**Acrida ungarica** Herbst, 1786 (Acrididae: Orthoptera)  
Karyotype Analysis

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**Summary** In this study, the karyotype of the *Acrida ungarica* species (chromosome number, chromosome morphology and chromosome lengths) belonging to the Acridinae subfamily of the Acrididae family was examined. As a result of these examinations, the species number of chromosomes was determined to be $2n\sigma^-=23$ (XO). It was found that all autosomal chromosomes as well as the X chromosome had an acrocentric structure. As a result of counting carried out on five individuals, the mean chiasma frequency was found to be 16.39.

**Key words** *Acrida ungarica*, Chromosome, Karyotype, Orthoptera, Chiasma frequency.

Karyotype analysis is considerably important especially for the characterisation of the populations. Orthoptera comprises of more than 24000 species and is one of the insect orders that has been systematically well studied in Turkey (Karabağ 1983, Demirsoy 1974, Ünal 2000, Çiplak 2003, Sevgili and Heller 2003, Mol and Zeybekoğlu 2013). According to recent studies, 682 species and subspecies belonging to Orthoptera together with five species belonging to the Acridinae subfamily of the Acrididae family have been listed in Turkey (Ünal 2014).

There have been few studies conducted on Orthopteras cytogenetically in Turkey (Koca 1993, Türkoğlu 2001, Türkoğlu and Koca 2002a, 2002b, Türkoğlu et al. 2003, Koca and Tunçbaş 2006, Çakmak and Koca 2014).

In chromosome studies conducted with grasshoppers belonging to Acridinae subfamily, it was generally determined that the chromosome number was $2n=23$, XO $\sigma^-=24$, XX $\sigma^-$, and the chromosomes were acrocentric in structure (Seino and Dongmo 2013a, Seino et al. 2012, Chadha and Mehta 2011, Seino and Akongnuı 2010, Seino et al. 2008).

Chiasmata are the points in which two of the four chromatids in bivalent are joined together in one or more than one place when homologous chromosomes separate from each other (John 1990). The presence of chiasma indicates the event of genetical crossing over (part replacement).

This study intends to contribute to the literature by firstly determining the chromosome number, structure and chiasma distribution of the *Acrida ungarica* Herbst 1786 genus belonging to the Orthoptera order from the Aydın region.

**Materials and methods**

After grasshoppers were collected from various places in Aydın, they were brought into the laboratory, their testes were removed and stored in a colchicine-hypotonic solution for 2 h at room temperature. At the end of this period, the testes were preserved in ethyl alcohol–glacial acetic acid (3 : 1) and at examination, the testes were stained with 2% aceto-orcein and a squashed preparation was made with 45% acetic acid.

Photographs of cells which showed good distributions in preparations to analyse and measure, whose chromosome morphologies were clearly visible and whose chromosomes were located on a same plane were taken using a Olympus (Model BX51) microscope, 40º and 100º objective glass. Chromosome lengths of 10 cells were determined by using ocular and objective micrometer directly with microscope measurements.

The classification of chromosomes was performed according to the Levan et al. (1964). Among the examined samples prepared, chiasmata were counted in 25 diplo- tene cells of five individuals in bivalents and the mean chiasma frequency of the species was determined.

**Results**

As a result of cytogenetic examinations made on the *Acrida ungarica* species, the chromosome number was found to be $2n\sigma^-=23$, XO (Fig. 1). All autosomes and the X chromosomes were acrocentric. Chromosome lengths varied between 1.90–11.00 µm. The relative length were between 2.66–15.44. The length of the X chromosome was determined to be 8.00 µm. It was the third largest chromosome of the karyotype and covered 11.23% of the genome.
A karyogram representing *Acrida ungarica* is shown in Fig. 2 while the idiogram is provided in Fig. 3. The measurements of chromosome morphology are given in Table 1. The mechanism of sex determination is of XX (♀)/XO (♂) type. Eleven bivalents and one univalent (X chromosome) were observed in *Acrida ungarica* (Fig. 4a–e). While generally two chiasmata were observed in long bivalents, in a few, three chiasmata, and in even fewer, four chiasmata was observed (Fig. 4c). Mean chiasma frequency and distribution of genes are given in Table 2. Only one chiasma occurred in short bivalents (Fig. 4).

**Discussion**

Karyology and genetic structure are specific for genus and provide an identity to a genus. Karyotype analysis between species provide a better understanding of evolutionary relationship and separation between these species (Meera Rao 1990). Further, karyotype evolutionary studies in different animal groups show that karyotype is generally not constant and structural alterations may occur on an evolutionary time scale. While White (1973) asserts that chromosome differences between species are a key factor at the beginning of speciation, John and Miklos (1988) point out that structural chromosomal changes provide important clues in the prediction of the history and phylogeny of speciation.

The species in the Acrididae family generally show a fixed situation in terms of their number and morphology (John and Hewitt 1968). This family is 2*n*=23, XO with 2*n*=24, XX and its karyotype consists of acro- or subacrocentric chromosomes. Some karyological changes in same species of the Acrididae family have been observed. It is thought that the observed karyotype changes result from same chromosomal arrangements causing small changes in the chromosome numbers and morphologies (Hewitt 1979, Camacho 1980, Cabrero and Camacho 1982).

It is determined that the Acridinae subfamily members of the Acrididae family types, *Acrida turrita*, *Chirista compta*, *Caryphosima stenoptera producta*, *Oxyctantops spissus*, have 2*n*=23 XO(♂)/XX(♀) chromosome numbers and all chromosomes possess an acrocentric structure. It is determined that karyotype differences among the types occur when acrocentric structure

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**Table 1. Morphometric characteristics of the chromosomes of *Acrida ungarica***

<table>
<thead>
<tr>
<th>Number of chromosome pairs</th>
<th>Chromosome length (µm) Mean+S.D.</th>
<th>Relative length (% T.C.L.)</th>
<th>Chromosome morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>11.00±1.74</td>
<td>15.44</td>
<td>a</td>
</tr>
<tr>
<td>II</td>
<td>9.00±1.74</td>
<td>12.64</td>
<td>a</td>
</tr>
<tr>
<td>III</td>
<td>7.9±1.50</td>
<td>11.09</td>
<td>a</td>
</tr>
<tr>
<td>IV</td>
<td>6.8±1.11</td>
<td>9.55</td>
<td>a</td>
</tr>
<tr>
<td>V</td>
<td>6.3±1.09</td>
<td>8.84</td>
<td>a</td>
</tr>
<tr>
<td>VI</td>
<td>5.3±0.60</td>
<td>7.44</td>
<td>a</td>
</tr>
<tr>
<td>VII</td>
<td>5.1±0.33</td>
<td>7.16</td>
<td>a</td>
</tr>
<tr>
<td>VIII</td>
<td>4.2±0.83</td>
<td>5.89</td>
<td>a</td>
</tr>
<tr>
<td>IX</td>
<td>3.3±1.20</td>
<td>4.63</td>
<td>a</td>
</tr>
<tr>
<td>X</td>
<td>2.4±0.60</td>
<td>3.37</td>
<td>a</td>
</tr>
<tr>
<td>XI</td>
<td>1.9±1.39</td>
<td>2.66</td>
<td>a</td>
</tr>
<tr>
<td>XII (X)</td>
<td>8.00±0.87</td>
<td>11.23</td>
<td>a</td>
</tr>
</tbody>
</table>

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chromosome are classified as long, short, and medium (Seino and Dongmo 2013a). Chadha and Mehta (2011) found that the chromosome number of the species was $2n^o=23$ in their study carried out on Acrida turittta, A. exallata, Phlaeoba infumata and P. antennata and sexual mechanism determined to be XO//XX. They observed that all types of chromosome morphologies are acrocentric in structure and the X chromosome is the largest are of all types (Table 3). Yadav and Yadav (1986) also reported the similar results between chromosome num-

**Table 2.** Mean chiasma frequency and distribution of Acrida ungarica.

<table>
<thead>
<tr>
<th>Individual</th>
<th>Counted cell</th>
<th>Mean chiasma frequency ±S.D.</th>
<th>1 Chi</th>
<th>2 Chi</th>
<th>3 Chi</th>
<th>4 Chi</th>
<th>Bivalent number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>16.52±0.29</td>
<td>165</td>
<td>84</td>
<td>26</td>
<td>—</td>
<td>275</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>16.36±0.28</td>
<td>171</td>
<td>75</td>
<td>29</td>
<td>—</td>
<td>275</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>15.44±1.87</td>
<td>187</td>
<td>66</td>
<td>22</td>
<td>—</td>
<td>275</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>16.6±0.27</td>
<td>165</td>
<td>83</td>
<td>27</td>
<td>—</td>
<td>275</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>17.04±0.20</td>
<td>161</td>
<td>79</td>
<td>34</td>
<td>1</td>
<td>275</td>
</tr>
<tr>
<td>Mean</td>
<td>25</td>
<td>16.39</td>
<td>849</td>
<td>387</td>
<td>138</td>
<td>1</td>
<td>1375</td>
</tr>
</tbody>
</table>

S.D.: Standard Deviation; Chi: Chiasma.

**Table 3.** Karyotype characteristic features of Acridinae (Tryxalinae) subfamily belonging to the species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sub-family</th>
<th>Ch. number</th>
<th>Sex determining mechanism</th>
<th>Number of chromosome per size group</th>
<th>Morphology of chromosomes</th>
<th>Length of X chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. compta (Walker)</td>
<td>Acridinae</td>
<td>23</td>
<td>XX-XO</td>
<td>S 4 2 4</td>
<td>Acrocentric</td>
<td>7.3±0.52</td>
</tr>
<tr>
<td>C. s. producta (Walker)</td>
<td>Acridinae</td>
<td>23</td>
<td>XX-XO</td>
<td>S 4 2 4</td>
<td>Acrocentric</td>
<td>5.60±0.56</td>
</tr>
<tr>
<td>A. turritta Linnaeus</td>
<td>Acridinae</td>
<td>23</td>
<td>XX-XO</td>
<td>S 4 2 4</td>
<td>Acrocentric</td>
<td>5.00±0.08</td>
</tr>
<tr>
<td>O. spissus (Walker)</td>
<td>Acridinae</td>
<td>23</td>
<td>XX-XO</td>
<td>S 4 2 4</td>
<td>Acrocentric</td>
<td>6.60±0.00</td>
</tr>
<tr>
<td>A. ungarica (Herbst 1786)</td>
<td>Acridinae</td>
<td>23</td>
<td>XX-XO</td>
<td>S 4 2 4</td>
<td>Acrocentric</td>
<td>8.00±0.87</td>
</tr>
<tr>
<td>A. turritta (Chadha and Mehta 2011)</td>
<td>Tryxaline</td>
<td>23</td>
<td>XX-XO</td>
<td>S 4 2 4</td>
<td>Acrocentric</td>
<td>199.0±22.26</td>
</tr>
<tr>
<td>P. antennata (Chadha and Mehta 2011)</td>
<td>Tryxaline</td>
<td>23</td>
<td>XX-XO</td>
<td>S 4 2 4</td>
<td>Acrocentric</td>
<td>141.0±1.18</td>
</tr>
</tbody>
</table>

Ch.: Kromozom, S: Short, M: Medium, L: Long.
bers and sexual mechanism. Sharma and Gautam (2002) acquired similar results carried out with 11 grasshopper types in Simla. Therefore, chromosome number and sex determination mechanism of short-horned grasshoppers show sameness even in different regions.

In a study carried out with *Acrida turitita*, *Paracrina luculenta* and *Morphecris fasciata* of the Acrididae family, Li et al. (2008) stated that the chromosome number of the species was $2n=23$ (22A+XO) and that chromosome morphologies are acrocentric in structure. They note that all chromosomes are acrocentric in structure and while 4 pairs of chromosomes are long, 5 pairs of chromosomes are medium and 2 pairs of chromosomes are short in *Acrida turitita*, 6 pairs of chromosomes are long, 2 pairs are medium and 3 pairs are short in other two types. It is determined that while X chromosome is medium height in *A. turitita* and *M. fasciata*, it is long in *P. luculenta*.

The XX$^P$/XO$^P$ sex determination mechanism has been observed in grasshoppers in general. XX/XO$^-^P$ sex determination mechanism has been detected in many species belonging to Acrididae family (White 1968, 1973, Camacho and Cabrero 1983, Warchalowska-Sliwa 1984, Warchalowska-Sliwa et al. 1993, Bugrov 1996). However, the neo XY sex development mechanism (XX/neO-XYe$^-^P$) has also been observed in some Acrididae species (White 1973, Hewitt 1979, John 1983).

It is stated that the X chromosomes of species belonging to Acrididae family are generally acrocentric in structure (Santos et al. 1983, Gusachenko et al. 1992). But it has been determined that the same species carries metacentric X chromosome. Pericentric inversion and geographical differences can be indicated as a reason for the change arising from the structure of the X chromosome (Türköğlu 2001). The fact that the X chromosome of the *Acrida ungarica* species has an acrocentric structure has been determined in this study.

The chromosome arm number, also known as NF (Fundamental Number) value, is of great importance in cytogenetic studies as it gives the genetical index of a chromosome. Even if the chromosome number may change by means of events such as centric fission and fusion, the arm number remains unchanged. The chromosome number of *Chorthippus bornhalmi* species belonging to Acrididae family is $2n=17$ XO (NF=23). Three pairs of autosomes are in the structure of submetacentric, five pairs of autosomes and X chromosome are in the structure of acrocentric (Çakmak and Koca 2014). However, the *Chorthippus schimidi* species is reported to have $2n=23$, XO (NF=23) acrocentric chromosome in Bugrov’s (1996) study. Although the *Ch. schimidi* species has chromosome number greater than those of *Ch. bornhalmi*, the arm number is not changed and remains fixed. Arm number of *Acrida ungarica* having 23 acrocentric chromosomes has been detected as NF=23 in this study.

It is know that chiasma frequency is of great importance in terms of reflecting genetic exchange ratio and many internal and external factors affect this exchange (Sybenga 1975). Chiasma frequencies in 25 diplotene cell five individuals of *Acrida ungarica* collected from Aydin province vary between 15.44 and 17.04 in our study. The average chiasma frequency of this species was determined to be 16.39. It is thought that differences between chiasma frequencies may result from genetic differences between individuals. There are also chromosome aberrations that affect chiasma frequency in both positive and negative ways (Teoh and Yang 1983, Viseras and Camacho 1984, Goni et al. 1985). Although researchers have not detected differences between individuals in terms of karyotype, it is possible that some small changes may result in differences in chiasma frequency.

Seino et al. (2012) found that average chiasma frequencies were respectively 12.20±0.77 and 16.20±0.72 in the scope of their study performed on *Coryphosoma stenoptera producta* and *Christa compta* species collected from Cameroon. It was found that chiasma frequency of *C. compta* is significantly higher than chiasma frequency of *C. stenoptera producta*. This difference may be explained that while *C. stenoptera producta* has two long bivalents, *C. compta* has four long bivalents. Researchers encountered three chiasma bivalent in *C. compta* which they did not observe in *C. stenoptera producta*. Seino et al. (2008) states that there is a positive correlation between the chromosome lengths and chiasma frequency of Acrididae grasshoppers. Researchers explain that long bivalents in *C. compta* contribute to a chiasma frequency significantly higher than in *C. stenoptera producta*.

By considering that chiasma frequency is under the influence of both genetic and enviromental factors, the mean chiasma frequencies of *Chorthippus loratus* individuals collected from three different regions (Sinop, Tokat, İzmir) were detected as being 14.23 in Sinop region, 14.20 in Tokat region, 14.91 in İzmir region (Koca 1993). According to statistical research, there is no difference in terms of chiasma frequency between Sinop and Tokat, while there are significant differences between Sinop-İzmir and Tokat-İzmir. This difference may be explained in geographical terms. Furthermore, it is stated that differences in bivalent size may affect chiasma number considerably and short bivalents have one or two chiasma whereas long bivalents have three or more chiasmata (Koca 1993). In our survey, one or two chiasmata were seen in short bivalents while three or rarely four chiasmata were encountered in long bivalents. Effects of heat implementation in *Ch. dorsatus* and *Ch. brunneus* collected from Sivas, on chiasma frequency were also researched in the same survey. While no effect of heat implementation has been determined on chiasma frequency of *Ch. dorsatus*, a decline was
determined in the chiasma frequency of Ch. brunnneus individuals held at 4°C for 24 h when compared to the control group (Koca 1993).

Seino and Dongmo (2013b) state that mean chiasma frequency is higher in the dry than wet season population. While chiasma frequency of Tophronoto thalephora between dry and wet season population is not important statistically, that the mean chiasma frequency of Zonocerus variegatus is higher in dry season than wet season populations is important statistically. Researchers state that T. thalephora and Z. variegatus have rod and ring-shaped bivalents with 1–3 chiasmata and there is no seasonal effect on the number of chiasmata of these bivalents.

Oyidi (1968) found that in the study carried out on wet and dry season generations of Zonocerus variegatus, dry season generation has higher chiasma frequency than wet season generation. Wet season insect generations of this species raised in laboratory are bigger than dry season generations and it was argued that body size causes them to have high chiasma frequency. In the study carried out with Zonocerus variegatus (Iheagwam and Ene-Obong 1985), it has been found that climate change may affect chiasma frequency.

As the study of only morphological differences remains insufficient in systematic studies, studies at cytogenetic, biochemical and molecular levels are also needed. The chromosome number and structure of A. ungarica has been defined for the first time and karyological differences have been analysed in this study. This study contributes to the literature available for this species in the future and also can be used to determine the evolutionary relationship between species that are similar in morphology and chromosome number.

Acknowledgements

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idae) in Nigeria, with particular reference to the relationship


