

# Comparative study of the karyotypes of two species of grasshoppers in the genera *Chorthippus* Fieber and *Euchorthippus* Tarbinsky (Orthoptera: Arcypteridae)

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**Abstract:** The chromosome karyotypes of *Chorthippus albomarginatus* (De Geer) which represents the genus *Chorthippus* Fieber and *Euchorthippus unicolor* (Ikonnikov) which represents the genus *Euchorthippus* Tarbinsky were compared. The chromosome numbers for these two species fit  $2n (\text{♂}) = 17 = 16 + XO$ , in which five pairs of autosomal and sex chromosomes are terminal chromosomes, and the other three pairs of autosomes are metacentric chromosomes. This similarity between the two genera indicates that they have a close phylogenetic relationship. However, these two species can be distinguished by their different chromosome formula (CF) and the relative sex chromosome length (RL). The CF in *C. albomarginatus* is  $K (2n, \text{♂}) = 6m + 11t = 8L + 4M + 4S + XO$ , whereas that of *E. unicolor* is  $K (2n, \text{♂}) = 6m + 11t = 8L + 6M + 2S + XO$ . The relative lengths of sex chromosomes in the two species are different. The sex-chromosome of *C. albomarginatus* is located at the ninth position and its relative length is 2.96% whereas that of *E. unicolor* is at the eighth position and its relative length is 4.26%. These results show that significantly different genetic features exist for the two genera.

**Key words:** Arcypterinae; Chromosomes; karyotype

雏蝗属与异爪蝗属二个近缘属蝗虫的染色体核型比较（直翅目：网翅蝗科）

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**摘要:** 采用常规染色体制片方法, 对雏蝗属的模式种白边雏蝗 *Chorthippus albomarginatus* (De Geer) 和异爪蝗属的模式种素色异爪蝗 *Euchorthippus unicolor* (Ikonnikov) 的染色体核型进行了分析比较。结果表明, 两种蝗虫的染色体数目均为  $2n (\text{♂}) = 17 = 16 + XO$ ; 常染色体为中部着丝粒 ( $m$ , 6 条) 和端部着丝粒 ( $t$ , 10 条) 两种类型, 性染色体类型为端部着丝粒。二者的相似性显示出 2 属具有较近的亲缘关系, 并且在进化过程中处于较近的发展阶段。但 2 种蝗虫的核型公式和染色体的相对长度组成则不相同, 白边雏蝗  $K (2n, \text{♂}) = 6m + 11t = 8L + 4M + 4S + XO$ ; 素色异爪蝗  $K (2n, \text{♂}) = 6m + 11t = 8L + 6M + 2S + XO$ ; 且 2 种蝗虫性染色体的位次也有明显差别, 白边雏蝗的性染色体为第 9 位, 而素色异爪蝗则为第 8 位。由此看出, 该 2 属的蝗虫存在的不同遗传特征。

**关键词:** 网翅蝗亚科; 染色体; 核型分析

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## Introduction

*Chorthippus albomarginatus* (De Geer) and *Euchorthippus unicolor* (Ikonnikov) are grasshoppers belonging to the Arcypterinae (Orthoptera: Arcypteridae) (Zheng & Xia 1998). Comparison of chromosome karyotypes in grasshoppers not only reveals their evolutionary history and trends, but also reveals the phylogeny and taxonomic status of the species. In addition, it may provide a scientific basis for pest control (Zhang *et al.* 2003; Ma *et al.* 2000). According to the literature, there are about 10,000 species of grasshoppers worldwide. So far, over 1000 species of grasshoppers' chromosome have been analyzed (Ma & Zheng 1989). So far, chromosome karyotypes for some species in the Pamphagidae, Pyrgomorphidae, Oedipodidae and Arcypteridae have been reported in China (Yang *et al.* 2008; Yan 2001). No analysis of chromosome karyotypes comparing species in the genus *Chorthippus* Fieber with members of the genus *Euchorthippus* Tarbinsky has been reported. In this paper, we compare the chromosome karyotypes of *C. albomarginatus* and *E. unicolor* and discuss implications for cytotaxonomy.

## Material and methods

Specimens of adult grasshoppers were identified by Nonnaizab and are preserved at the specimen museum of Inner Mongolia Normal University. *Chorthippus albomarginatus* (De Geer) was collected from meadows near forests in Bindert suom of Mongolia (05-VIII-2009). *Euchorthippus unicolor* (Ikonnikov) was collected from meadows near the forests of Daqingshan Mountain in Inner Mongolia (05-VII-2009).

After dissecting the live male adult grasshoppers, their testes were put into Canoy stationary liquid (Ethanol : acetic acid = 3 : 1) for 8–12 hours. They were then transferred into 70% alcohol and stored under 4°C until use. Some seminiferous tubules from the testes were stained with Fe-aceto carmine by a traditional squash method for 25 minutes and then prepared as glass slides for further observation.

These glass slides were observed under an Olympus optical microscope. After 50 cells from the glass slides of each species were collected, final chromosome numbers were determined based on 85% cell division phase as cardinal number. From these, 5 cells were selected randomly. Pictures were taken by a Motic-BA200 digital photomicrography system. Chromosome karyotypes and chromosome length were analyzed by Motic Images Advanced 3.2 software. The karyotypes and the idiograms of the two species of *Chorthippus* were obtained using Video Test-Karyo 3.1 software. The relative lengths and the arm ratios were calculated based on the following formula. The relative length (%) = measured length of every chromosome / total measured length of genome × 100%. The arm ratio = length of long arm / length of short arm.

## Results

Morphological characteristics of the two type species

*Euchorthippus* Tarbinsky. Body small; fastigial foveola present; antennae filiform, surpassing behind posterior margin of pronotum; lateral carinae almost parallel or slightly

curved; sulcus in the middle or behind middle of pronotum; tegmina and wings long or short; claws unequal; tympanal organ developed. A specimen is shown in Fig. 1.



Figures 1, 2. 1. *Euchorthippus unicolor* (Ikonnikov) ♂; 2. *Chorthippus albomarginatus* (De Geer) ♂. Body, dorsa view.

*Chorthippus* Fieber. Body small or medium-sized; fastigial foveola present; antennae filiform, surpassing behind posterior margin of pronotum; median carina low; lateral carinae almost parallel or slightly curved; tegmina long or short; claws equal; tympanal organ developed. A specimen is shown in Fig. 2.

By comparing the morphological characteristics of these two genera, morphological variations are not obvious; only in *Chorthippus* are the claws equal while in *Euchorthippus*, they are unequal.

#### Chromosome numbers of the two type species

According to the XO sex-determining mechanism, the chromosome numbers of 96.4% metaphase cells of *C. albomarginatus* were  $2n$  (♂) = 17 = 16 + XO. The chromosome numbers of 97.3% of *E. unicolor* were  $2n$  (♂) = 17 = 16 + XO.

#### Karyotypes of the two species

After measuring the length of short arms and long arms of chromosomes from 5 testicular cells for each species, the relative lengths and the arm ratios were calculated and the divisions of the chromosome karyotype were determined by the method of Levan *et al.* (1964) and Kuo *et al.* (1972).

The karyotype parameters including the absolute length, the relative length, and the arm ratios of the two species are shown in Table 1.

According to Levan's method, the two species have the same chromosome type. Both of these chromosomes #1–3 were metacentric whereas chromosomes #4–8 and the sex-chromosome were telocentric.

**Table 1. The karyotype parameters of the two type species**

Species	No. of chromo-somes	Absolute length ( $\mu\text{m}$ )			Relative length (%)	Arm ratio	Variation range	Chromosome type	
		Long arm	Short arm	Total length				Levan	Kuo
<i>Chorthippus</i>	1	6.78	4.7	11.48	25.33	1.44		m	L
<i>albomarginatus</i>	2	5.59	3.81	9.4	20.74	1.47		m	L
(De Geer)	3	4.6	3.85	8.45	18.64	1.19		m	L
	4			4.55	10.04	$\infty$		t	L
	5			3.62	7.99	$\infty$	11.67	t	M
	6			2.85	6.29	$\infty$		t	M
	7			1.87	4.13	$\infty$		t	S
	8			1.77	3.90	$\infty$		t	S
	X			1.34	2.96	$\infty$		t	S
<i>Euchorthippus</i>	1	6.02	3.32	9.34	22.10	1.81		m	L
<i>unicolor</i>	2	5.18	3.09	8.27	19.57	1.68		m	L
(Ikonnikov)	3	4.51	3.28	7.79	18.43	1.38		m	L
	4			4.28	10.13	$\infty$		t	L
	5			3.94	9.32	$\infty$	17.56	t	M
	6			2.92	6.91	$\infty$		t	M
	7			2.28	5.40	$\infty$		t	M
	8			1.64	3.88	$\infty$		t	S
	X			1.80	4.26	$\infty$		t	S

According to the Kuo's method, the chromosomes of the two species can be divided into four groups.

(1) Group L: The relative lengths of chromosomes are more than 10%, which are considered large chromosomes. #1–4 chromosomes belonged to this category for both species, in which #1–3 were metacentric whereas chromosome #4 was telocentric. The relative lengths of #1–4 chromosomes in *C. albomarginatus* were 25.33%, 20.74%, 18.64%, and 10.04% whereas for *E. unicolor*'s, they were 22.10%, 19.57%, 18.43%, and 10.13%.

(2) Group M: The relative lengths of chromosomes are between 5% and 10%, which are designated medial chromosomes. #5–6 chromosomes in *C. albomarginatus* with relative length of 7.99% and 6.29%. The #5–7 chromosomes in *E. unicolor* had relative lengths of 9.32%, 6.91%, 7.16%, and 5.40% and are designated medial chromosomes. They were all telocentric.

(3) Group S: The relative lengths of these chromosomes are less than 5%, which designates them as small chromosomes. #7–8 chromosomes of *C. albomarginatus* had relative lengths of 4.13% and 3.90%. The #8 chromosome of *E. unicolor* had a relative length of 3.88% and belongs to this category.

(4) Group X represents the sex chromosome in both species. There were considerable differences in the relative length and position of sex chromosomes between these two species. The sex chromosome of *C. albomarginatus* was located in the ninth position and its relative length was 2.96% whereas that of *E. unicolor* was located in the eighth position and its relative

length is 4.26%.

### Chromosome karyotypes

The chromosome plates and idiogram of the karyotypes of the two type species are shown in Figs. 3–6. The results indicate that both of these species have the same chromosome number of 17 with the same karyotype formula of  $K(2n, \text{♂}) = 6m + 11t$  using Levan's method. However, in accordance with Kuo's method, there is a considerable difference between the group type formulas for the two species. The group type formula for *C. albomarginatus* is  $K(2n, \text{♂}) = 8L + 4M + 4S + XO$ , while *E. unicolor* is  $K(2n, \text{♂}) = 8L + 6M + 2S + XO$ .

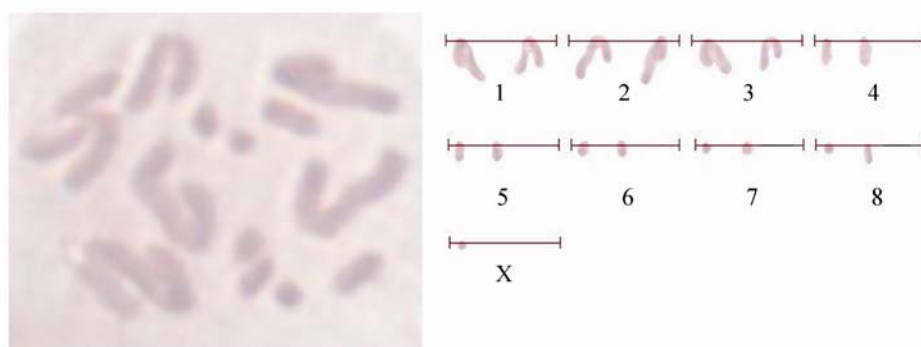


Figure 3. The anaphase and karyogram for *Chorthippus albomarginatus*.

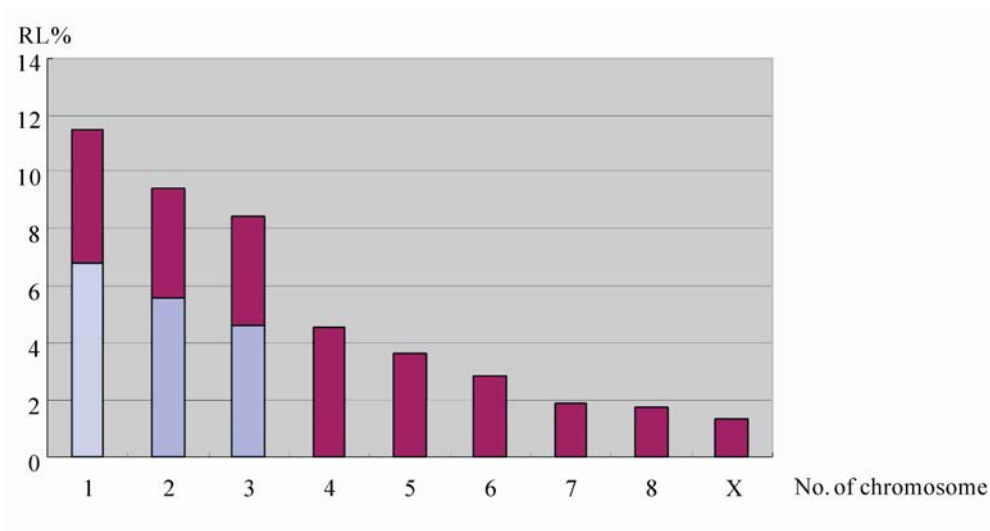
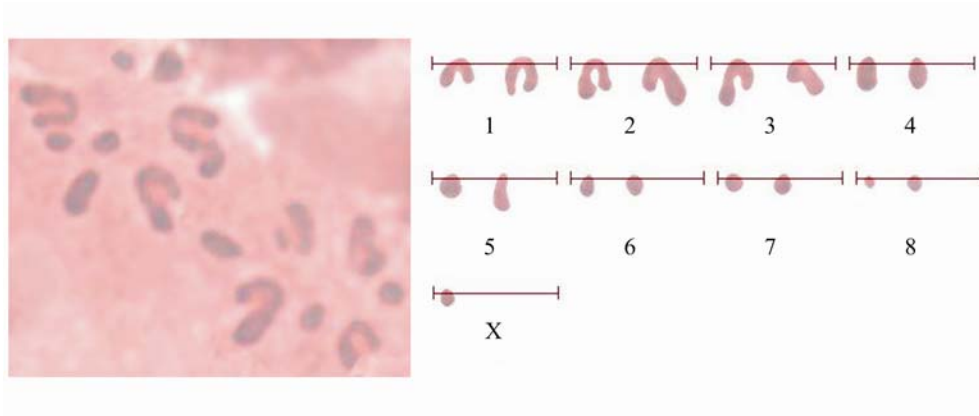
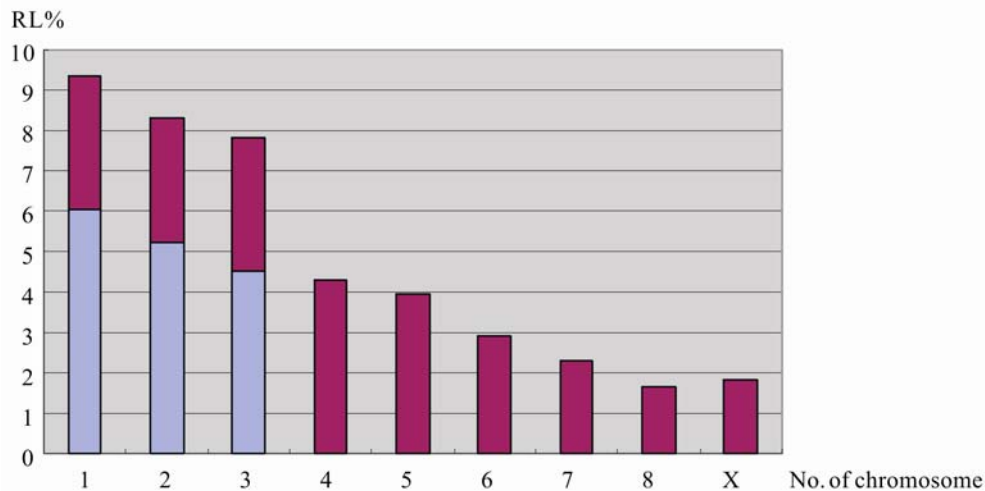


Figure 4. The karyotype of *Chorthippus albomarginatus* (De Geer).

Figure 5. The anaphase karyogram of *Euchorthippus unicolor*.Figure 6. The karyotype of *Euchorthippus unicolor* (Ikonnikov).

### Conclusion

The chromosome karyotypes of the two species representing the genera *Chorthippus* and *Euchorthippus* are compared for the first time. According to Levan's method, we found that the chromosome karyotypes of the two type species are  $2n (\text{♂}) = 17$  in both species, in which 3 pairs are metacentric and the other 5 pairs and sex chromosome are telocentric, showing the common features of the two genera. However, according to Kuo's method, we found that the relative lengths of every chromosome were different. For example, the relative length of chromosome  $L_1$  of *Chorthippus albomarginatus* was longer than that of *Euchorthippus unicolor* (25.33% vs. 22.1%).

Meanwhile, the chromosome group type of *Chorthippus albomarginatus* was  $K (2n, \text{♂}) = 6m + 11t = 8L + 4M + 4S + XO$  and in *Euchorthippus unicolor* this was  $K (2n, \text{♂}) = 6m + 11t = 8L + 6M + 2S + XO$ .

Although the sex-chromosomes of the two species are telocentric in morphology, their locations and the relative lengths are different. The sex chromosome of *Chorthippus*

*albomarginatus* is located in the ninth position and its relative length is 2.96% whereas that of *Euchorthippus unicolor* is located in the eighth position and its relative length is 4.26%.

Our results show that there are significant differences in the relative lengths of both autosomal and sex-chromosomes, and the locations of the sex-chromosome in the two species indicate different hereditary features exist in these two genera.

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