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Research

First Cytogenetic Analysis of *Eratigena agrestris* (Araneae: Agelenidae) From Turkey

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Abstract

In this study, chromosomal characteristics of *Eratigena agrestris* (Agelenidae) were investigated for the first time. Karyotype features including diploid chromosome number, sex chromosome system, chromosome morphology, and meiotic behavior were obtained from specimens collected in two localities of Mediterranean region. A spreading method including dissection, hypotonization, fixation, and staining was used to prepare the chromosome slides. In a total, 10 adult males were used due to having high numbers of dividing cells. Cytogenetical results showed that the diploid chromosome number and sex chromosome system was 2n♂ = 42 (X_1X_20). The sex chromosomes were identified tentatively. All chromosomes were telocentric. Relative chromosome lengths of autosomal pairs ranged between 5.65 and 3.32%, and relative chromosome lengths of X_1 and X_2 were 5.33 and 4.19%, respectively. In the first meiotic division stages, bivalents usually had one chiasma, but some had two chiasmata. At the end of the meiosis, two kinds of nuclei, with or without sex chromosomes, have occurred. These results contribute to a better characterization of the Agelenidae cytogenetic.

Key words: karyotype, chromosome, meiosis

The order Araneae is a diverse group of arachnids that are widely distributed all over the world. Current taxonomic systems show that more than 48,000 spider species are found and divided into three main clades, such as Mesothelae, Mygalomorphae, and Araneaomorphae (Coddington and Levi 1991). Among them, the last one represents the most derived taxonomic group including more than 40,000 species (World Spider Catalog 2019). However, only almost 850 spider species belonging to 70 families have been studied by cytogenetics previously (Araujo et al. 2019).

Agelenidae is one of the family belongs to the Araneomorphae infraorder, including 82 genera and 1,307 species and the genus *Eratigena* Bolzern, Burckhardt & Hänggi, 2013 comprises 37 species, mainly from Europe, North America, and part of Asia (World Spider Catalog 2019). Among them, *Eratigena agrestris* (Walckenaer 1802) is a member of the funnel-web spiders that have usually long legs, swift-runners that build funnels or tube-shaped retreats in grass, billet piles, rock piles, and various area around the home and yard (Davis 2016).

Among the species of Agelenidae, only 17 species were cytogenetically studied and most studied taxa belong to the genus *Tegenaria* Latreille, 1804 with six karyotyped species (Araujo et al. 2019). Within the genus *Eratigena*, *E. atrica* (C.L. Koch 1843) is the only species of the whole genus that was chromosomally analyzed, presenting a karyotype formula of 2n♂ = 42, X_1X_20 (Revell 1947).

Most spider species have a multiple X chromosome system called X_1X_20 system (X_1X_2 in males and $X_1X_1X_2X_2$ in females) (Král 2007) which has been noticed as a plesiomorphic character in spiders because it is present in representatives of the phylogenetically basal family Liphistiidae (Mesothelae) (Suzuki 1954).

This study reports the karyotype of *E. agrestris* based on diploid chromosome number, sex chromosome system, and meiotic characteristics, for Turkish populations. This study will introduce new data on the genus *Eratigena* and fill a gap in cytogenetics of the family Agelenidae.

Materials and Methods

Biological Material

Male specimens of *Eratigena agrestis* (Walckenaer 1802) were collected during 4 May 2018 from Mediterranean part of Turkey: 1) Pozantı/Adana: (3♂; 37°25′19.98″N, 34°52′46.55″E; Collection Numbers: ZKPA1.18–ZKPA3.18), 2) Soğucak/Mersin: (7♂; 36°58′01.30″N, 34°32′05.35″E; ZKSM1.18–ZKSM7.18) (Permission from Turkish Environment and Urban Ministry, Provincial Directorate of Environment and Urbanization, Permission Number: 51512229-3807). All of the collected spiders were placed in separate plastic tubes and transferred to the laboratory. Subadult samples were kept in the laboratory until they were adults and fed

with *Drosophila melanogaster* twice a week. A wet cotton was put into plastic tubes for the moisture requirement. The specimens used in the study were preserved in the Genetics Laboratory of Department of Molecular Biology and Genetics, Faculty of Art and Science, Nevşehir Hacı Bektaş Veli University (Nevşehir, Turkey).

Experimental Steps of Chromosome Preparation

Chromosome preparations were made according to the protocol of Dolejš et al. (2011), a classical spreading technique. Testes were used due to providing high numbers of dividing cells, which are useful to determine the karyotype and analyze the meiotic behavior. Chromosome preparations were made by three main steps as follows:

First step: Testes were dissected out in a physiological solution for invertebrates (Lockwood 1961) and then put into a hypotonic solution (0.075 M KCl) for 12–15 min at room temperature (RT). For fixation, freshly prepared Carnoy's solution (3:1; ethanol:acetic acid) was used twice for 10 and 20 min at RT.

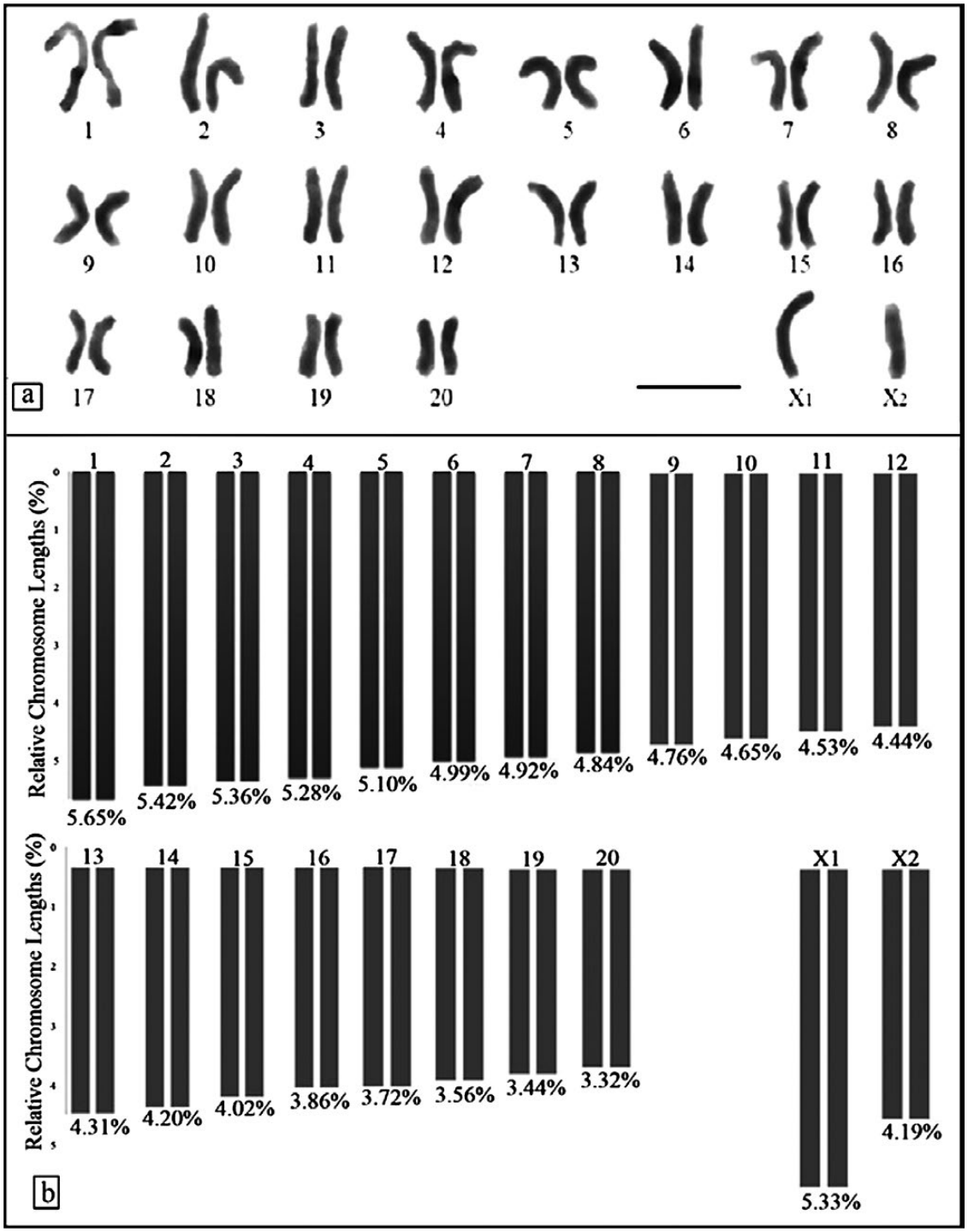

Fig. 1. Karyology of *Eratigena agrestris* from spermatogonial metaphases (2n♂ = 42, $X_1X_2$0): (a) karyogram; (b) ideogram (bar = 10 µm).

Second step: A drop of acetic acid (60%) was put on a slide and a piece of tissue was put into the acetic acid, then immediately cell suspension was prepared using a pair of tungsten needles. The preparations were placed on a histological plate (surface temperature was 42°C). Cell suspension was moved until the drop had almost evaporated. The preparations were air-dried for 24 h and inspected under a phase-contrast microscope (Leica DM500) to choose best quality preparations with chromosomes.

Third step: The preparations were stained with 5% Giemsa solution in Sörensen buffer (pH = 6.8) for 27 min at RT. The preparations were then washed with tap water and then washed with distilled water. They were placed in special boxes after being dried in RT and kept in 4°C.

Microscope Analysis and Karyotyping

Chromosome preparations were investigated under a light microscope CX21 (Olympus) and chromosome photographs including mitotic and meiotic cells were taken at 100× magnification with a BX53 (Olympus) microscope equipped with DP26 digital camera by CellSens (Olympus) program. Some chromosomal parameters such as the short arm (p), long arm (q), total chromosome length (C), centromeric index (CI = p/C × 100), relative chromosome length (RCL%), and X_2/X_1 ratio of spermatogonial metaphases were measured and calculated using CellSens (Olympus) program from 10 well-spread metaphases. Karyotype was prepared by arranging chromosomes in pairs based on their size using images of mitotic metaphases using Adobe Photoshop CS3 program and ideogram was made by Excel software (Microsoft Office). Chromosome measurements followed Levan et al. (1964) criteria (metacentric = 1.0–1.69, submetacentric = 1.7–2.99, subtelocentric = 3.0–6.99, and telocentric = 7.0–∞).

Results

Karyotype of *Eratigena agrestris*

Karyotype of *E. agrestris* comprises 42 telocentric chromosomes, 20 autosomal pair, and two sex chromosomes (Fig. 1). The sex chromosome system was $♂X_1X_20/♀X_1X_1X_2X_20$. Karyotype formula for males was $2n♂ = 42, X_1X_20$. RCL of the largest autosomal pair was 5.65% and the smallest autosomal pair was 3.32%. Chromosomes decreased gradually in size. RCL of the sex chromosomes, X_1 and X_2, were 5.33 and 4.19%, respectively (Table 1). The X_2/X_1 ratio was obtained from 10 spermatogonial metaphases as 78.61 (%).

Meiotic Characteristics of *Eratigena agrestris*

Stages of meiosis

At diplotene, 20 autosomal bivalents and two positively heteropycnotic univalent sex chromosomes were observed (Fig. 2a). Some bivalents had two chiasmata; in general, the bivalents had only one chiasma. At anaphase II, the sister chromatids were moved to the opposite poles and there were two kinds of nuclei: n = 22 (20 autosomes and two isopycnotic sex chromosomes) and n = 20 (20 autosomes) (Fig. 2b).

Discussion

Chromosomal studies on the genus *Eratigena* are represented by *E. atrica*, that presents 2n♂ = 18–24 according to Carnoy (1885) and 2n♂ = 42 according to Revell (1947). This latter being coincident with the 2n♂ = 42 found in the present paper. Carnoy (1885) did not provide information about the sex chromosome system and chromosome morphology of the species however, Revell (1947) clarified the sex chromosome system as X_1X_20 type and the telocentric morphology of the chromosomes, as also found in the present study. The probable reason for the different diploid number found by Carnoy (1885) may be pointing the methodological problems of the studies in that time that conduct to erroneous interpretations.

When the karyotype characteristics are evaluated, the gradual decrease of relative chromosome lengths, the absence of very large or very small chromosomes is a common feature in entelegyne spiders (Poyraz 2017). At the same time, when meiotic division properties

Table 1. Measurements from 10 spermatogonial metaphases of *Eratigena agrestris*

Pair no.	Long arm (q) (µm)	Short arm (p) (µm)	Total length (µm)	Centromeric index (CI)	Relative length (RCL%)	Chromosome morphology
1	11.56 ± 0.22	0	11.56 ± 0.22	0	5.65	t
2	11.08 ± 0.36	0	11.08 ± 0.36	0	5.42	t
3	10.97 ± 0.14	0	10.97 ± 0.14	0	5.36	t
4	10.80 ± 0.32	0	10.80 ± 0.32	0	5.28	t
5	10.44 ± 0.20	0	10.44 ± 0.20	0	5.10	t
6	10.21 ± 0.27	0	10.21 ± 0.27	0	4.99	t
7	10.06 ± 0.42	0	10.06 ± 0.42	0	4.92	t
8	9.90 ± 0.18	0	9.90 ± 0.18	0	4.84	t
9	9.73 ± 0.26	0	9.73 ± 0.26	0	4.76	t
10	9.52 ± 0.34	0	9.52 ± 0.34	0	4.65	t
11	9.26 ± 0.15	0	9.26 ± 0.15	0	4.53	t
12	9.08 ± 0.12	0	9.08 ± 0.12	0	4.44	t
13	8.82 ± 0.40	0	8.82 ± 0.40	0	4.31	t
14	8.60 ± 0.10	0	8.60 ± 0.10	0	4.20	t
15	8.22 ± 0.38	0	8.22 ± 0.38	0	4.02	t
16	7.90 ± 0.14	0	7.90 ± 0.14	0	3.86	t
17	7.62 ± 0.21	0	7.62 ± 0.21	0	3.72	t
18	7.28 ± 0.40	0	7.28 ± 0.40	0	3.56	t
19	7.04 ± 0.33	0	7.04 ± 0.33	0	3.44	t
20	6.80 ± 0.16	0	6.80 ± 0.16	0	3.32	t
X_1	10.90 ± 0.27	0	10.90 ± 0.27	0	5.33	t
X_2	8.58 ± 0.12	0	8.58 ± 0.12	0	4.19	t

t = telocentric; ± = standard deviation.

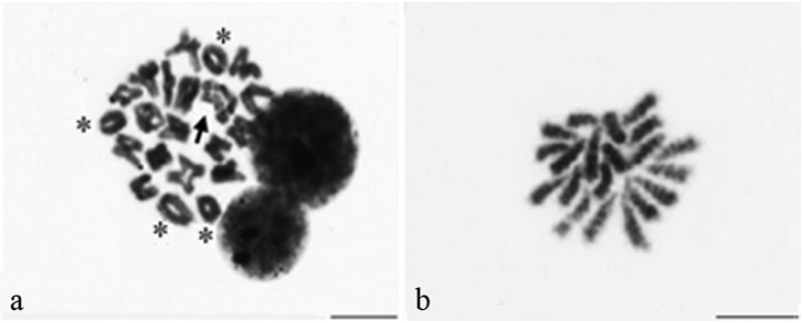

Fig. 2. Meiosis of *Eratigena agrestris* from adult males: (a) diplotene, 20 autosomal bivalents and two univalent sex chromosomes (arrow shows sex chromosomes and asterisks show bivalents that have two chiasmata); (b) anaphase II (20 autosomes, without sex chromosomes) (bars = 10 µm).

are considered, a positive heteropycnosis of sex chromosomes in meiosis I and isopycnosis in meiosis II, usually having only one chiasma (sometimes two chiasmata) on diplotene bivalents and migration to the same pole of sex chromosomes on anaphase I are frequently encountered in entelegyne spiders (Araujo et al. 2012). Thus, it can be concluded that the findings obtained in this study are consistent with genus characteristics.

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References Cited

Araujo, D., M. C. Schneider, E. Paula-Neto, and D. M. Cella. 2012. Sex chromosomes and meiosis in spiders: a review. pp. 87–108. *In* A. Swan (ed.), Meiosis – molecular mechanisms and cytogenetic diversity. InTech, Rijeka, Croatia. doi:10.5772/31612

Araujo, D., M. C. Schneider, E. Paula-Neto, and D. M. Cella. 2019. The spider cytogenetic database. http://www.arthropodacytogenetics.bio.br.spiderdatabase

Carnoy, J. B. 1885. La cytodiérèse chez les Arthropodes: (Mémoire deposé le 1. avril 1885.). Aug. Peeters, Louvain.

Coddington, J. A., and H. W. Levi. 1991. Systematics and evolution of spiders (Araneae). Annu. Rev. Ecol. Syst. 22: 565–592.

Davis, R. S. 2016. Hobo spider *Eratigena agrestis*. Utah State University Extension and Utah Plant Pest Diagnostic Laboratory. ENT-86-08.

Dolejš, P., T. Kořínkova, J. Musılová, V. Opatová, L. Kubcová, J. Buchar, and J. Král. 2011. Karyotypes of central European spiders of the genera Arctosa, Tricca and Xerolycosa (Araneae: Lycosidae). Eur. J. Entomol. 108: 1–16.

Král, J. 2007. Evolution of multiple sex chromosomes in the spider genus *Malthonica* (Araneae: Agelenidae) indicates unique structure of the spider sex chromosome systems. Chromosome Res. 15: 863–879.

Levan, A. K., K. Fredga, and A. A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. Hereditas. 52: 201–220.

Lockwood, A. P. 1961. 'Ringer" solutions and some notes on the physiological basis of their ionic composition. Comp. Biochem. Physiol. 2: 241–289.

Poyraz, H. 2017. Cytogenetic investigations on some spider species belonging to Gnaphosidae family. M.Sc. thesis, Nevşehir Hacı Bektaş Veli University, Science Institute, Nevşehir, Turkey.

Revell, S. H. 1947. Controlled X-segregation at meiosis in Tegenaria. Heredity. 1: 337–347.

Suzuki, S. 1954. Cytological studies in spiders III. Studies on the chromosomes of fifty-seven species of spiders belonging to seventeen families, with general considerations on chromosomal evolution. J. Sci. Hiroshima Univ., Ser. B, Div. 1. 15: 23–136.

World Spider Catalog. 2019. The world spider catalog, version 20.0. Natural History Museum Bern. doi:10.24436/2. http://wsc.nmbe.ch