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Cytogenetic Analysis of *Tegenaria elysii* (Araneae: Agelenidae)

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Abstract

There are 61 species of agelenid spiders in our country and the genus *Tegenaria* represents the widest group in terms of species number. In this study, it was aimed to investigate the cytogenetic properties of *Tegenaria elysii*. Chromosomes were obtained by making some modifications to the method of Pekár and Král (2001). Hypotonic, fixation and staining steps were applied to the gonads respectively. As a result, the number of diploid chromosomes and the sex chromosome system were found as 2n=42, ♂X_1X_20/♀$X_1X_1X_2X_2$. Total relative lengths of chromosomes decreased gradually from 6,83% to 3,31%. Sex chromosomes which positive heteropycnotics in the stage of meiosis. I have been detected to be isopycnic in the stage of meiosis II. 20 autosomal bivalent and two sex chromosomes were identified in which diplotene and diakinesis stages.

Keywords: Agelenidae, chromosome, karyotype

1. INTRODUCTION

Spiders are one of the largest animal group, and contain almost 47 000 species all around the world [1]. In our country, a total of 1117 spider species belonging to 52 families were determined [2]. Until now, although studies have been carried out in systematics, fauna and ecology, the cytogenetic investigations is scarce. The fact that tiny chromosome morphology, the presence of different sex chromosome systems and the requirement of modification in the method for each species limits the investigations on spider cytogenetics [3].

Spiders are divided into three subclasses namely Mesothelae, Mygalomorphae and Araneomorphae [4]. Among them, Agelenidae family is belonging to the subclass Araneomorphae and easily distinguished from other families by its characteristics posterior spinneret which has two-segments and long, thin, tipped toward each other. *Tegenaria* Latreille 1804 differs from other genera by the number and position of gonadal teeth, the structure of the tibial and median apophyses, the shape of the conductor, the shape of the vulva only having a spiral duct, and the scleroid structure [5]. The araneomorph karyotypes are characterized by a predominance of acrocentric chromosomes, X_1X_20 sex chromosome system [6], relatively low diploid chromosome numbers (ranges from 10 to 49, [7]), and chiasmatic meiosis. Acrocentric/telocentric karyotypes of entelegynes with lower chromosome numbers could be derived from ancestral karyotypes by tandem fusions [8] or by cycles of centric fusions and subsequent pericentric inversions [7]. The latter hypothesis is

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supported by the fact that centric fusions are the most frequent source of chromosome polymorphism found in populations of entelegyne spiders [7, 9]. In this study, karyotype characteristics and meiotic behavior of *Tegenaria elysii* Brignoli, 1978 belonging to Agelenidae family, which is found in our country, were investigated for the first time.

2. EXPERIMENTAL

The male specimens of *Tegenaria elysii* were collected from Nevşehir, Kayseri and Kahramanmaraş between March to May in the year 2015 from different habitats and localities. The collection were made by hands or pitfall traps (Table 1). During the field study, no treatment was applied to the spiders, and each one was taken to falcon tubes and placed in the laboratory. Male gonads were used in obtaining chromosomes due to the lots of cells contained. Therefore, it was possible to determine various mitotic and meiotic stages from males. However, females were not used because of the low division frequency.

Table 1. Data of collection date, locality information and number of specimens used in this study

Collection Date	Number of species	Localities	
		Region	Coordinates
14.03.2015	2♂♂	Nevşehir, Acıgöl	38°32'24.58"N 34°32'51.72"E
11.04.2015	1♂	Kayseri, Pınarbaşı	38°42'47.93"N 36°22'49.75"E
19.04.2015	3♂♂	Kahramanmaraş, Göksun	38°00'42.27"N 36°28'15.80"E
23.05.2015	7♂♂	Kayseri, Pınarbaşı	38°42'45.51"N 36°23'26.84"E

The chromosome preparations were performed according to the method of [10] with some modifications. Alive male specimens were squeezed from the prosoma and separated from the opistosoma. Prosoma and pedipalps were kept in 70% ethanol for systematic diagnosis. The gonads were dissected out in physiological solution for invertebrates. Then three steps were used; hypotonic solution in 0.075 M KCl (for 15 min), twice fixation in methanol:glacial acetic acid (3:1 for 10 and 20 min); spreading in 60% glacial acetic acid solution on heating plate that surface temperature 42 °C (for 20 min). The slides were air dried for overnight and stained with 5% Giemsa solution in Sorensen phosphate buffer (ph=6.8) for 50 min. Chromosome slides were investigated an BX53 microscope (Olympus) and well spread stages were photographed using DP26 digital camera with CellSens software (Olympus). The diploid chromosome number were determined by the mitotic metaphases or diplotene stages. Relative chromosome lengths (RCL) including standard deviations were calculated as a percentage of the total chromosome length of the diploid set by CellSens software. Classification of chromosome morphology was made by the protocol of (Table 2) [11].

Tablo 2. Determination of chromosome morphology according to the centromere position (C) and arm ratio (q/p)

Centromere position	Arm ratio (q/p)	Chromosome morphology
Median	1.00-1.70	Metacentric
Submedian	1.71-3.00	Submetacentric
Subterminal	3.01-7.00	Subtelocentric
Terminal	7.01-∞	Acrocentric

3. RESULTS

In this study, cytogenetic structure, diploid number, sex chromosome system and meiosis characteristics of *Tegenaria elysii* Brignoli, 1978 were determined for the first time.

1.1. Karyotype and sex chromosome system of *T. elysii*

The chromosome set of male *T. elysii* (2n=42, X_1X_20) contained 42 chromosomes with telocentric morphology (Fig.1, 2).

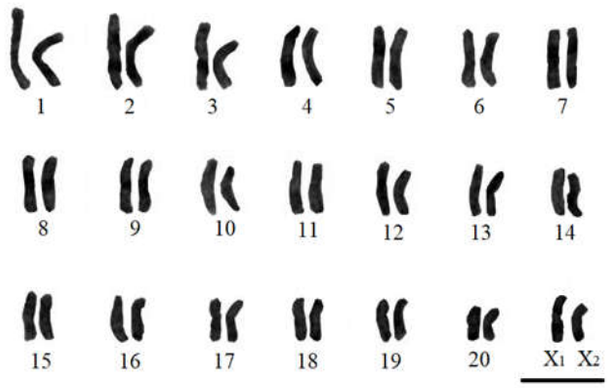

Fig.1. Karyotype of *T. elysii* based on spermatogonial metaphases (Scale=10 µm)

Autosome pairs decreased gradually in size from 6,83% to 3,31% of total chromosome length (TCL). Relative length of X_1 and X_2 were 4,24% and 3,92% of TCL, respectively (Table 3). X_1 was longer than the 14th autosome pair and X_2 was longer than the 16th autosomal pair. X_1 and X_2 were in similar size.

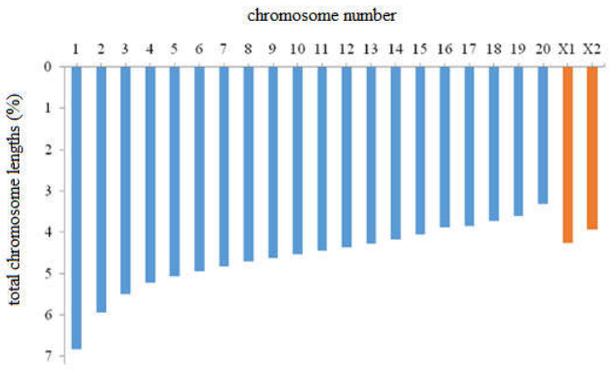

Fig. 2. Idiogram of *T. elysii* based on the haploid set of chromosomes

Table 3. Chromosome length measurements of *T. elysii*: short arm (p); long arm (q); relative lengths (p+q); arm ratio (q/p); TCL: total chromosome lengths (%); CM: chromosome morphology; T: telocentric ; ± standart deviation)

	p µm	q µm	(p+q)	q/p	TCL (%)	CM
1	0	10,88±0,67	10,88±0,67	∞	6,83	T
2	0	9,46±0,4	9,46±0,4	∞	5,93	T
3	0	8,77±0,37	8,77±0,37	∞	5,5	T
4	0	8,32±0,36	8,32±0,36	∞	5,22	T
5	0	8,07±0,4	8,07±0,4	∞	5,06	T
6	0	7,87±0,43	7,87±0,43	∞	4,94	T
7	0	7,69±0,33	7,69±0,33	∞	4,82	T
8	0	7,51±0,35	7,51±0,35	∞	4,71	T
9	0	7,37±0,29	7,37±0,29	∞	4,62	T
10	0	7,22±0,32	7,22±0,32	∞	4,53	T
11	0	7,08±0,28	7,08±0,28	∞	4,44	T
12	0	6,97±0,25	6,97±0,25	∞	4,37	T
13	0	6,81±0,3	6,81±0,3	∞	4,27	T
14	0	6,66±0,28	6,66±0,28	∞	4,18	T
15	0	6,45±0,23	6,45±0,23	∞	4,05	T
16	0	6,19±0,42	6,19±0,42	∞	3,88	T
17	0	6,13±0,44	6,13±0,44	∞	3,85	T
18	0	5,93±0,5	5,93±0,5	∞	3,72	T
19	0	5,76±0,55	5,76±0,55	∞	3,61	T
20	0	5,27±0,62	5,27±0,62	∞	3,31	T
X_1	0	6,76±0,35	6,76±0,35	∞	4,24	T
X_2	0	6,25±0,39	6,25±0,39	∞	3,92	T

1.2. Meiotic characteristics of *T. elysii*

The sex chromosomes were stained positively heteropycnotic during the first substages of prophase I (i.e. leptotene, zygotene and pacythene) (Fig. 3.a). 20 autosomal biavelents and two univalent sex chromosomes were determined in diplotene, diakinesis and metaphase I. The bivalents had one chiasma (or sometimes two chiasmata) with terminal, interstitial and proximal type (Fig. 3.b). All chromosomes including sex chromosomes were “V” shaped in anaphase I and sex chromosomes were located on the periphery of nucleus (Fig. 3.c). During the second meiotic stages (i.e. prophase II, metaphase II and anaphase II), the sex chromosomes were isopycnotic but

were easily distinguished because of their earlier condensation (Fig. 3.d).

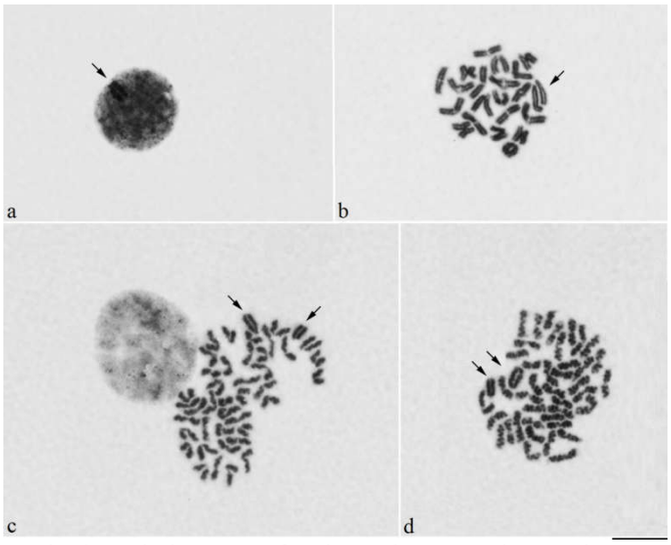

Fig. 3. Meiotic stages of male *T. elysii* a. Pachytene, b. Diplotene, c. Anaphase I, d.Prophase II (arrows indicate sex chromosomes) (Scale=10 µm)

DISCUSSION

Upto now, 77 genera and 1272 species belonging to the family Agelenidae were determined [1]. 11 genera (i.e. *Agelena* Walckenaer, 1805; *Agelescape* Levy, 1996; *Allagelena* Zhang, Zhu ve Song, 2006; *Coelotes* Blackwall, 1841; *Eratigena* Bolzern, Burckhardt ve Hänggi, 2013; *Lycosoides* Lucas, 1846; *Maimuna* Lehtinen, 1967; *Pireneitega* Kishida, 1955; *Tegenaria* Latreille, 1804; *Textrix* Sundevall, 1833; *Urocoras* Ovtchinnikov, 1999) and 61 species are spread out in our country [2].

Cytogenetic studies on spiders are scarce due to the necessity for the spider to keep alive, specific applications for individuals, the low frequency of mitotic metaphases and tiny chromosome morphology. Despite all these difficulties, seven genera and 15 species belonging to the Agelenidae family were studied, previously. These species are listed as *Agelena auclandi* Burman; *Agelena gautami* Tikader, 1962; *Agelena labyrinthica* (Clerck, 1757); *Agelena limbata* Thorell, 1897; *Agelenopsis naevia* (Walckenaer, 1841); *Allagelena difficilis* (Fox, 1936); *Allagelena opulenta* (L. Koch, 1878); *Eratigena atrica* (C.L. Koch, 1843); *Pireneitega luctuosa* (L. Koch, 1878); *Tegecoelotes corasides* (Bösenberg & Strand, 1906); *Tegenaria campestris* (C.L. Koch, 1834); *Tegenaria domestica* (Clerck, 1757); *Tegenaria ferruginea* (Panzer, 1804); *Tegenaria parietina* (Fourcroy, 1785) ve *Tegenaria silvestris* L. Koch, 1872 [12]. According to these previously studies, the diploid chromosome number were determined between 2n♂=18 (*E. atrica* [13]) and 2n♂=52 (*A. naevia* [14]). However the most common diploid chromosome number is 2n♂=42-43 in the family [12]. Although the family has a great diversity in the number of diploid chromosomes, the results obtained in our study were found to be consistent with family features.

The X_1X_20 sex chromosome system seen in most of the spiders is also characteristic for the agelenid spiders. It is known that this sex chromosome system is often encountered in spiders. It is assumed that the X_1X_20 system is generated from the X0 system either by centric fission or by the duplication of the X chromosome. However, other sex chromosome systems that are rarely seen in the family are $X_1X_2X_30$ and $X_1X_2X_3X_4X_5Y$ [15]. The presence of the Y chromosome is explained by the disruption of a fragment from the large X chromosome [16]. In our study, the sex chromosome system of *T. elysii* was found to be compatible with other family members.

In primitive spider groups, chromosomal morphology is heterogeneous and has chromosomes of metacentric, submetacentric, acrocentric and telocentric type. However, modern spiders, including agelenid spiders, usually have a homogenous structure. Taxa usually have chromosomes of either acrocentric or telocentric type. Morever, in the first meiotic division phases, the positive heteropycnotic structure of sex chromosomes, to be located on the nucleus surface, to move together, and the isopycnotic structure in the stage of second meiotic division, were also observed in other members of the family. In addition, at the stages of diplotene, diakinesis and metaphase I, the bivalents generally have one chiasma possesses a hypothesis, which leads to the idea that chromosome behavior is preserved in the family. In conclusion; these cytogenetic characters obtained for the Agelenidae family are not sufficient to distinguish the taxa alone and additional molecular based studies are needed.

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