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Research Article/Araştırma Makalesi

A New Record of Reduced Chromosome Number in Tenebrionidae (Coleoptera)

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Abstract: The karyological features of *Akis subtricostata* was determined for the first time with conventional and silver nitrate staining. The diploid number 2n=16 and meioformula 7+neoXY represents a deviation from the modal karyotype of Coleoptera. The pericentromeric heterochromatin was detected with both Giemsa and silver nitrate staining. In addition to determining a single possible NOR on prophase I nuclei, $AgNO_3$ revealed that several telomeric regions of mitotic metaphase chromosomes were slightly more argyrophilic.

Keywords: Karyotype, NOR, neoXY, *Akis subtricostata*, heterochromatin.

Tenebrionidae'de (Coleoptera) İndirgenmiş Kromozom Sayısına Yeni Bir Kayıt

Öz: *Akis subtricostata*'nın karyolojik özellikleri geleneksel ve gümüş nitrat boyama ile ilk kez belirlenmiştir. 2n=16 diploid sayısı ve 7+neoXY formülü, Coleoptera takımının model karyotipinden bir sapma temsil etmektedir. Perisentromerik heterokromatin hem Giemsa hem de gümüş nitrat boyamaları saptanmıştır. Profaz I nukleuslarında olası tek bir NOR belirlemenin yanı sıra, $AgNO_3$ birkaç mitotik metafaz kromozomunun telomerik bölgelerinin biraz daha arjirofilik olduğunu ortaya çıkartmıştır.

Anahtar kelimeler: Karyotip, NOR, neoXY, *Akis subtricostata*, heterokromatin.

1. Introduction

Tenebrionidae is the seventh largest family of Coleoptera with more than 20.000 described species (Fattorini, 2000; Bouchard et al., 2005; Lillig et al., 2012; Tezcan et al., 2012; Slipinsky et al, 2011, McKenna & Farrell, 2009, Iwan & Löbl, 2020). Beetles belonging to the family Tenebrionidae occupy a great array of diverse habitats and show considerable species diversity mainly in arid and semiarid environments. Due to having a worldwide distribution and comprising several agriculturally and economically important species, Tenebrionidae has been the focus of evolutionary biology (Papadopoulou et al., 2009, 2010; Condamine et al., 2013; Lamb & Bond, 2013; Kergoat et al., 2014), biogeography (Juan et al., 1995, 1996a, 1996b; Rees et al., 2001) and ecology studies (Los Santos et al., 2000; Carrara & Flores, 2012; Fattorini & Ulrich, 2012; Fattorini, 2013). However, cytogenetic studies and chromosomal information about the group are still insufficient to represent this diverse family. Possessing small chromosomes is one of the reasons that makes conventional banding techniques and fluorescence labelling difficult and therefore cytogenetic studies scarce (Dutrillaux et al., 2006).

While the chromosome number in Tenebrionidae ranges between 14 and 38, the most prominent diploid number within the family is 2n=20. Considering the studied species, there is a tendency of a decrease in chromosome number in Pimelinae subfamily. However, the species with increased chromosome number are members of the subfamily Tenebrioninae (Juan & Petitpierre, 1991). Even though it has been reported in two genera (*Akis* and *Morica)* so far, 2n=16 is the second most common record of reduced diploid number in Pimelinae (Blackmon & Demuth, 2015). *Akis* is a genus which comprises approximately 34 species in the palearctic region yet only four species (*Akis acumita*, *A. bacarozzo*, *A. bremeri* and *A. discoidea*) have been studied cytogenetically (Juan & Petitpierre, 1991). All four species possess the meioformula of 7+neoXY. It is stated that neoXY system is derived from autosome-gonosome translocations (Schneider et al, 2006; Dutrillaux & Dutrillaux, 2009; Lira-Neto et al., 2012) and it has been frequently reported in Coccinellidae, Chrysomelidae and Scarabaeidae families (Blackmon & Demuth, 2015).

In this study, *Akis subtricostata,* a new record from Türkiye (Keskin & Yağmur, 2008), has been analyzed cytogenetically in order to corroborate the reduced diploid number reported from the genus. Furthermore, we provide the first cytogenetic information about the species by analyzing mitotic and meiotic spreads using conventional and differential staining.

2. Material and Methods

Adult *A. subtricostata* specimens that were collected from the Harran Ruins, Şanlıurfa brought alive to our laboratories in Ege University (İzmir). Mitotic and meiotic plates wereobtained from the male gonads applying the microspreading (Chandley et al., 1994) and splashing (Murakami & Imai, 1974) methods. The slides were stained with 4% Giemsa solution for 20 minutes for conventional staining. The silver impregnation method (Patkin & Sorokin, 1983) was applied in order to determine the possible NOR regions.

The chromosome spreads were photographed and

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analyzed with Zeiss Axioscope light microscope using ZEN software. The male karyotype and chromosomal measurements were made with Image J software (Rasband, 1997-2015) and Levan plugin (Sakamoto & Zacaro, 2009).

3. Results

Spermatogonial plates of *A. subtricostata* showed 2n=16 diploid number with meioformula 7+neoXY. The karyotype is composed of 2 subtelocentric, 2 submetacentric, and 3 metacentric pairs of autosomes. The submetacentric neoX is the second largest chromosome of *A. subtricostata* while the neoY is subtelocentric (Fig. 1). In metaphase I the heteromorphic bivalent can be determined as neoXY (Fig. 2a). Giemsa-stained prophase nuclei presented dark stained pericentromeric regions (Fig. 2b).

Silver nitrate revealed a single possible NOR (Fig. 3a) and predominantly stained the pericentromeric regions of the chromosomes at prophase I (Fig. 3b). In silver-stained mitotic metaphase plates, telomeric regions of at least 3 pairs of chromosomes gave relatively high argyrophilic signals along with centromeric regions (Fig. 4).

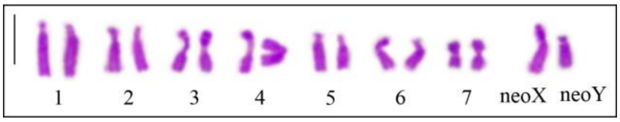

Figure 1. Karyotype of *Akis subtricostata* (bar = 5µm).

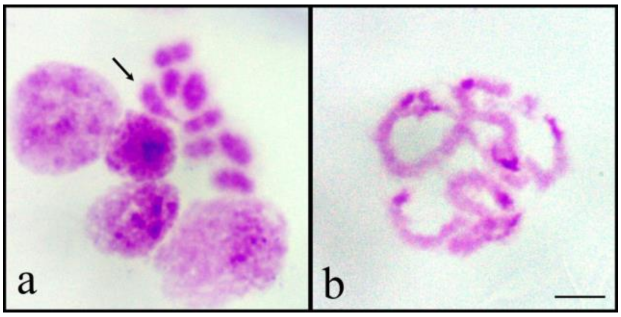

Figure 2. Giemsa stained a) metaphase I, b) prophase I stages (arrow indicates neoXY bivalent, bar = 5µm).

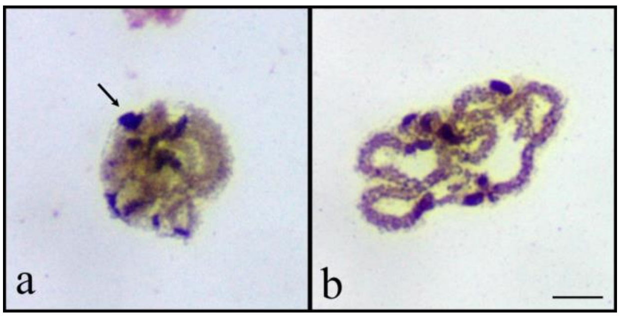

Figure 3. Silver nitrate stained prophase I nuclei a) possible NOR showed with arrow, b) heterochromatin distribution (bar = 5µm).

4. Discussion

2n=20 is considered to be the modal karyotype for Coleoptera as it is the conserved diploid number among its species. Xy_p sex determination system and meta-submetacentric morphology of the chromosomes are two other conserved features of the order. However, different chromosome numbers and morphologies resulting from chromosomal rearrangements like translocations, fusions, and fissions have been reported in various families and subfamilies (Smith & Virkki, 1978; Petitpierre et al., 1991; Cabral-de-Mello et al., 2008; Lira-Neto et al., 2012). The most common reduced diploid number is 2n=18 since it only requires the fusion of two autosomal pairs. It is followed by 2n=16 which is mostly reported in Chrysomelidae and Coccinellidae families. In Tenebrionidae, this diploid number is only present in the tribe Akidini and it represents 2.4% of the studied Tenebrionids (Blackmon & Demuth, 2015).

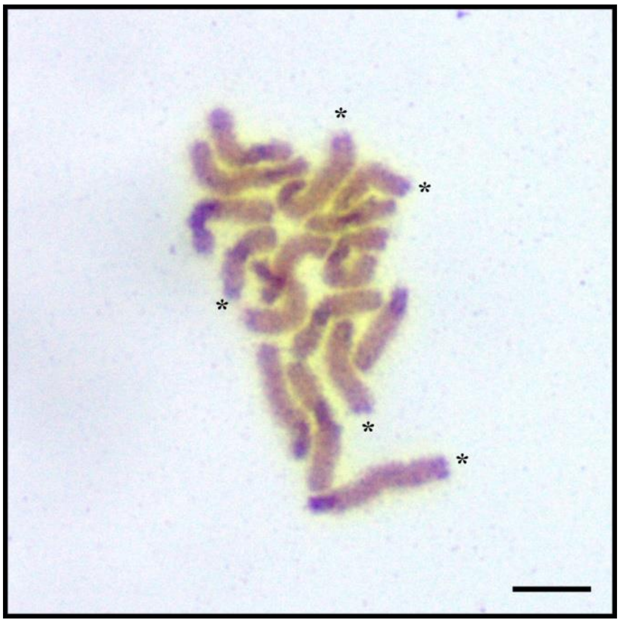

Figure 4. Silver stained mitotic metaphase plate shows argyrophilic telomeric regions (asterisks indicate telomeric heterochromatin, bar = 5µm).

Sex chromosome morphologies and determination systems also vary among the families of Coleoptera. While Xy_p is the most common system; XO, neoXY, and multiple sex chromosomes have been reported within the order (Pons, 2004; Karagyan et al., 2012; Lira-Neto et al., 2012; Blackmon & Demuth, 2015). In addition to being common in Coccinellidae, Chrysomelidae, and Scarabaeidae, neoXY system has been reported in Tenebrionidae as well (Blackmon & Demuth, 2015). This sex determination system along with the reduced diploid number is present in all studied Akidini representatives (Juan & Petitpierre, 1991).

This study demonstrated that *A. subtricostata* corroborated with the 2n=16 reduced chromosome number and neo-sex determination system of the tribe. In addition to reduced diploid number, chromosome morphology is quite deviated from Coleoptera that frequently has metacentric and submetacentric chromosomes (Petitpierre, 1996). In *A. subtricostata* two autosomal pairs along with the neoY chromosome were determined as subtelocentric. It is evident that multiple chromosomal rearrangements such as Robertsonian translocations, pericentric inversions, and fusions are at work in the genome evolution of the genus.

The heterochromatin in Coleoptera can be detected on pericentromeric regions (Rozek, 1998; Pons et al., 2004; Bione et al., 2005; Lachowska et al., 2005; Şendoğan & Alpagut Keskin, 2016, Çalışan & Alpagut-Keskin, 2023) as well as on telomeric regions (Bione et al., 2005; Colomba et al., 2006; Dutrillaux & Dutrillaux, 2009; Şendoğan et al., 2019). In *A. subtricostata* pericentromeric blocks were demonstrated with silver nitrate and Giemsa staining. On the other hand, high argyrophilic signals were also detected on several chromosomal ends. These dark stained chromosomal regions can be associated with the heterochromatin as they represent more condensed and predominantly stained areas of the chromosomes. Silver nitrate staining also revealed a possible NOR regions on prophase nuclei. It is stated that silver particles highlight the nucleolar protein around the rDNA and; thus, determine the transcriptionally active NOR (Medina et al., 1983; Jordan, 1987; Vitturi et al., 1999; Kavalco & Pazza, 2004; Dutrillaux et al., 2007).

In conclusion, the diploid number (2n=16) and meioformula (7+neoXY) of *A. subtricostata* was demonstrated for the first time in this study. Karyological findings of the species resembled those of other Akidini yet the sex determination system and reduced diploid number are what make this species intriguing. It is necessary to increase the cytogenetic studies on beetles in order to broaden the data available. Further comparative studies are important in order to understand the karyotype evolution in beetles. Completely understanding the karyotype evolution of the group needs further comparative studies.

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Conflict of interest: The author declares that there is no conflict of interest.

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