

## Cytological Studies on Six Species of Spiders from Taiwan (Araneae: Theridiidae, Psechridae, Uloboridae, Oxyopidae, and Ctenidae)

Shyh-Hwang Chen

Department of Biology, National Taiwan Normal University, Taipei, Taiwan 116, R.O.C.

Tel: 886-2-29326234 ext. 231. Fax: 886-2-29312904. E-mail: biofv023@scc.ntnu.edu.tw

(Accepted July 13, 1999)

**Shyh-Hwang Chen (1999)** Cytological studies on six species of spiders from Taiwan (Araneae: Theridiidae, Psechridae, Uloboridae, Oxyopidae, and Ctenidae). *Zoological Studies* 38(4): 423-434. An improved newly introduced air-dried method was to prepare spider chromosomes. Chromosomal data are reported for 6 species of 5 families of spiders from Taiwan. The number of diploid chromosomes ( $2n$ ) in both male ( $\delta$ ) and female ( $\text{♀}$ ), the number of autosomal bivalents and the univalent sex chromosomes ( $X$ 's) in the 1st meiotic division ( $n(\text{I})$ ) in male, the number of chromosomes in the 2nd meiotic division ( $n(\text{II})$ ) in male, and the type of sex-determining mechanism of each species were determined as follows: *Octonoba spinosa* (Uloboridae)  $2n = 18$   $\delta$  /  $20$   $\text{♀}$ ,  $n(\text{I}) = 8 + X_1X_2$ ,  $n(\text{II}) = 8$  and  $10$ ,  $X_1X_2O$  type; *Achaearanea tepidariorum* (Theridiidae)  $n(\text{I}) = 10 + X_1X_2$ ,  $X_1X_2O$  type; *Psechrus sinensis* (Psechridae)  $2n = 24$   $\delta$ ,  $n(\text{I}) = 11 + X_1X_2$ ,  $n(\text{II}) = 11$  and  $13$ ,  $X_1X_2O$  type; *Oxyopes macilentus* (Oxyopidae)  $2n = 21$   $\delta$  /  $22$   $\text{♀}$ ,  $n(\text{I}) = 10 + X$ ,  $n(\text{II}) = 10$  and  $11$  in the Taipei population, and  $2n = 23$   $\delta$ ,  $n(\text{I}) = 11 + X$ ,  $n(\text{II}) = 11$  and  $12$  in the Hualien population, both  $XO$  type; *Oxyopes sertatus* (Oxyopidae)  $2n = 21$   $\delta$  /  $22$   $\text{♀}$ ,  $n(\text{I}) = 10 + X$ ,  $n(\text{II}) = 10$  and  $11$ ,  $XO$  type; and *Anahita fauna* (Ctenidae)  $2n = 29$   $\delta$ ,  $n(\text{I}) = 13 + X_1X_2X_3$ ,  $n(\text{II}) = 13$  and  $16$ ,  $X_1X_2X_3O$  type. The chromosome data of *Octonoba spinosa*, *Psechrus sinensis*, *Oxyopes macilentus*, and *Anahita fauna* are reported for the first time. In addition, chromosome data of the families Psechridae and Ctenidae represented by *Psechrus sinensis* and *Anahita fauna*, respectively, are also reported for the first time. *Oxyopes macilentus* is reported to exhibit chromosome polymorphism among populations, and the diploid chromosome number  $2n = 23$  with 11 pairs of autosomes in 1 male is reported for the first time among all species of *Oxyopes* studied.

**Key words:** Araneae, Chromosome, Sex-determining mechanism, Taiwan.

Studies on chromosomes of spiders are few. Up to the present, about 400 out of 34 000 spider species have been studied (Hackman 1948, Suzuki 1951 1952 1954, Datta and Chatterjee 1983, Srivastava and Shukla 1986, Gorlova et al. 1997). The chromosome numbers of most spiders are relatively stable in contrast to those of harvestmen in arachnids (Tsurusaki and Cokendolpher 1990). Although there are some exceptions, most spiders of the same genus, or even the same family that have been investigated usually contain the same number of chromosomes (Hackman 1948, Suzuki 1952 1954). For example, the chromosome numbers are  $2n = 28$  in males and 30 in females in 7 out of 8 pisaurid spiders (Pisauridae) investigated, and  $2n = 22$  in male and 24 in female in 18 out of 20 species of theridiid spiders (Theridiidae). Exceptions provide useful information to clarify cryptic species or to test their

phylogeny. Another remarkable characteristic of spider chromosomes, frequently shown, is the presence of multiple sex chromosomes. Generally, there is only 1 pair of sex chromosomes in the great majority of other animals, such as  $XX \text{♀} / XY \text{♂}$  in the  $XY$  type, and  $ZW \text{♀} / ZZ \text{♂}$  in the  $ZW$  type. In spiders, 3 major types of sex-determining mechanisms have been reported, i.e.,  $XO$ ,  $X_1X_2O$ , and  $X_1X_2X_3O$  types. Spiders with the  $XO$  type have 2 homologous  $X$ 's in female but only 1 unpaired  $X$  chromosome in male ( $XX \text{♀} / X \text{♂}$ ). In the same way, sex chromosomes are  $X_1X_1X_2X_2 \text{♀} / X_1X_2 \text{♂}$  and  $X_1X_1X_2X_2X_3X_3 \text{♀} / X_1X_2X_3 \text{♂}$  for spiders with the  $X_1X_2O$  and  $X_1X_2X_3O$  types. The evolution of multiple sex chromosomes in spiders is complicated, and many hypotheses have been proposed (White 1973). However, the  $X_1X_2O$  type seems to be the most primitive in present day spiders as stated by Suzuki (1954). This type may

have evolved from a remote XO-type ancestor by centric fragmentation accompanying inversion following breakage. The  $X_1X_2X_3O$  type was derived from the  $X_1X_2O$  type by the same process described above, and the modern XO type evolved by the gradual elimination of one of the 2 X's from the  $X_1X_2O$  type (Suzuki 1954). In addition, some other rare sex-determining mechanisms have also been reported. Datta and Chatterjee (1983) reported the  $X_1X_2X_3X_4O$  type in *Heteropoda sikkimensis*, *Meta segmentata*, and *Parassus* sp. from India; Maddison (1982) reported the XXXY sex chromosomes in males of *Pellenes*; and Rowell (1985) reported a sex-linked complex heterozygosity in *Delena cancerides* from Australia. Except for a few families, the  $X_1X_2O$  type of sex-determining mechanism has been reported from nearly all families studied. However, species of the same family generally have the same major sex-determining mechanism (Table 1). The exceptions can be simply explained by sex chromosome evolution that certainly has occurred in these families, or can be used to test the phylogeny of these families. Therefore, both chromosome number and sex-determining mechanism are important in the study of spider phylogeny and mode of chromosome evolution. No chromosome numbers or sex-determining mechanisms of spiders have previously been reported from Taiwan. In the present paper, 6 species of spiders of 5 families are studied cytologically. Chromosome data of *Octonoba spinosa*, *Psechrus sinensis*, *Oxyopes macilentus*, and *Anahita fauna* are reported for the first time. In addition, chromosome data of the families Psechridae and Ctenidae represented by *Psechrus sinensis* and *Anahita fauna*, respectively, are also reported for the first time.

## MATERIALS AND METHODS

Spiders were collected from Hualien (eastern Taiwan) and Taipei (northern Taiwan) from February to April 1998. All vouchers were preserved in 70% ethanol and deposited in the Arachnological Collections of the Department of Biology, National Taiwan Normal Univ. (NTNUB-Ar).

An improved air-dried method for preparing spider chromosomes was devised, following the method of Luykx (1983), with minor modifications by the author. The abdomen of a spider anesthetized in ethyl ether was cut open on a wax plate using fine scissors. Gonads were removed, placed onto a slide, and soaked in 2 drops of 0.075 M potassium chloride solution in a humid chamber for about 35

min. Any excessive solution was removed from the slide by forceps, and the gonads were fixed by adding a few drops of fixative I (methyl alcohol: glacial acetic acid: water = 3: 3: 4, by volume) across the inclined slide. With the slide lying flat, gonads were immediately macerated with fine needles, and then 10 drops of fixative II (methyl alcohol: glacial acetic acid = 1: 1, by volume) were added to the tissues. This was let to stand for 15 s, the slide was drained

**Table 1.** Types of sex-determining mechanisms in all studied spider families. Data are based on Datta and Chatterjee (1983), Gorlova et al. (1997), Hackman (1948), Srivastava and Shukla (1986), Suzuki (1952 1954), and Tugmon et al. (1990). Rare and occasional cases are in parentheses

Family	Sex-determining mechanism
Suborder Mesothelae	
Liphistiidae	$X_1X_2O$
Suborder Mygalomorphae	
Atypidae	$X_1X_2O$
Dipluridae	$X_1X_2O$
Theraphosidae	$X_1X_2O$
Suborder Araneomorphae	
Agelenidae	$X_1X_2O$ $X_1X_2X_3O$
Amaurobiidae	$X_1X_2O$
Anyphaenidae	$X_1X_2O$
Araneidae	(XO) $X_1X_2O$
Clubionidae	$X_1X_2O$ $X_1X_2X_3O$
Corinnidae	$X_1X_2O$
Cybaeidae	$X_1X_2O$
Dictynidae	$X_1X_2O$
Dysderidae	XO
Eresidae	$X_1X_2O$
Gnaphosidae	XO $X_1X_2O$
Hahniidae	$X_1X_2O$
Hersiliidae	$X_1X_2O$
Heteropodidae	(XO) ( $X_1X_2O$ ) $X_1X_2X_3O$ ( $X_1X_2X_3X_4O$ )
Linyphiidae	(XO) $X_1X_2O$ $X_1X_2X_3O$
Lycosidae	$X_1X_2O$
Mimetidae	$X_1X_2O$
Miturgidae	$X_1X_2O$
Nesticidae	$X_1X_2O$
Oecobiidae	$X_1X_2O$ $X_1X_2X_3O$
Oxyopidae	XO ( $X_1X_2O$ )
Philodromidae	(XO) $X_1X_2O$
Pholcidae	XO $X_1X_2O$
Pisauridae	$X_1X_2O$
Salticidae	XO $X_1X_2O$ ( $X_1X_2X_3O$ )
Segestriidae	XO
Selenopidae	$X_1X_2X_3O$
Sicariidae	$X_1X_2O$
Tetragnathidae	$X_1X_2O$ ( $X_1X_2X_3O$ ) ( $X_1X_2X_3X_4O$ )
Theridiidae	$X_1X_2O$
Thomisidae	XO ( $X_1X_2O$ )
Trochanteriidae	$X_1X_2O$
Uloboridae	(XO) $X_1X_2O$ ( $X_1X_2X_3O$ )
Zodariidae	$X_1X_2O$

briefly, and then the slide was placed into a Coplin jar containing fixative III (methyl alcohol: glacial acetic acid = 3: 1, by volume) for 30 min. The slide was removed and several drops of glacial acetic acid were added across the inclined slide, after which the specimen was drained again, and dried over a flame briefly. Chromosomes were stained by adding 10-15 drops of 10% Giemsa solution to the macerated tissues, then covering with a cover slide for 6 min, rinsing in distilled water to wash off the cover slide and excess Giemsa solution, and drying by using bibulous paper. An average of 20 well-spread cells for each animal were counted and photographed under oil immersion without adding any cover slide. Photographed slides were permanently mounted with Permount after rinsing the slides in 3 changes of Xylene solution and labeled for long-term storage.

*Specimens examined:* *Octonoba spinosa* (Uloboridae)—NTNUB-Ar 2212, 1 ♀ 2 ♂♂, 15-III-1998, Shihting, Taipei Co. *Achaearanea tepidariorum* (Theridiidae)—NTNUB-Ar 2214, 1 ♂, 19-II-1998, campus of National Taiwan Normal Univ., Taipei. *Psechrus sinensis* (Psechridae)—NTNUB-Ar 2209, 1 ♂, 19-II-1998, Pinglin, Taipei Co. *Oxyopes macilentus* (Oxyopidae)—NTNUB-Ar 2219, 2 ♀♀ 1 ♂, 2-IV-1998, Tsantsushan, Taipei; NTNUB-Ar 2220, 1 ♂, 18-IV-1998, Wulai, Taipei Co.; NTNUB-Ar 2205, 1 ♂, 17-II-1998, Antung, Hualien Co.

*Oxyopes sertatus* (Oxyopidae)—NTNUB-Ar 2216, 3 ♂♂, 29-III-1998, Hsienchihiyen, Taipei; NTNUB-Ar 2218, 2 ♀♀, 2-IV-1998, Tsantsushan, Taipei. *Anahita fauna* (Ctenidae)—NTNUB-Ar 2210, 1 ♂, 18-II-1998, Antung, Hualien Co. All specimens were collected by the author.

## RESULTS

Table 2 is a summary of both mitotic and meiotic chromosome numbers of the 6 species of Taiwanese spiders.

### *Octonoba spinosa* Yoshida, 1982

The male spermatogonial mitotic plate consists of 18 chromosomes ( $2n = 18$ ) (Fig. 1), and the female oogonial mitotic plate consists of 20 chromosomes ( $2n = 20$ ) (Fig. 2). All chromosomes are telocentric.

In the 1st meiotic division, 2 X-chromosomes were much condensed and more heavily stained than autosomes at the stages from leptotene (Fig. 3) to pachytene (Fig. 4). Eight autosomal bivalents and 2 univalent sex chromosomes were observed at the diakinesis stage (Fig. 5). The 2 X's are the longest ones remaining parallel and in association with each

**Table 2.** Diploid chromosome number ( $2n$ ), 1st and 2nd meiotic chromosome counts ( $n$  (I) and  $n$  (II)), and locales (Loc) in 6 species of Taiwanese spiders

Taxa	Sex	$2n$	$n$ (I)	$n$ (II)	Loc <sup>a</sup>
Family Uloboridae					
<i>Octonoba spinosa</i>	M	18	8 + X <sub>1</sub> X <sub>2</sub>	8, 10	ST
	F	20	–	–	ST
Family Theridiidae					
<i>Achaearanea tepidariorum</i>	M	–	10 + X <sub>1</sub> X <sub>2</sub>	–	NTNU
Family Psechridae					
<i>Psechrus sinensis</i>	M	24	11 + X <sub>1</sub> X <sub>2</sub>	11, 13	PL
Family Oxyopidae					
<i>Oxyopes macilentus</i>	M	23	11 + X	11, 12	AT
	M	21	10 + X	10, 11	TS
	F	22	–	–	TS
	M	21	10 + X	10, 11	WL
<i>Oxyopes sertatus</i>	M	21	10 + X	10, 11	CM
	F	22	–	–	TS
Family Ctenidae					
<i>Anahita fauna</i>	M	29	13 + X <sub>1</sub> X <sub>2</sub> X <sub>3</sub>	13, 16	AT

Loc<sup>a</sup>: AT = Antung, Hualien Co.; CM = Hsienchihiyen, Taipei; NTNU = NTNU campus, Taipei; PL = Shihpai, Pinglin, Taipei Co.; ST = Shihting, Taipei Co.; TS = Tsantsushan, Taipei; WL = Wulai, Taipei Co.

other.  $X_1$  is slightly longer than the other ( $X_2$ ). Neither is heavily stained. Either 8 or 10 chromosomes were observed in the anaphase II plate (Fig. 6). The sex-determining mechanism is  $X_1X_2O$  type.

#### ***Achaearanea tepidariorum* (C. Koch, 1841)**

Only 1 male is available in this study. In the 1st meiotic division, 10 autosomal bivalents and 2 univalent sex chromosomes were shown at the diplotene and diakinesis stages (Figs. 7, 8). The 2 X's are the shortest ones, but are unequal in size. Both X's and one end of the autosomal bivalents were strongly condensed and heavily stained at the diplotene stage (Fig. 7). Although a spermatogonial mitotic plate was not found, the male diploid chromosome number calculated from the diakinesis stage should be 22 ( $2n = 22$ ). The sex-determining mechanism is

$X_1X_2O$  type.

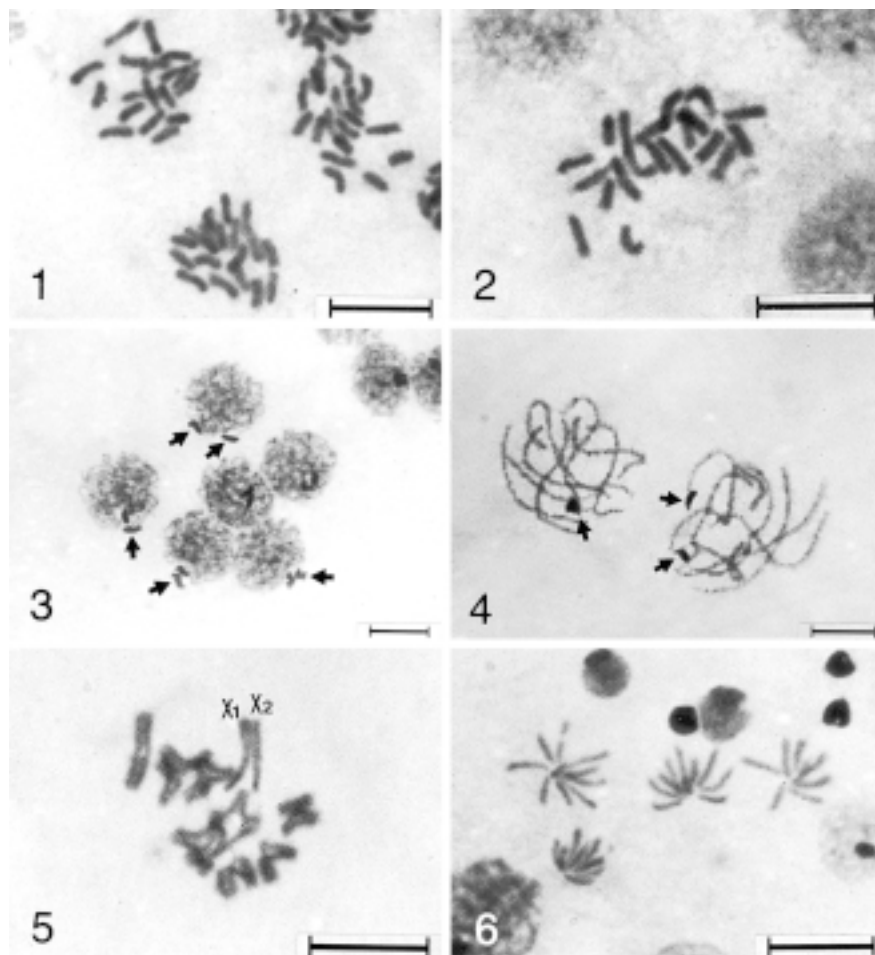
#### ***Psechrus sinensis* Berland, 1914**

Only 1 male is available in this study. The male spermatogonial mitotic plate shows 24 chromosomes ( $2n = 24$ ) (Fig. 9). All chromosomes are telocentric.

There are 11 autosomal bivalents and 2 univalent sex chromosomes in the metaphase of the 1st meiotic division (Fig. 10). Two univalent X-chromosomes are parallel and associated with each other. The sex-determining mechanism is  $X_1X_2O$  type.

#### ***Oxyopes macilentus* L. Koch, 1878**

Two distinct chromosome numbers were found among populations of *Oxyopes macilentus* in north-



**Figs. 1-6.** *Octonoba spinosa*. 1. Spermatogonial mitotic metaphase plate,  $2n = 18$ . 2. Oogonial mitotic metaphase plate,  $2n = 20$ . 3. Two heavily stained sex chromosomes (arrows) situated in the margin of leptotene plates. 4. Pachytene plates with 2 separated sex chromosomes (arrows), which are often in contact with each other (left plate). 5. Diakinesis plate with 8 bivalents and 2 large univalent sex chromosomes ( $X_1$ ,  $X_2$ ). 6. Anaphase II plates with either  $n = 8$  or 10. Bar = 10  $\mu$ .

ern and eastern Taiwan. In the Taipei population (northern Taiwan: Tsantsushan and Wulai), the male spermatogonial mitotic plate consists of 21 chromosomes ( $2n = 21$ ), and the female oogonial mitotic plate consists of 22 chromosomes ( $2n = 22$ ) (Figs. 11, 12). All chromosomes are telocentric.

During the 1st meiotic division, an X-chromosome situated in the margin of the pachytene plate was much condensed and heavily stained (Fig. 13). Ten autosomal bivalents and 1 univalent sex chromosome were observed at the diakinesis stage (Fig. 14). Either 10 or 11 chromosomes were found in the anaphase II plates (Fig. 15). The sex-determining mechanism is XO type.

However, the only individual of *Oxyopes macilentus* from the Hualien population (eastern Taiwan: Antung) shows a unique chromosome number. Its spermatogonial mitotic plate consists of 23 chromosomes ( $2n = 23$ ) (Fig. 16). All are telocentric. There were 11 autosomal bivalents and 1 univalent sex chromosome clearly shown at the diakinesis stage (Fig. 17). Either 11 or 12 chromosomes were observed in the metaphase II plate (Fig. 18). The sex-determining mechanism of *Oxyopes macilentus* from the Hualien population is also the XO type.

There is a proximal secondary constriction

clearly visible in the male mitotic metaphase plate of the Taipei population (Fig. 11) and in 1 autosomal bivalent of the male diakinesis plate of both populations (Figs. 14, 17). The same secondary constriction is also found in *O. sertatus* but not in the other species of the present study.

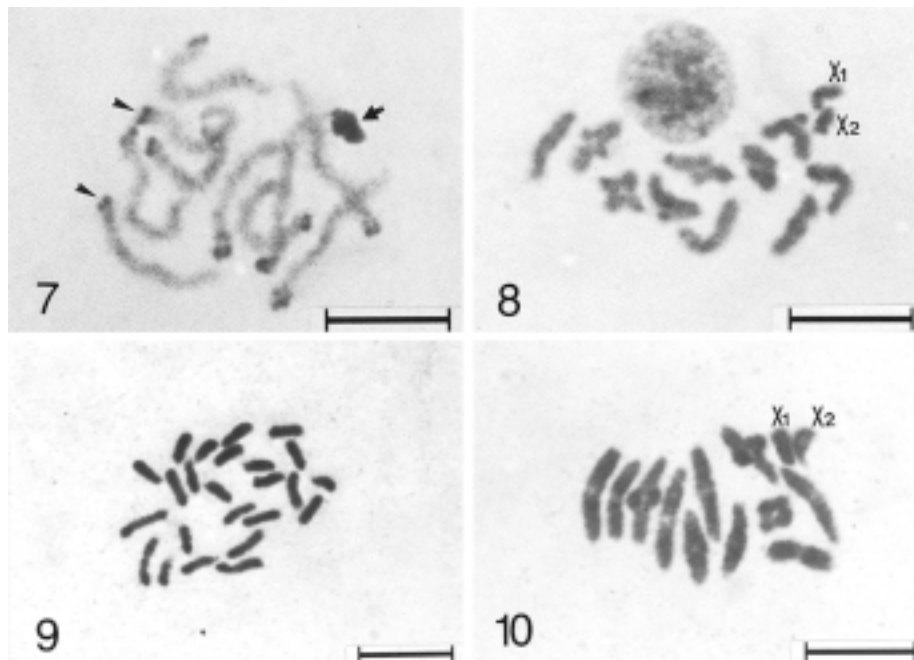
#### *Oxyopes sertatus* L. Koch, 1877

The male spermatogonial mitotic plate consists of 21 chromosomes ( $2n = 21$ ), and the female oogonial mitotic plate consists of 22 chromosomes ( $2n = 22$ ) (Figs. 19, 20). All chromosomes are telocentric.

During the 1st meiotic division, 2 X-chromosomes were much condensed and heavily stained at the pachytene stage (Fig. 21). Ten autosomal bivalents and 1 univalent sex chromosome were clearly shown at the diakinesis stage (Fig. 22). In addition, a proximal secondary constriction is clearly visible in 1 bivalent. Either 10 or 11 chromosomes were observed in the anaphase II plate (Fig. 23). The sex-determining mechanism is XO type.

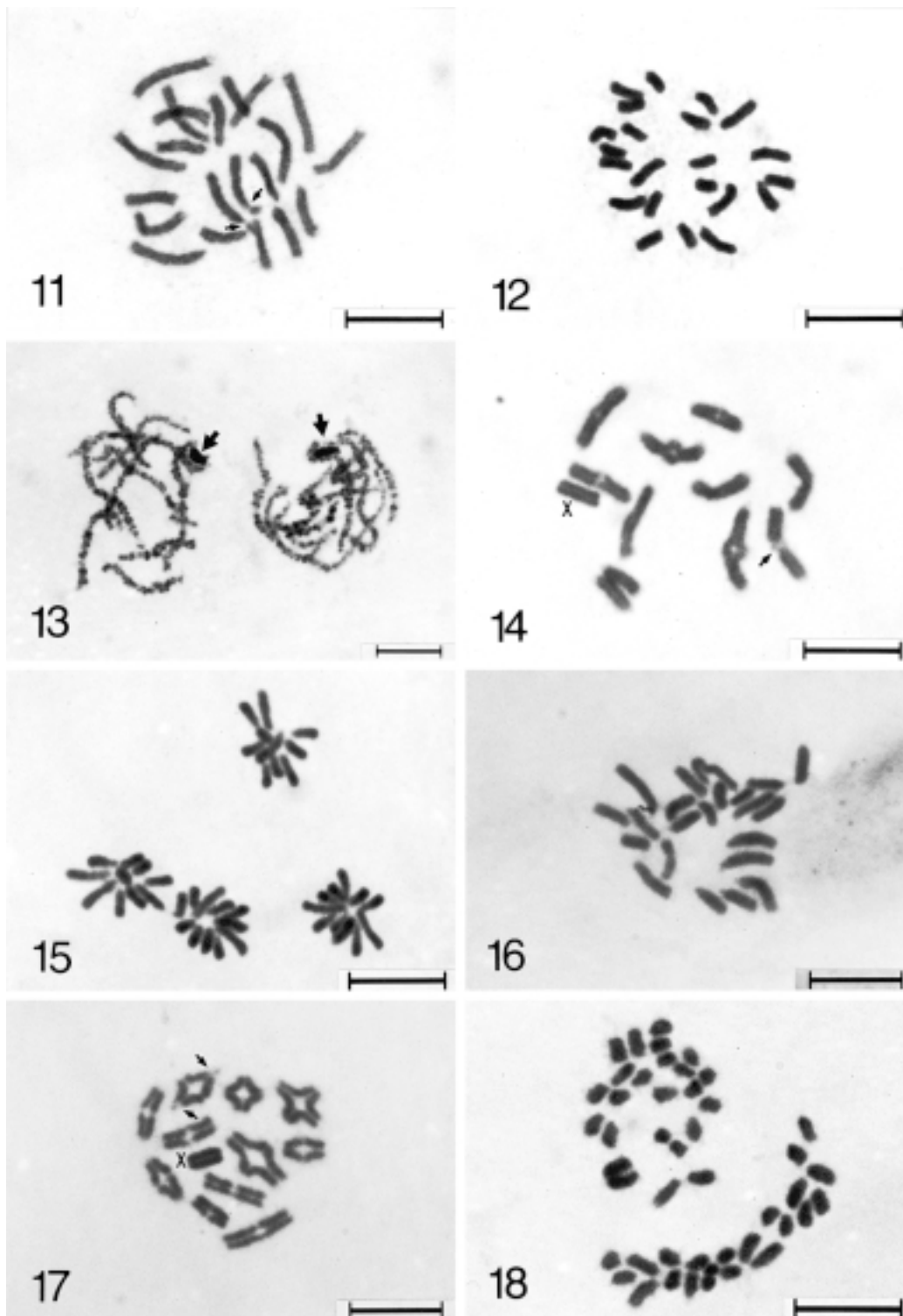
#### *Anahita fauna* Karsch, 1879

Only 1 male is available in this study. The male spermatogonial mitotic plate consists of 29 chromo-



**Figs. 7-8.** *Achaearanea tepidariorum*. 7. Pachytene plate with both sex chromosomes (arrow) and one end of each autosome (arrowhead) heavily stained. 8. Diakinesis plate with 10 bivalents and 2 univalent sex chromosomes ( $X_1$ ,  $X_2$ ). Bar = 10  $\mu$ .

**Figs. 9-10.** *Psecchrus sinensis*. 9. Spermatogonial mitotic metaphase plate,  $2n = 24$ . 10. Metaphase plate with 11 bivalents and 2 univalent sex chromosomes ( $X_1$ ,  $X_2$ ). Bar = 10  $\mu$ .



**Figs. 11-18.** *Oxyopes macilentus*. Figs. 11-15. Taipei population. 11. Spermatogonial mitotic metaphase plate,  $2n = 21$ . A distinctly secondary constriction (arrow) is visible proximally in 1 pair of both homologues. 12. Oogonial mitotic metaphase plate,  $2n = 22$ . 13. A heavily stained sex chromosome (arrow) situated in the margin of each pachytene plate. 14. Diakinesis plate with 10 bivalents and 1 univalent sex chromosome (X). There is a proximal crossover (arrow) in the bivalent, making the constriction appear as a gray area in the middle. 15. Anaphase II plates with either  $n = 10$  or  $11$ . Figs. 16-18. Hualien population. 16. Spermatogonial mitotic metaphase plate,  $2n = 23$ . 17. Diakinesis plate with 11 bivalents and 1 univalent sex chromosome (X). Secondary constrictions (arrows) are visible near the centromeres of both chromosomes in 1 bivalent. 18. Metaphase II plates with either  $n = 11$  or  $12$ . Bar =  $10 \mu$ .

somes ( $2n = 29$ ) (Fig. 24). Chromosomes are generally arranged in a radial configuration with a hollow center in the metaphase. However, there are still a few chromosomes present in the hollow center of the plate. No interconnective strands between 2 adjacent chromosomes are visible in any metaphase plates examined. All chromosomes are telocentric.

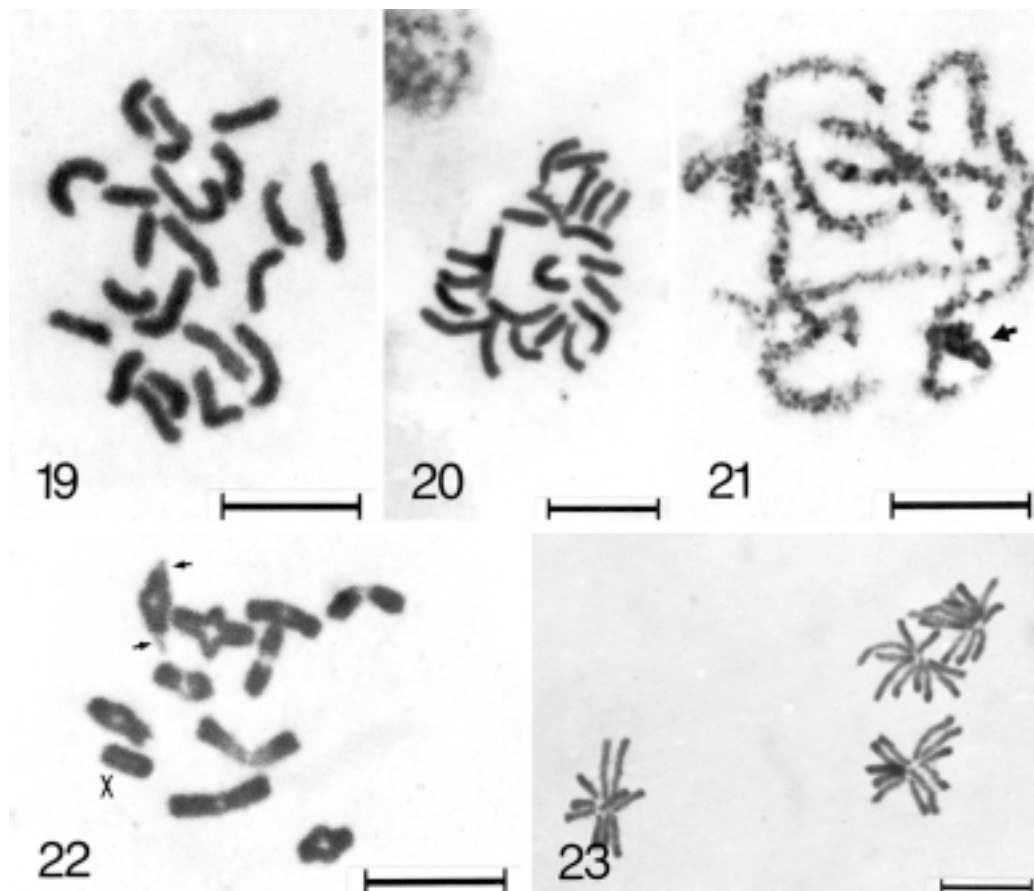
During the 1st meiotic division, 3 X-chromosomes were heavily stained and closely arranged, and they often formed a long bar in the margin of the cell plate (Fig. 25). There were 13 autosomal bivalents and 3 unequal univalent sex chromosomes associated with each other at the diakinesis stage (Fig. 26). One X-chromosome ( $X_1$ ) is distinctly longer than the others ( $X_2$  and  $X_3$ ). Either 13 or 16 chromosomes were found in the anaphase II plate (Fig. 27). All chromosomes were also arranged in a radial configuration with a hollow center at this stage. Sex chromosomes are indistinguishable from auto-

somes. The sex-determining mechanism is  $X_1X_2X_3O$  type.

## DISCUSSION

### Chromosome preparation

Gonads are excellent materials, providing good mitotic metaphase plates and various stages of meiotic plates. In all mitotic metaphase plates examined in this study, sex chromosomes are indistinguishable from autosomes. Therefore, meiotic preparations are necessary for determination of the sex-determining mechanism (Tugmon et al. 1990). However, nonhomologous sex chromosomes are often arranged together forming a heavily stained sex-chromosomal complex during the 1st meiotic division which makes counting the sex chromosomes



**Figs. 19-23.** *Oxyopes sertatus*. 19. Spermatogonial mitotic metaphase plate,  $2n = 21$ . 20. Oogonial mitotic metaphase plate,  $2n = 22$ . 21. A heavily stained sex chromosome (arrow) situated in the margin of the pachytene plate. 22. Diakinesis plate with 10 bivalents and 1 univalent sex chromosome (X). Secondary constrictions (arrows) are visible near the centromeres of both chromosomes in 1 bivalent. 23. Anaphase II plates with chromosomes arranged in a solid radial configuration,  $n = 10$  or  $11$ . Bar =  $10 \mu$ .

difficult. Suitable stages for counting actual haploid chromosome numbers are metaphase II and anaphase II plates of the 2nd meiotic division, from which two kinds of the cell plates can be found in having different chromosome numbers. One contains the autosomal chromosomes only, and the other contains sex chromosomes in addition to autosomes. Thus, a precise sex-determining mechanism is available.

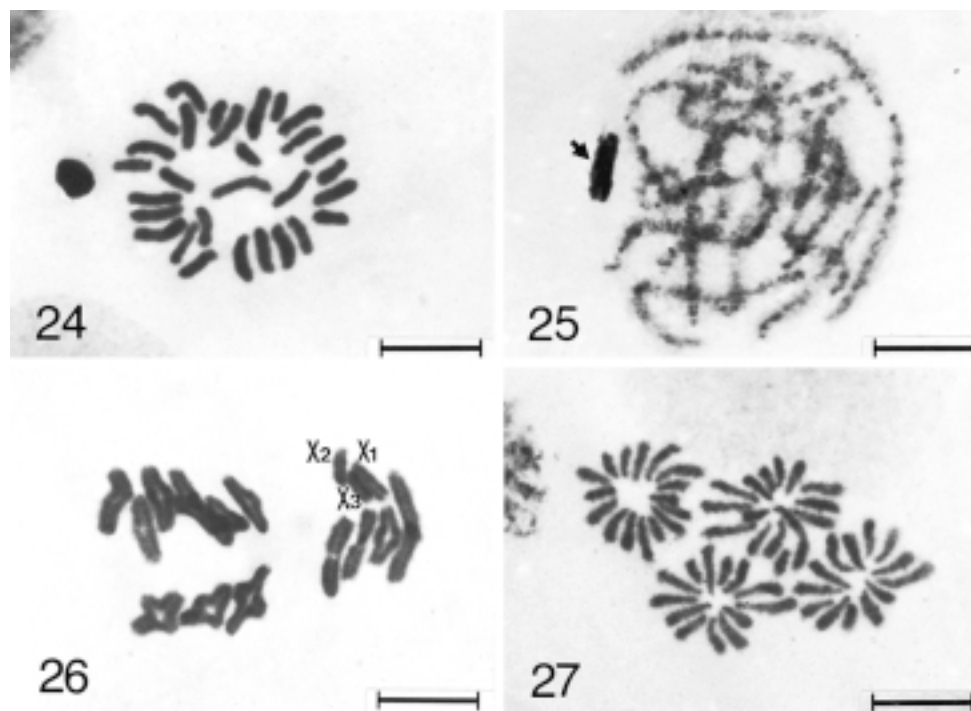
There are many methods for preparing spider chromosomes. However, the thin section of paraffin method used by Sharma (1950) is too complicated, and the squash method used by Maddison (1982) is not convenient for further staining techniques. The embryonic cell suspension method (splash method) used by Matsumoto (1977) and Rowell (1985) can only determine the  $2n$  number, but not the sex-determining mechanism. All these methods are unsuitable for field use. Cokendolpher and Brown (1985) developed an air-dried method, which has been used by many authors (Tugmon et al. 1990, Gorlova et al. 1997). It is a modified "cell suspension method" but avoids dropping the cell. Instead, a glass rod is used to macerate the tissue and spread the dissociated cells by tilting the slide back and forth. No true smear

method is used in the study of chromosomes. The improved air-dried method using fine needles to macerate (smear) the tissues developed by Imai et al. (1977) with Australian ants and followed by Luykx (1983) with the wood-roach is considered a "needle smear method" in contrast to that of Cokendolpher and Brown (1985). The present study followed the procedures of Luykx (1983) but removed and transferred the gonads to a slide before the hypotonic treatment in a humid chamber. Chromosomes of 6 species belonging to 5 families of spiders in Taiwan have been successfully investigated by this modified "needle smear method" for the first time. Besides, it is also time saving, suitable for use in the field, especially much easier to perform, and allows further banding techniques to be used on the samples.

#### Family Uloboridae

*Octonoba spinosa* is endemic to Taiwan, and is restricted to low mountain areas in northern Taiwan (Yoshida 1982). Chromosome data are reported for the first time.

Five other uloborid species have been studied cytologically, i.e., *Miagrammopes orientalis* and



**Figs. 24-27.** *Anahita fauna*. 24. Spermatogonial mitotic metaphase plate with 29 chromosomes ( $2n = 29$ ) arranged in a hollow radial configuration. 25. Three condensed sex chromosomes arranged closely to form a long bar (arrow) situated in the margin of a pachytene plate. 26. Diakinesis plate with 13 bivalents and 3 univalent sex chromosomes ( $X_1X_2X_3$ ). 27. Anaphase II plates with either 13 or 16 chromosomes ( $n = 13$  or  $16$ ) arranged in a hollow radial configuration. Bar =  $10 \mu$ .



*Octonoba varians* (= *Uloborus varians*) from Japan (Suzuki 1954) and *Uloborus khasiensis*, *U. danolius*, and *U. krishnae* from India (Datta and Chatterjee 1983). The diploid chromosome number is reported as  $2n = 22$  in males of *O. varians* and  $2n = 22$  ♂ /  $24$  ♀ in *M. orientalis*. However, the male diploid chromosome numbers in the 3 Indian species of *Uloborus* are 18, 17, and 19, respectively. The meiotic metaphase I plates of the 2 Japanese uloborid species show 10 autosomal bivalents and 2 univalent sex chromosomes which undoubtedly are the  $X_1X_2O$  type of sex-determining mechanism (Suzuki 1954). In contrast, males of the 3 Indian *Uloborus* species showed 8 pairs of autosomes and 1 to 3 univalent sex chromosomes, i.e.,  $XO$ ,  $X_1X_2O$ , and  $X_1X_2X_3O$  types. Obviously, the chromosome data of *Octonoba spinosa* are not congruent with those of Suzuki's *O. varians*,  $2n = 22$  ♂ /  $24$  ♀, but are the same as those of *U. khasiensis* in India. Morphologically, the male palpal organ bearing a large projection continuing from the median apophysis of the bulb is present in *Octonoba spinosa*, but is not in *O. varians*. Although the male palpal organ of Indian uloborid spiders can not be compared so far, the chromosome data seem to agree with the morphology of the palpal organ, and against the monophyly of the genus *Octonoba*.

Nine species of uloborid spiders in 5 genera: *Hyptiotes*, *Miagrammopes*, *Octonoba*, *Philoponella*, and *Zosis* have been recorded in Taiwan (Chen 1996). To understand their phylogenetic relationships, further cytological investigations on all species of the genus *Octonoba* and all Taiwanese uloborid spiders are needed.

### Family Theridiidae

*Achaearanea tepidariorum* is a cosmopolitan species from Finland (Hackman 1948) and Japan (Suzuki 1954, Igarashi and Akio 1977, Kageyama and Seto 1979) which has been studied cytologically. The present study, using Taiwanese material, also shows a diploid chromosome number of  $2n = 22$  in male with the  $X_1X_2O$  type of sex-determining mechanism, both of which are the same as those of previous reports.

Until the present, 20 species in 8 genera of theridiid spiders have been studied cytologically. Except for *Chrysso venusta* from Japan and *Argyrodes gazingenensis* from India, which both have a diploid chromosome number with  $2n = 24$  in male and 26 in female, the other species contain a typical chromosome number with  $2n = 22$  in male and 24 in female, and the sex-determining mecha-

nism is like that of *Achaearanea tepidariorum*. The chromosome number seems relatively conservative in this family.

### Family Psechridae

*Psechrus sinensis* is mainly distributed in mainland China and in the low mountain areas of northern Taiwan (Chen unpubl. data). No chromosome data of the family Psechridae have been previously reported.

The Psechridae belongs to the superfamily Lycosoidea by having a grate-shaped tapetum, a synapomorphic character of Lycosoidea (Coddington and Levi 1991, Griswold 1993). In contrast to other families in the Lycosoidea, psechrid spiders bear a cribellum in front of the spinnerets, have the calamistrum on the 4th leg, and have 3 claws at the end of the legs with a tuft of hairs under the 3rd claw. They also construct a funnel web with a retreat hidden in the crevice. Phylogenetically, the Psechridae is a sister group to the family Oxyopidae (Coddington and Levi 1991) or a sister group to the families Stiphidiidae, Senoculidae, and Oxyopidae (Griswold 1993). The close relationships between Psechridae and Oxyopidae are also supported by DNA comparisons of mt16S rDNA (Fang et al. unpubl. data). However, no chromosome data of Stiphidiidae and Senoculidae have been studied. The model chromosome number of the Psechridae represented by *P. sinensis* shows 11 autosomal bivalents and 2 univalent X-chromosomes in the 1st meiotic division, which is not the same as those of any lynx spiders studied, but is within the range of chromosomal variations of the entire Lycosoidea (Hackman 1948, Suzuki 1952 1954, Srivastava and Shukla 1986, Gorlova et al. 1997). The Psechridae having the  $X_1X_2O$  type of sex-determining mechanism is the same as most families of Lycosoidea. Therefore, the present chromosome data cannot support the phylogeny of Psechridae suggested by both Coddington and Levi (1991) and Griswold (1993).

### Family Oxyopidae

Eleven species including 2 unidentified ones of *Oxyopes* have been studied cytologically (Hackman 1948, Suzuki 1952, Datta and Chatterjee 1983, Srivastava and Shukla 1986, Tugmon et al. 1990). Except for *Oxyopes salticus*, all have a model chromosome number  $2n = 21$  in male and 22 in female, contain 10 autosomal bivalents and 1 univalent sex chromosome in the diakinesis stage, and have a haploid chromosome number of either 10 or 11. The

sex-determining mechanism is XO type. Although Painter (1914) reported the haploid chromosome data of *O. salticus* to be 10 bivalents and 2 X-chromosomes in the 1st meiotic division, his data were questioned by Hackman (1948) and Suzuki (1954) and probably were erroneous.

Both *O. sertatus* and *O. macilentus* are common in Taiwan. Only *O. sertatus* has been previously studied cytologically by workers from Japan (Suzuki 1952, Igarashi and Akio 1977). The chromosome data obtained from Taiwanese materials also contain the same model chromosome number as those reported by previous workers.

Chromosome data of *O. macilentus* are reported for the first time. Two different chromosome numbers were obtained from 2 remote populations in Taiwan. Spiders from Tsantsushan as well as Wulai (Taipei population) have the model chromosomes. But an individual from Antung (Hualien population) having 1 additional pair of autosomal chromosomes is distinctly different from those of other congeneric species. Therefore, the status of Taiwanese *O. macilentus* is unclear and may need to be revised in the future. In addition, a more detailed survey on chromosome variations of the genus *Oxyopes* from various locales in Taiwan is also needed to reveal its geographical distribution and chromosomal evolution.

However, *Peuceitia viridans* has 28 chromosomes in male, and has 13 autosomal bivalents and 2 univalent X-chromosomes at the diakinesis stage (Bole-Gowda 1950). Therefore, the sex-determining mechanism of *P. viridans* is  $X_1X_2O$  type. These chromosomal features are distinctly different from those of *Oxyopes* but resemble those of most genera in the families Lycosidae and Pisauridae (Suzuki 1954) which may support the Oxyopidae being a member of the superfamily Lycosoidea. In addition, the XO type of sex-determining mechanism in *Oxyopes* seems more advanced than the  $X_1X_2O$  type in *Peuceitia*.

### Family Ctenidae

*Anahita fauna* is distributed in low mountain areas of Taiwan. No chromosomal data of the family Ctenidae were reported before. *Anahita fauna* in the present study shows a diploid chromosome number of  $2n = 29$ , a haploid chromosome number of either 13 or 16, and an  $X_1X_2X_3O$  type of sex-determining mechanism, all of which are reported for the first time.

Coddington and Levi (1991) placed the Ctenidae in the superfamily Lycosoidea. It is inter-

esting to note that the sex-determining mechanism in nearly all genera and families of Lycosoidea previously studied is  $X_1X_2O$  type, except in the genus *Oxyopes* (Oxyopidae) which is XO type. In addition, *Anahita fauna* (Ctenidae) in this paper showing 3 X-chromosomes is another exception. The phylogenetic relationship of the Ctenidae is somewhat ambiguous. Coddington and Levi (1991) considered that the Ctenidae was an unresolved lineage, which was a trivial sister group of the clade containing the Acanthoctenidae and Zoropsidae and the clade containing all the remaining families of Lycosoidea. DNA sequence data indicated that the Ctenidae is not monophyletic (Huber et al. 1993). Moreover, DNA sequences of mt16S rDNA among the families Lycosidae, Pisauridae, Ctenidae, Oxyopidae, and Psecridae belonging to the superfamily Lycosoidea were compared, and it was concluded that the Ctenidae and Pisauridae were the most closely related taxa (Fang et al. unpubl. data). There are 13 pairs of autosomes and 2 subequal X-chromosomes in pisaurid spiders (Suzuki 1954). If one of the sex chromosomes of pisaurid spiders is involved in an accidental inversion followed by centromeric fission as Suzuki hypothesized (1954), 1 large and 2 small X-chromosomes as in those of *Anahita* will evolve. The chromosome data seem to support the phylogenetic hypothesis suggested by DNA molecular data. However, the known chromosome data in both Ctenidae and Pisauridae are very poor, and the evolution of multiple sex chromosomes has not been critically tested. It is still hard to reach any conclusion at the present time, and further studies are required.

**Acknowledgments:** I am very grateful to Dr. P. Luykx at the Univ. of Miami, FL for his protocol in chromosome preparation, to Dr. Jenn-Che Wang and Dr. Lu-Hsi Chang at the Department of Biology, National Taiwan Normal Univ. for providing facilities for photography. I also thank Yu-Yuan Liaw for her assistance in the field. Thanks are also extended to the anonymous referees for their valuable comments.

### REFERENCES

- Bole-Gowda BN. 1950. The chromosome study in the spermatogenesis of two lynx-spiders (Oxyopidae). Proc. Zool. Soc. Bengal **3**: 95-107.
- Chen SH. 1996. A checklist of spiders in Taiwan. Ann. Taiwan Mus. **39**: 123-155.
- Coddington JA, HW Levi. 1991. Systematics and evolution of spiders (Araneae). Ann. Rev. Ecol. Syst. **22**: 565-592.
- Cokendolpher JC, JD Brown. 1985. Air-dry method for studying

- chromosomes of insects and arachnids. *Entomol. News* **96**: 114-118.
- Datta SN, K Chatterjee. 1983. Chromosome number and sex-determining system in fifty-two species of spiders from north-east India. *Chromos. Inform. Serv.* **35**: 6-8.
- Gorlova OY, IP Gorlov, E Nevo, DV Logunov. 1997. Cytogenetic studies on seventeen spider species from Israel. *Bull. Br. Arachnol. Soc.* **18**: 249-252.
- Griswold CE. 1993. Investigations into the phylogeny of the lycosoid spiders and their kin (Arachnida: Araneae: Lycosoidea). *Smithsonian Contrib. Zool.* **539**: 1-39.
- Hackman W. 1948. Chromosomenstudien an Araneen mit besonderer Berücksichtigung der Geschlechtschromosomen. *Acta Zool. Fenn.* **54**: 1-101.
- Huber KC, TS Haider, MW Muller, BA Huber, RJ Schweyen, FG Barth. 1993. DNA sequence data indicates the polyphyly of the family Ctenidae (Araneae). *J. Arachnol.* **21**: 194-201.
- Igarashi H, K Akio. 1977. The chromosome observation techniques for spiders. *Acta Arachnol.* **27** (Spec. no.): 157-166.
- Imai HT, RH Crozier, RW Taylor. 1977. Karyotype evolution in Australian ants. *Chromosoma (Berl.)* **59**: 341-393.
- Kageyama A, T Seto. 1979. Chromosomes of seven species of Japanese theridiid spiders. *Chromos. Inform. Serv.* **27**: 10-11.
- Luyckx P. 1983. XO-XX sex chromosomes and Robertsonian variation in the autosomes of the wood-roach *Cryptocercus punctulatus* (Dictyoptera: Blattaria: Cryptocercidae). *Ann. Entomol. Soc. Am.* **76**: 518-522.
- Maddison WP. 1982. XXXY sex chromosomes in males of the jumping spider genus *Pellenes* (Araneae: Salticidae). *Chromosoma (Berl.)* **85**: 23-37.
- Matsumoto S. 1977. An observation of somatic chromosomes from spider embryo-cells. *Acta Arachnol.* **27** (Spec. no.): 167-172.
- Painter TS. 1914. Spermatogenesis in spiders. *Zool. Jahrb., Anat. Ontog. Tiere* **38**: 509-576.
- Rowell DM. 1985. Complex sex-linked fusion heterozygosity in the Australian huntsman spider *Delena cancerides* (Araneae: Sparassidae). *Chromosoma (Berl.)* **93**: 169-176.
- Sharma GP. 1950. Spermatogenesis in the spider, *Plexippus paykulli*. *Res. Bull. East Panjab Univ. (Zool.)* **5**: 67-80.
- Srivastava MDL, S Shukla. 1986. Chromosome number and sex-determining mechanism in forty-seven species of Indian spiders. *Chromos. Inform. Serv.* **41**: 23-26.
- Suzuki S. 1951. Cytological studies in spiders. I. A comparative study of the chromosomes in the family Argiopidae. *J. Sci. Hiroshima Univ. (ser. B.)* **12**: 67-98.
- Suzuki S. 1952. Cytological studies in spiders. II. Chromosomal investigation in the twenty-two species of spiders belonging to the four families, Clubionidae, Sparassidae, Thomisidae and Oxyopidae, which constitute Clubionoidea with special reference to sex chromosomes. *J. Sci. Hiroshima Univ. (ser. B.)* **13**: 1-52.
- 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.)* **15**: 23-136.
- Tsurusaki N, JC Cokendolpher. 1990. Chromosomes of sixteen species of harvestmen (Arachnida, Opiliones, Caddidae and Phalangidae). *J. Arachnol.* **18**: 151-166.
- Tugmon CR, JD Brown, NV Horner. 1990. Karyotypes of seventeen USA spiders (Araneae, Araneidae, Gnaphosidae, Loxoscelidae, Lycosidae, Oxyopidae, Philodromidae, Salticidae and Theridiidae). *J. Arachnol.* **18**: 41-48.
- White MJD. 1973. *Animal cytology and evolution*. London: Cambridge Univ. Press.
- Yoshida H. 1982. Spiders from Taiwan. I. Two new species of the genus *Octonoba* (Araneae: Uloboridae). *Acta Arachnol.* **30**: 71-74.

## 六種臺灣產蜘蛛染色體之研究（蜘蛛目：姬蜘蛛科、樓網蜘蛛科、渦蜘蛛科、貓蜘蛛科及絞蜘蛛科）

陳世煌<sup>1</sup>

本文以蜘蛛生殖腺為材料，依 Luykx (1983) 之方法並加以改良，首次應用在蜘蛛染色體之製作。六種臺灣產蜘蛛雌（♀）雄（♂）染色體之二倍體（ $2n$ ）數目、雄蜘蛛第一次減數分裂（ $n(I)$ ）之二價體數目和性染色體（X's）、第二減數分裂（ $n(II)$ ）之染色體數目，以及各種蜘蛛之性別決定機制分別為：棘渦蜘蛛 *Octonoba spinosa*（渦蜘蛛科） $2n = 18$  ♂ /  $20$  ♀， $n(I) = 8 + X_1X_2$ ， $n(II) = 8$  或  $10$ ， $X_1X_2O$  型性別決定機制；大姬蜘蛛 *Achaearanea tepidariorum*（姬蜘蛛科） $n(I) = 10 + X_1X_2$ ， $X_1X_2O$  型；中國樓網蜘蛛 *Psechrus sinensis*（樓網蜘蛛科） $2n = 24$  ♂， $n(I) = 11 + X_1X_2$ ， $n(II) = 11$  或  $13$ ， $X_1X_2O$  型；條紋貓蜘蛛 *Oxyopes macilentus*（貓蜘蛛科）之臺北族群為  $2n = 21$  ♂ /  $22$  ♀， $n(I) = 10 + X$ ， $n(II) = 10$  或  $11$ ，而花蓮族群為  $2n = 23$  ♂， $n(I) = 11 + X$ ， $n(II) = 11$  或  $12$ ，二者均為  $XO$  型性別決定機制；斜紋貓蜘蛛 *Oxyopes sertatus*（貓蜘蛛科） $2n = 21$  ♂ /  $22$  ♀， $n(I) = 10 + X$ ， $n(II) = 10$  或  $11$ ， $XO$  型；絞蜘蛛 *Anahita fauna*（絞蜘蛛科） $2n = 29$  ♂， $n(I) = 13 + X_1X_2X_3$ ， $n(II) = 13$  或  $16$ ， $X_1X_2X_3O$  型。其中棘渦蜘蛛、中國樓網蜘蛛、條紋貓蜘蛛和絞蜘蛛等四種臺灣常見蜘蛛之染色體數目及性別決定機制為首次記錄。而中國樓網蜘蛛和絞蜘蛛分別代表的樓網蜘蛛科和絞蜘蛛科之染色體也是首次報導。條紋貓蜘蛛具有染色體多型性，其花蓮族群之雄性個體染色體數目  $2n = 23$  為本屬蜘蛛之首例。

**關鍵詞：**蜘蛛，染色體，性別決定機制，臺灣。

<sup>1</sup> 國立臺灣師範大學生物學系