocentric (rod-shaped) chromosomes. When the chromosomes in the complement are arranged according to their size, they showed a size decrement with small variation between chromosome pairs, i.e., they decrease in a very gradual manner. Unlike the chromosome members of family Acrididae considered earlier, no distinctly smaller pairs were observed.

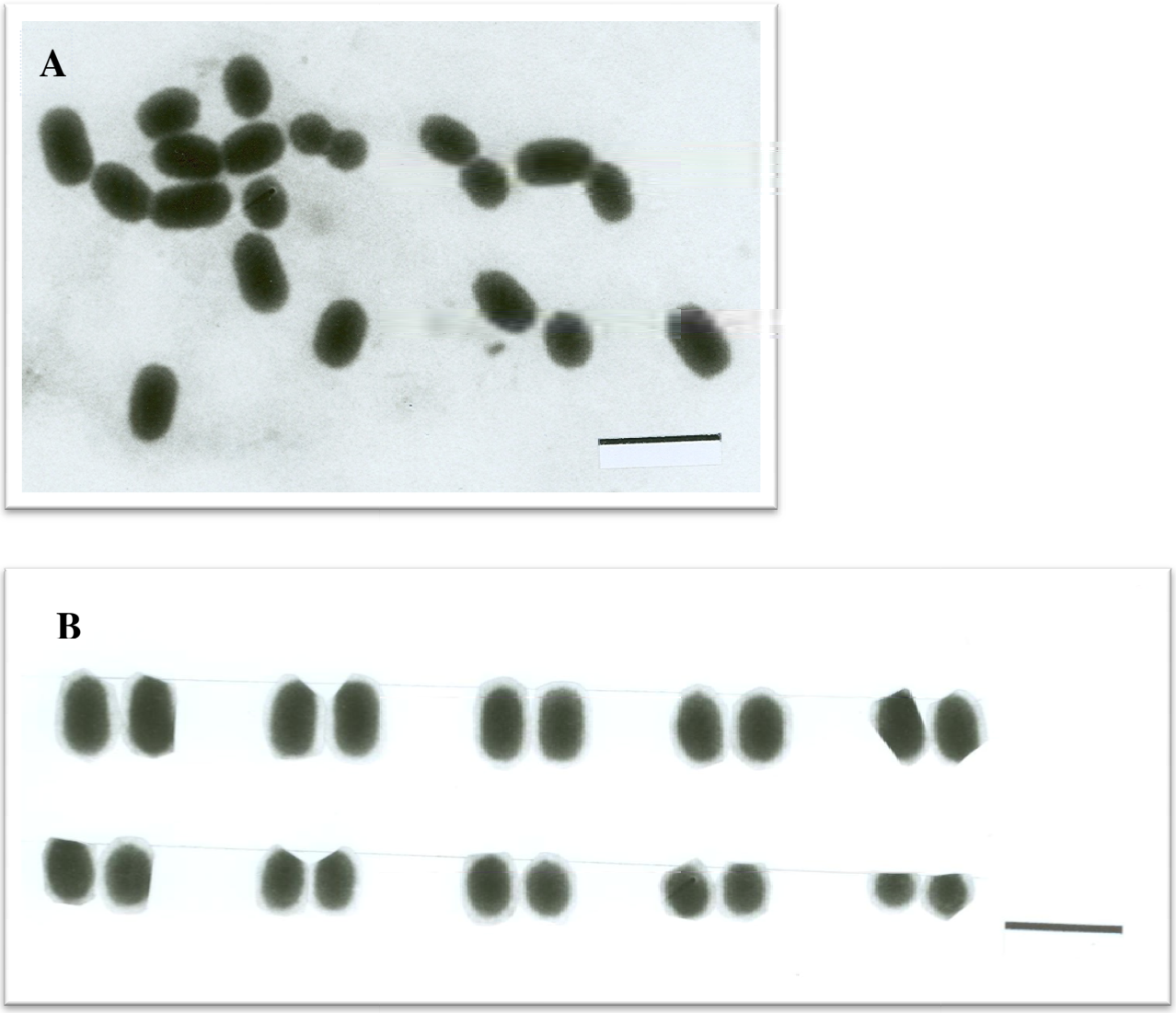


Fig. 16. Mitotic metaphase chromosome spread (A) and karyotype (B) of female *paratettix* spp. from Nazreth. Bar = 10 µm

Table 5. A summary of karyological data (i.e., karyotypic formula, diploid chromosome number (2n) and autosomal fundamental number (FNa)) of the studied grasshopper specimens.

Family	Sub family	Grasshopper taxa	Sex	Karyotypic formula	2n	FNa
Acrididae	Cyrtacanthacridinae	*Acanthacris* spp.	M	23t	23*	22
	Oedipodinae	*Paracinema tricolor* (AA)	F	24t	24	22
		Paracinema tricolor (DB)	F	24t	24	22
		Gastrimargus spp. (Z)	F	24t	24	22
		Gastrimargus spp. (M)	F	24t	24	22
		Gastrimargus spp. (DB)	F	24t	24	22
		Gastrimargus spp. (DB)	M	23t	23	22
		Acrotylus spp. (N)	F	24t	24	22
		Pardalophora spp. (N)	M	23t	23	22
		Pardalophora spp. (N)	F	24t	24	22
	Acridinae	*Acrida* spp.-1 (DZ, S &M)	F	24t	24	22
		Acrida spp.-2 (M)	F	24t	24	22
	___	Acrididae MU	F	24t	24	22
		Acrididae WDZU	F	24t	24	22
Tetrigidae	Tetriginae	*Paratettix* spp. (N)	F	20t	20	18

Note: AA = Addis Ababa; DB = Debre Birhan ; DZ = Debre Zeit; M = Melkasa; N = Nazreth; S= Sheno; Z = Zeway

***** Some cells showed variable chromosome number which is 2n > 23 or 2n < 23 chromosomes

6. DISCUSSION

In spite of the presence of large number of grasshopper species in Ethiopia, none of them had any chromosomal reports to date. Moreover, studies on the species composition of Ethiopian grasshoppers are inadequate because studies have not been carried out and published on their species composition and taxonomic identification, except for the two papers published by Stretch-Lilija (1977) and Tibebu Habtewold and Landin (1992). Hence, the identification of the specimens was done to the level of our expertise and available scarce literatures.

Nine of the ten grasshopper taxa (*Acanthacris* spp., *Paracinema tricolor*, *Gastrimargus* spp., *Acrotylus* spp., *Pardalophora* spp., *Acrida* spp.-1, *Acrida* spp.-2, Acrididae MU and Acrididae WDZU) possess a standard chromosome number of 2n = 23 in males and 2n = 24 in females, with telocentric chromosome morphology. In all these species the karyotypes are asymmetrical and show slight bimodality. According to Stebbins (1971), an asymmetrical karyotype contains chromosomes with subterminal or terminal centromeres and with different relative size.

The present result is in conformity with what have been described for the family Acrididae. Different studies have indicated that the species of the family Acrididae have a uniform/ conserved karyotype. They have a predominance of karyotype with 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, in the present study all the species, with some exceptions, manifest karyotypic conservatism in both the morphology and number of chromosomes, which is the characterstic of the family. 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, that 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 patterns of interstitial heterochromatin blocks including the number, location, distribution and size of heterochromatin blocks in the karyotype.

In the present study, hyper-diploid (2n = 24, 27) and hypo-diploid (2n = 22) conditions relative to the normal 2n = 23 chromosome was observed in somatic cells of *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 it to be due to aneuploidy, but as to what causes the aneuploidy was not indicated.

In addition to the variability in somatic chromosome number, in all the analyzed meiotic cells of *Acanthacris* spp. an extra unpaired chromosome was observed. This chromosome was largely heterochromatic and stains heavily except at its ends. In size, it is comparable to X chromosome. Like the X-chromosome, it is positively heterochromatic and sometimes it bends on itself as the single X chromosomes of male grasshopper sometimes does. Although it is not possible to identify this chromosome among the somatic chromosomes, it must 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. 5B). The nature of this extra chromosome is not clear. If it were an additional copy (trisomy) of one of the somatic chromosomes, some trivalent associations would be expected to be formed. However, in these meiotic cells, no trivalent association was observed, and thus it is unlikely that this chromosome is an ordinary somatic chromosome. Also one would expect its other two homologues to be heterochromatic and behave in the same manner as this particular chromosome, but no such phenomenon was observed. In this regard it behaves like a B-chromosome. It has been observed that this chromosome is sometimes associated with the X chromosome. This implies that the two have some homology and probably this chromosome might have been derived from the X chromosome or share some homologous segment in common.

Studies 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.

Cabrero *et al.* (2003) have indicated cases in which the B-chromosomes can be derived from the X chromosome.

As it is described earlier, the *Gastrimargus* spp. examined in the present study has a diploid chromosome number of 2n = 23 (males) and 24 (females), with telocentric chromosomes, and the autosomal fundamental number is 22. This result is in concordance with the karyotype reported by Channaveerappa and Ranganath (1997) for *Gastrimargus africanus orienatlis*, although they observed some numerical instability in germ line cells of some individuals within the studied species of the genus *Gastrimargus*. They concluded that, this chromosome numerical inconsistency in some of the male germ line cells is due to aneuploidy. Unlike their result, no chromosomal instability was observed in the specimens of *Gastrimargus* spp. in the present study.

Furthermore, in *Gastrimargus* spp. in the present study the two sister chromatids of the dyads at anaphase-I stage of meiosis widely opened apart to about 180° while still held together at one end by the centromere. These dyads apparently looked-like a metacentric chromosome having its centromere exactly at the middle. In animals, normally, at dyad stage, the arms of sister chromatids separate as the result of dissociation of cohesin complex (Gimenez-Abian *et al.,* 2004). However, a dyad of telocentric chromosome may only have V shape. In the present study, however, it was observed that the sister chromatids have opened such that individual dyads look like a single metacentric chromosome. Although the exact mechanism 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 for what was observed in the present study would remain for future investigation.

The chromosome number of *Acrotylus* spp., as observed in the present study, is in agreement with the one reported by Turkoglu and Koca (2002) for a different species of the same genus, *Acrotylus insbricus*. Regardless of the chromosome number similarity, the two differ in their autosomal fundamental number which is 22 in the present *Acrotylus* spp. and 44 in *Acrotylus insbricus*. The fundamental number change is the result of chromosome morphological alteration. In the *Acrotylus* species used in the present study all the chromosomes are telocentric, whereas the chromosome complement of *Acrotylus insbricus* is composed of metacentric

chromosomes. The possible cause for the morphological deviation in *Acrotylus insbricus* might be pericentric inversion, that converted the ancestral telocentrics to metacentrics.

The karyotype of the female *Paratettix* spp. was found to consist of 2n = 20 telocentric chromosomes. 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). 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. According to Roberts (1941), the first group comprises only two families: Pamphagidae and Pyrgomorphidae, whereas the second contains the remaining families. Those families under Chasmosacci contains a karyotype of 2n = 19 (male) and 20 (female) telocentric chromosomes and considered to be 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) cited in Mesa and Fontanetti (1983) considered the karyotype with 2n = 23 (X0)/ 24(XX) (Cryptosacci) as a primitive karyotype, while the 2n = 19 (X0)/ 20 (XX) (Chasmosacci) as a derivative.

The studied 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.

Generally, a slight variation can be perceived when the karyotype of the studied species are compared. The two examined species belonging to the subfamily Acridinae possess more or less similar karyotypes with two pairs of comparably small chromosomes. In contrast, out of the three species studied under subfamily Oedipodinae, *Paracinema tricolor* illustrates somewhat different karyotype having three pairs of comparably smaller chromosomes. This karyotype is much more similar with the karyotype of Acrididae WDZU. Similarly to the karyotype of *Gastrimargus* spp. and *Pardalophora* spp., the chromosome complements of *Acanthacris* spp. (subfamily

Cyrtacanthacridinae) also had two pairs of smaller chromosomes but, unlike them there is no distinct difference between the 10^{th} and the other two smaller chromosomal pairs. Additionally, the karyotypes of *Gastrimargus* spp. and *Pardalophora* spp. from subfamily Oedipodinae had an enormous resemblance with that of *Acrida* spp.-1 and *Acrida* spp.-2 from subfamily Acridinae by having two pairs of smaller chromosomes where there is quite distinct size difference between the 10^{th} chromosomal pair and the next two pairs of smaller chromosomes. On the contrary, in Acrididae MU, the whole chromosomes in the karyotype seem to decrease in a gradual manner. In comparison with family Acrididae, family Tetrigidae, in addition to the chromosome numerical variation, had only one pair of smaller chromosome.

7. CONCLUSION AND RECOMMENDATION

7.1. Conclusion

The karyotypes of the studied grasshopper species showed high degree of similarity in terms of their diploid chromosome number and morphology. Irrespective of the intercellular karyotypic instability shown in *Acanthacris* spp., all species in the family Acrididae possess a chromosome number of 2n = 23 (22 autosomes + X) in males and 2n = 24 (22 autosomes + XX) in females. The chromosomes of these species are exclusively telocentric. In all the species studied, the chromosome varied in size in gradual manner, but the last two, or in some cases the last three, pairs of chromosomes are sharply smaller than the rest of chromosome pairs. This size difference coupled with the telocentric nature of the chromosome make the karyotype an asymmetric one with a slight tendency towards bimodalism.

Contrariwise, *Paratettix* spp. (family Tetrigidae) has a different karyotype with 2n = 20 telocentric chromosomes. This shows that this family is chromosomally distinct from the acridid family. This species possesses the karyotype of the Chasmosacci group (2n = 19 (X0)/ 20 (XX)) while resembling the Cryptosacci ones morphologically.

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 studies to clarify the systematics of these insects.

7.2. Recommendation

From the aforepresented results of the chromosomal study on Ethiopian grasshoppers, it is evident that several lines of research are need to be undertaken. Hence, the following points are recommended:

- As this is the first study on Ethiopian grasshoppers and also confined to small number of sites, further studies of chromosomes of the grasshoppers of Ethiopia is needed in view of the large diversity and economic significance of grasshoppers in Ethiopia.

- Further studies should include additional cytological techniques such as C-banding and silver staining in order to generate more cytological information.

- As species may vary in their genome size despite their similarity in chromosome number and morphology, determination of the genome size would provide additional information about the genetic variability among the grasshopper species.

- In the future studies, cytomolecular techniques such as *in situ* hybridization would be useful to reveal variation in the amount and distribution of various classes of repetitive DNA sequences in the genomes of different species as this would reveal the repatterning of chromosomes in the evolution of different grasshopper species.

- Further study in the chromosomes of *Acanthacris* spp. is necessary to understand the nature of chromosome numerical instability as well as explain the nature of the extra chromosome which in the present study is tentatively assumed to be a B chromosome.

- Taxonomic identification of the specimens was a problem in the present study. If taxonomic characterization of Ethiopian grasshoppers is undertaken by entomologists, it would facilitate the cytogenetic study of Ethiopian grasshoppers.

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APPENDICES

Appendix 1. Some species of grasshoppers recorded in Ethiopia (Source: Tibebu Habtewold and Landin, 1992).

Superfamily/ Family	Subfamily	Species
Acrididae	Oedipodinae	*Aiolopus longicornis* Sjostedt
		Aiolopus thalassinus (Fabricius)
		Acrotylus patruelis Herrich-Shaffer
		Gastrimargus africanus Saussure
		Morphacris fasciata Thunberg
		Paracinema tricolor (Thunberg)
		Trilophidia conturbata (Walker)
	Acridinae	*Acrida bicolor* Thunberg
		Acrida herbacea I Bolivar
		Coryphosima species
		Duronia chloronota Stal
		Pycnodictyia galinieri Reiche and Fairmaire
	Eyprepocneminae	*Cataloipus abyssinicus* Uvarov
		Eyprepocnemis noxia Dirsh
		Tylotropidius dydimus (Thunberg)
	Catantopinae	*Catantops momboensis centralis* Jago
		Diabolocatantops axillaris (Thunberg)
		Epacrocatantops curvicercus Miller
	Cyrtancanthacridinae	*Acanthacris ruficornis* Fabricius
		Cyrtacanthacris tatarica Linnaeus
	Calliptaminae	*Acorypha johnstoni* (Kirby)
Pyrgomorphidae	Pyrgomophinae	*Chortogonus senegalensis abyssinicus* I Bolivar
		Dictyophorus griseus (Reiche and Fairmaire)

CONTINUED

Superfamily/ Family	Subfamily	Species
		Parasphena abyssinica Uvarov
		Phymateus aegrotus Gerstaecker
		Pyrgomorpha cognate Krauss
Tettigonoidea	Conocephalinae	*Conocephalus conocephalus* (Linnaeus)
	Copiphorinae	*Ruspolia flavoviren* (Karny)
Tetrigidae		*Paratettix* species

Appendix 2. Length of each chromosome (µm), Total chromosome length for a set, relative chromsome length (%) and chromosome morphology of nine grasshopper species.

Note: CL = Chromosome length, RL = Relative length, CM = Chromosome morphology, AA = Addis Ababa, M = Melkasa, W = Wolenkomi

		Chromosome number																								
Taxa		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Total for set
Acanthacris spp. (2n = 23) ♦ male ♦ W	CL	7.37	6.84	6.32	6.32	5.79	5.53	5.00	4.74	4.74	4.47	4.21	3.95	3.68	3.42	3.42	3.16	2.89	2.63	2.37	2.11	2.11	1.84	1.84		94.75
	RL	7.78	7.22	6.67	6.67	6.11	5.84	5.28	5.00	5.00	4.72	4.44	4.17	3.88	3.61	3.61	3.34	3.05	2.78	2.50	2.23	2.23	1.94	1.94		
	CM	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t		
Paracinema tricolor (AA) ♦ female	CL	7.89	8.42	7.37	7.37	6.84	6.84	6.32	6.58	5.79	5.26	5.00	4.74	4.47	4.21	4.21	4.21	3.95	3.95	2.11	2.11	1.84	1.58	1.05	1.05	113.16
	RL	6.97	7.44	6.51	6.51	6.04	6.04	5.59	5.81	5.12	4.65	4.42	4.19	3.95	3.72	3.72	3.72	3.49	3.49	1.86	1.86	1.63	1.4.	0.93	0.93	
	CM	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	
Gastrimargus spp. ♦ M ♦ female	CL	7.63	7.89	7.37	7.37	6.32	6.32	6.05	6.05	5.26	5.26	4.74	4.74	4.47	4.21	4.21	3.95	3.68	3.95	3.42	3.42	1.58	1.58	1.32	1.58	112.37
	RL	6.79	7.02	6.56	6.56	5.62	5.62	5.38	5.38	4.68	4.68	4.22	4.22	3.98	3.75	3.75	3.52	3.27	3.52	3.04	3.04	1.41	1.41	1.17	1.41	
	CM	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	
Acrida spp.-1 ♦ female ♦ M	CL	7.63	7.63	6.84	6.58	6.05	6.32	5.79	5.79	4.74	5.26	4.74	4.47	4.47	4.21	4.21	4.21	3.95	3.95	3.68	3.68	2.11	2.11	1.84	1.84	112.10
	RL	6.81	6.81	6.10	5.87	5.40	5.64	5.17	5.17	4.23	4.69	4.23	3.99	3.99	3.76	3.76	3.76	3.52	3.52	3.28	3.28	1.88	1.88	1.64	1.64	
	CM	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	
Acrida spp.-2 ♦ female ♦ M	CL	6.58	6.05	5.79	5.79	5.26	5.53	5.00	4.74	4.47	4.47	3.95	3.68	3.68	3.68	3.68	3.42	3.16	3.16	2.63	2.63	1.84	1.84	1.58	1.58	94.19
	RL	6.99	6.42	6.15	6.15	5.58	5.87	5.31	5.03	4.75	4.75	4.19	3.91	3.91	3.91	3.91	3.63	3.35	3.35	2.79	2.79	1.95	1.95	1.68	1.68	
	CM	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	
Pardalophora spp. ♦ male	CL	7.5	7.75	7.25	7.00	6.00	6.00	5.25	5.25	5.00	4.75	4.75	4.75	4.75	4.50	4.50	4.25	4.25	4.00	4.00	2.50	2.25	1.75	1.50		109.50
	RL	6.85	7.08	6.62	6.39	5.48	5.48	4.79	4.79	4.57	4.34	4.34	4.34	4.34	4.11	4.11	3.88	3.88	3.65	3.65	2.28	2.05	1.60	1.37		
	CM	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t		
Acrididae MU ♦ female	CL	6.32	6.05	5.79	5.26	5.00	5.00	4.74	4.74	4.47	4.47	3.95	3.95	3.68	3.68	3.42	3.16	3.16	2.89	2.63	2.11	1.84	1.58	1.58	1.58	91.05
	RL	6.94	6.64	6.36	5.78	5.49	5.49	5.21	5.21	4.91	4.91	4.34	4.34	4.04	4.04	3.76	3.47	3.47	3.17	2.89	2.32	2.02	1.74	1.74	1.74	
	CM	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	
Acrididae WDZU ♦ W ♦ female	CL	6.84	6.58	6.32	5.79	5.79	5.53	5.53	5.26	4.21	4.47	4.21	3.95	3.68	3.68	3.42	3.16	3.16	2.89	1.58	1.58	1.58	1.32	1.05	1.32	92.90
	RL	7.36	7.08	6.80	6.23	6.23	5.95	5.95	5.66	4.53	4.81	4.53	4.25	3.96	3.96	3.68	3.40	3.40	3.11	1.70	1.70	1.70	1.42	1.13	1.42	
	CM	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	
Paratettix spp. ♦ female	CL	6.58	6.58	6.32	6.32	6.05	6.05	5.79	5.79	5.53	5.53	5.00	5.26	5.00	5.00	5.00	4.74	4.21	3.95	2.63	2.89					104.22
	RL	6.31	6.31	6.06	6.06	5.81	5.81	5.56	5.56	5.31	5.31	4.80	5.05	4.80	4.80	4.80	4.55	4.04	3.79	2.52	2.77					
	CM	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t	t					