

Comparative Study on the Karyotype of Two Species of *Megaulacobothrus* Caud., 1921 (Acridoidea)

Dorjsuren Altanchimeg¹, Chen Lin², Nonnaizab²

¹Laboratory of Entomology, Institute of Biology, Mongolian Academy of Science, Ulaanbaatar 210351, Mongolia. E-mail: d_altanchimeg@yahoo.com

²Institute of Life Science and Technology, Inner Mongolia Normal University, Huhhot 014022, China. E-mail: linchen310@126.com; nonnaizb@imnu.edu.cn

Abstract

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Correspondence:

d_altanchimeg@yahoo.com

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The karyotypes of *Chorthippus* (*Megaulacobothrus*) *aethalinus* (Zubovskiy, 1899) and *Chorthippus* (*Megaulacobothrus*) *chinensis* Tarbinsky, 1927 were compared by means of the conventional cytogenetic method. The results showed that chromosome numbers of two species were $2n(\text{♂})=17=16+XO$, in which three pairs of autosomal and sex chromosomes were terminal chromosomes, and the other five pairs of autosomal ones were metacentric chromosomes, which are the diagnostic characters of *Chorthippus*. However, these two species could be identified by the different formulae and the relative length of chromosomes. The chromosome formula of *Chorthippus* (*M.*) *aethalinus* is $K(2n, \text{♂})=6m+11t=6L+6M+4S+XO$, whereas that of *Chorthippus* (*M.*) *chinensis* is $K(2n, \text{♂})=6m+11t=6L+8M+2S+XO$. In addition, we found that the location and the relative length of sex chromosomes in the two species were different from each other. Sex chromosome of *Chorthippus* (*M.*) *aethalinus* located at fifth position and its relative length was equal to 8.33%, whereas that of *Chorthippus* (*M.*) *chinensis* was at eighth position and its relative length was equal to 5.53%. These results showed that significant different karyotype features exist in the two compared species of *Chorthippus*.

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Introduction

The subgenus *Megaulacobothrus* Caudell, 1921 belongs to the genus *Chorthippus* Fieber, 1852 (Catantopinae, Acrididae, Orthoptera). *Chorthippus* is one of the largest genera in Catantopinae, and believed to be the main group of grasshoppers in the northern China. More than 200 species of this genus were recorded from Europe, Asia, Africa and USA. Up to date, 76 species of the genus including 4 subgenera, such as *Megaulacobothrus*, *Glybtobothrus*, *Altrichorthippus*, *Chorthippus* were recorded in China (Zheng & Xi, 1998). The comparison of the karyotypes in grasshoppers not only displays their evolutionary process and trend, but also reveals the

phylogeny and taxonomic status of the species. In addition, it could also provide the scientific basis for the pest control (Zhang *et al.*, 2003; Ma *et al.*, 2000). According to literatures, there are about 10,000 species of grasshoppers worldwide, and the chromosomes of the over 1000 species have been analyzed (Ma & Zheng, 1989). Currently, karyotypes of only some species of the families Pamphagidae, Pyrgomorphidae, Oedipodidae and Arcypteridae in China have been reported (Yan, 2001; Yang *et al.*, 2008).

Members of *Chorthippus* are the main grassland pests, which emergence every year. They have high density and serious harmfulness

to grassland. Comparative study on karyotypes of *Chorthippus (M.) aethalinus* (Zubovsky, 1899) and *Chorthippus (M.) chinensis* Tarbinsky, 1927 have not been reported. In this paper, we compared the karyotypes of these two species for discussing the characteristics of cytotaxonomy and providing the theoretical basis to the classification of grasshoppers.

Material and Methods

The adult grasshoppers were identified by Nonnaizab, which preserved in the Museum of the Inner Mongolia Normal University. *Chorthippus (M.) aethalinus* was collected from the meadow near forest in Wulanmaodu soum of Keerqinyouyizhongqi of Xing an meng, Inner Mongolia (August 5, 2009), and *Chorthippus (M.) chinensis* was collected from the meadow near forest of Bogdkhan mountain in the vicinity of Ulaanbaatar city of Mongolia (July 5, 2009).

After dissecting the alive adult male grasshoppers, their testes were preserved in fixation solution (ethanol : acetic acid = 3 : 1) for 8-12 hours. Afterwards the tests were transferred to 70% ethanol and stored under 4°C until further processing. Some seminiferous tubules from the testes were stained with *Fe*-acetic carmine by traditional squash method for 25 minutes, and then prepared glass slides for further observation using the optical microscope (Olympus BH-2).

The final chromosome numbers were determined based on 85% cell division phase as cardinal number. From which, five cells were selected randomly and then took their pictures by digital photomicrography system (Motic-BA200). Furthermore, the chromosome types and lengths were analyzed using Motic Images Advanced 3.2

software. Finally, the karyotypes and ideograms of two species were obtained using Video Test-Karyo 3.1 software. The relative length and arm ratio of chromosomes were calculated by the following formula.

$$\text{The relative length (\%)} = \frac{\text{Measured length of every chromosome}}{\text{Total measured length of genome} \cdot n} \cdot 100\%$$

$$\text{The arm ratio} = \frac{\text{Length of long arm}}{\text{Length of short arm}}$$

After measuring the length of short and long arms of chromosomes in 5 cells of each species, the relative length and arm ratio were calculated, and the divisions of the chromosome type were determined by the method of Levan *et al.* (1964) and Kuo *et al.* (1972).

Results

According to the XO sex-determining mechanism, the chromosome numbers of 95.2% metaphase cells of *Chorthippus (M.) aethalinus* were $2n(\♂)=17=16+XO$, whereas the chromosome numbers of 97.3% of *Chorthippus (M.) chinensis* were $2n(\♂)=17=16+XO$.

The karyotype parameters including the absolute length, relative length and the arm ratio of two species are shown in Table 1. According to the Levan's method, two species of *Chorthippus* have the same chromosome types. The chromosomes #1-3 were metacentric, whereas the chromosomes #4-8 and sex chromosomes were telocentric in these two species (Table 1). The chromosome plates and karyotypes of two *Chorthippus* are as shown in Figures 1 and 2.

According to the method by Kuo *et al.* (1972),

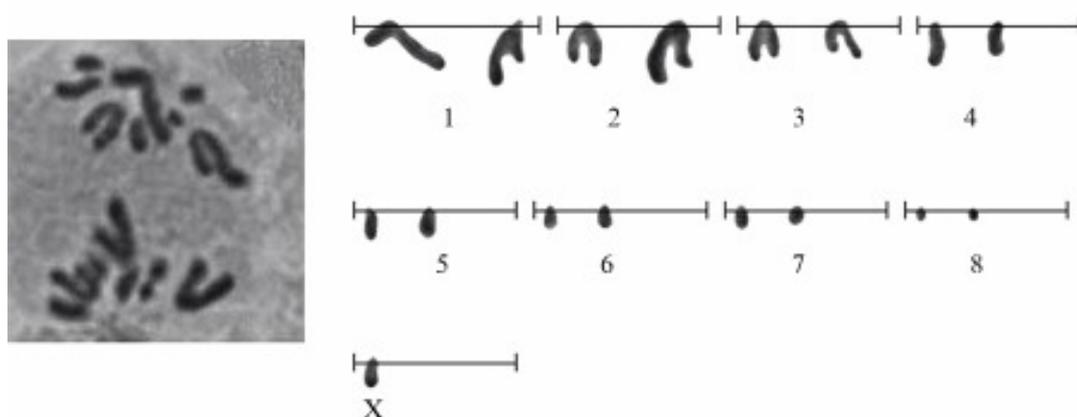


Figure 1. Karyotype of *Chorthippus (Megaulacobothrus) aethalinus*.

Table 1. The karyotype parameters of the two species of *Chorthippus* Fieb.

Species	Number of chromosomes	Absolute length (μm)			Relative length (%)	Arm ratio	Chromosome type	
		Long arm	short arm	Total length			According to Levan's method	According to Kuo's method
<i>Chorthippus (M.) aethalinus</i>	1	7.14	4.32	11.5	23.06	1.65	m	L ₁
	2	6.68	4.39	11.1	22.27	1.52	m	L ₂
	3	5.38	3.65	9.03	18.17	1.47	m	L ₃
	4	4.75			9.56		t	M ₄
	5	3.36			6.76		t	M ₅
	6	2.52			5.07		t	M ₆
	7	1.98			3.98		t	S ₇
	8	1.39			2.8		t	S ₈
	X	4.14			8.33		t	X
<i>Chorthippus (M.) chinensis</i>	1	6.43	4.81	11.2	21.82	1.34	m	L ₁
	2	6.27	3.87	10.1	19.69	1.62	m	L ₂
	3	5.02	4.35	9.37	18.19	1.15	m	L ₃
	4	4.52			8.77		t	M ₄
	5	3.97			7.71		t	M ₅
	6	3.69			7.16		t	M ₆
	7	3.18			6.17		t	M ₇
	8	2.55			4.95		t	S ₈
	X	2.85			5.53		t	X

the chromosomes of two studied species could be divided into four groups (Table 1).

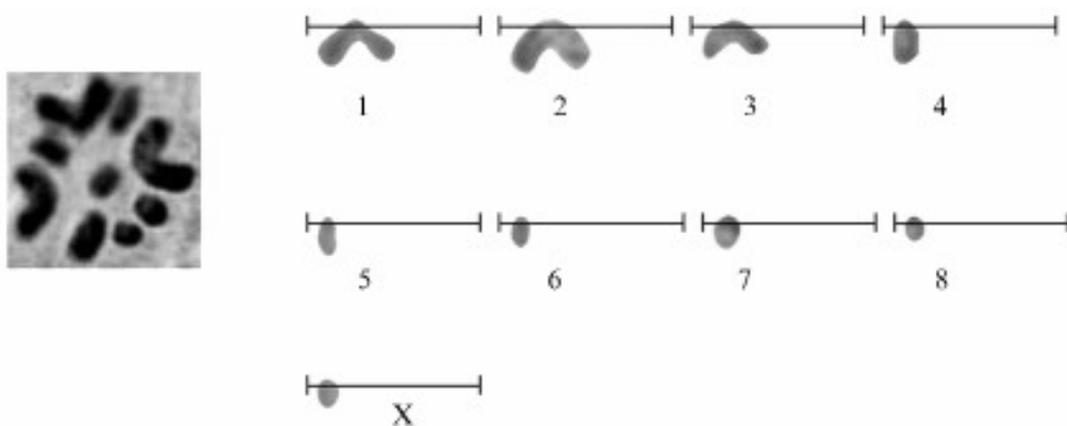
(1) Group L: Relative lengths of chromosomes are more than 10%, which called large chromosomes. #1-3 chromosomes of both species belong to this group.

(2) Group M: Relative lengths of chromosomes varied between 5% and 10%, which called medial chromosomes. #4-6 chromosomes in *Chorthippus*

(*M. aethalinus*, and #4-7 chromosomes in *Chorthippus (M.) chinensis* belong to this group.

(3) Group S: The relative lengths of chromosomes are less than 5%, which called small chromosomes. #7-8 chromosomes of *Chorthippus (M.) aethalinus*, and #8 chromosome of *Chorthippus (M.) chinensis* belong to this group.

(4) Group X represented sex chromosomes

Figure 2. Karyotype of *Chorthippus (Megaulacobothrus) chinensis*.

in both species. There were considerable differences in the relative lengths and positions of sex chromosomes between two species. The sex chromosome of *Chorthippus (M.) aethalinus* located in the fifth position and its relative length was equal to 8.33%, whereas that of *Chorthippus (M.) chinensis* located in the eighth position and its relative length was equal to 5.53%.

Our study result indicates the both species of *Chorthippus* have 17 chromosomes, with the same karyotype formula: $K(2n, \♂) = 6m + 11t$ according to Levan's method. However, in accordance with Kuo's method, there was considerable difference between group type formulas of two species. The group type formula of *Chorthippus (M.) aethalinus* was $K(2n, \♂) = 6L + 6M + 4S + 2XO$, while that of *Chorthippus (M.) chinensis* was $K(2n, \♂) = 6L + 8M + 2S + XO$.

Conclusion

The karyotypes of *Chorthippus (M.) aethalinus* and *Chorthippus (M.) chinensis* were compared for the first time. According to Levan's method, we found that the chromosome karyotypes were $2n(\♂) = 17$ in both species, in which 3 pairs of them were metacentric, whereas other 5 pairs and sex chromosomes were telocentric, which showing common features for the genus.

However, according to Kuo's method, the relative length of every chromosome was different from each other. For example, the relative length of chromosome L1 of *Chorthippus (M.) aethalinus* was apparently longer than that of *Chorthippus (M.) chinensis* (23.06% vs. 21.82%), whereas the relative length of chromosome S8 of *Chorthippus (M.) aethalinus* was shorter than that of *Chorthippus (M.) chinensis* (2.8% vs. 4.95%). The chromosome group type of *Chorthippus (M.) aethalinus* was $K(2n, \♂) = 6L + 6M + 4S + XO$, whereas that of *Chorthippus (M.) chinensis* was $K(2n, \♂) = 6L + 8M + 2S + XO$.

Although the sex chromosomes of two species of *Chorthippus* were telocentric in morphology,

their location and relative lengths were different. The sex chromosome of *Chorthippus (M.) aethalinus* located in fifth position and its relative length was equal to 8.33%, whereas that of *Chorthippus (M.) chinensis* located in eighth position and its relative length was equal to 5.53%.

Thus, our results showed that there are significant differences in the relative lengths of both autosomal and sex chromosomes, and the locations of sex chromosome in two species of *Chorthippus* indicating the different hereditary features in these species.

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