

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

By

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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*

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(With 5 Tables, 1 Diagram and 15 Plates)

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INTRODUCTION

The present paper is the third report of this series, which includes the description of chromosomes of 57 species of spiders belonging to 17 different families of all the 3 suborders in Araneae. In the previous papers (SUZUKI, '51a, '52a; SUZUKI and OKADA, '50) I have already reported the chromosomal results of 33 species, so that the numbers of spiders studied by me amount to 90 species of 21 families. When the species so far researched by other authors are added, the number totalled 181 species of 26 families.

The materials investigated in this paper comprise many important families or species such as *Heptathela kimurai* of Liphistiidae, *Atypus karschi* of Atypidae and some others. The first two species should be particularly noticed, because they are regarded as to be very old in phylogeny. If it were known that they have characteristic features in chromosomes, the results would serve certainly in resolving the problem of chromosomal evolution in spiders. In fact they revealed remarkable results as expected, especially in *Heptathela kimurai* an extremely high chromosome number was obtained, which was found to be an important key to interpret the evolution of chromosome numbers in these animals. Basing on these and other results the present author reached the conclusion that in spiders there exists correlation between the chromosome numbers and general phylogeny, in other words the chromosome number decreases according to the development of phylogeny.

The cytological survey in cribellate spiders such as Uloboridae and

Dictynidae may give some suggestions to arachnologists. But it seems to be overhasty to discuss the phylogeny of cribellate forms from cytological viewpoint, because not many characteristic chromosomal features have been obtained in this group. In Arachnomorphae *Ariadna lateralis*, *Pholcus crypticolens* and *Cryphoea silvicola* showed the notable results. In particular *Ariadna lateralis* is characteristic of cytological as well as phylogenetical standpoints, which contrary to its systematic position bears highly developed chromosomal condition. In *Pholcus crypticolens* was discovered metacentric element, and Finnish *Cryphoea silvicola* was found to bear XXXO-system of sex-determining mechanism.

Regarding sex-chromosomes XXO-system occurred in large majority of species studied. Single X and XXXO-system were found in only one species respectively.

In the section of observation the chromosomes of 57 species newly studied were stated, at the beginning of the discussion the general review of chromosomes in spiders was given based on the entire materials studied by me and other workers. Next to that the variation of number, shape and size of chromosomes was mentioned, which was followed by the statement of evolution of chromosome numbers, and finally the features of sex-chromosomes and their evolution were described.

MATERIALS AND METHODS

The materials employed in the present paper are 57 species of spiders belonging to 17 different families covering all three suborders of Araneae. The species names are listed in the Table I. Of these materials three species *Sitticus littoralis* (Salticidae), *Cryphoea silvicola* (Agelenidae) and *Crustulina guttata* (Theridiidae) are Finnish spiders, all the others are Japanese ones. Most of Japanese materials were collected in Hiroshima, its suburbs and Iwakuni. In Hiroshima, Mitaki situated in north-west of the city is the most important locality; Ujina and Nishihakushima are also in the same city. Funakoshi, Showa village, Fukuoji and Miiri village are located in the suburbs of the Hiroshima city.

Heptathela kimurai was secured in Kumamoto city and Yunoura village of Kumamoto Prefecture. The localities of Finnish spiders are noted in respective lines of the Table I.

Table I. Materials used in this paper.

Species	Sex	No. of indiv.	Stage	Month	Locality
Liphistiidae					
<i>Heptathela kimurai</i>	♂	2	immat.	May '48	Kumamoto
	♂	9	3 juv., 6 immat.	June '48	"
	♂	4	immat.	July '48	"
	♂	9	"	June '49	"
	♂	7	6 immat., 1 ad.	Aug. '49	"
	♂	6	immat.	July '50	Yunoura village
	♂	12	"	Aug. '50	" "
	♂	14	"	July '51	Kumamoto
	♂	8	"	Aug. '51	Yunoura village
Atypidae					
<i>Atypus karschi</i>	♂	7	immat.	June '48	Hiroshima Univ.
	♂	10	4 immat., 6 ad.	July '48	" "
	♂	3	juv.	Feb. '51	" "
	♂	many	"	Mar. '51	" "
	♂	10	6 immat., 4 juv.	Apr. '51	" "
	♂	3	immat.	May '51	" "
	♂	1	ad.	June '51	" "
Uloboridae					
<i>Miagrammopes orientalis</i>	♂	3	ad.	May '50	Iwakuni
	♂	1	immat.	Oct. '50	Showa village
	♀	2	juv.	Mar. '51	Iwakuni
	♂	4	immat.	Mar. '51	"
	♀	4	1 juv., 3 immat.	Apr. '51	"
<i>Uloborus varians</i>	♂	5	imrat.	May '50	"
	♂	3	"	Mar. '51	"
	♂	1	"	Apr. '51	Miiri village
Dictynidae					
<i>Dictyna foliicola</i>	♂	4	ad.	May '50	Mitaki
	♂	5	"	Mar. '51	"
	♂	5	"	May '51	"
Dysderidae					
<i>Ariadna lateralis</i>	♂	1	ad.	June '50	Iwakuni
Pholcidae					
<i>Pholcus crypticolens</i>	♂	1	ad.	Apr. '50	Showa village
	♂	2	"	May '50	Iwakuni
Drassidae					
<i>Drassodes</i> sp.	♂	1	immat.	Apr. '51	Nishihakushima
Salticidae					

Species	Sex	No. of indiv.	Stage	Month	Locality
<i>Myrmarachne japonica</i> ...	♂	1	juv.	Apr. '50	Mitaki
	♂	6	2 juv., 4 immat.	May '50	Iwakuni
	♀	1	immat.	May '50	▷
	♀	1	▷	June '50	▷
	♂	3	juv.	Apr. '51	▷
	♀	6	3 juv., 3 immat.	Apr. '51	▷
	♂	1	immat.	May '51	Mitaki
	♂	5	1 ad., 4 immat.	May '51	Iwakuni
	♂	2	immat.	Apr. '50	Mitaki
	♂	1	▷	Apr. '51	▷
<i>Hasarius albarius</i>	♀	1	▷	Apr. '51	▷
	♂	1	▷	May '51	Iwakuni
	♂	5	▷	Apr. '50	Mitaki
<i>H. adansoni</i>	♂	4	▷	May '50	▷
	♂	1	▷	June '51	▷
	♂	1	▷	July '50	Funakoshi
<i>Plexippus paykulli</i>	♂	2	▷	May '49	Mitaki
<i>Pl. setipes</i>	♂	1	▷	June '49	▷
	♂	1	ad.	Oct. '49	▷
	♂	5	immat.	Apr. '50	▷
	♂	1	▷	May '50	Iwakuni
	♂	1	▷	June '50	▷
	♂	1	▷	May '51	Mitaki
<i>Pl. crassipes</i>	♂	1	▷	May '50	▷
<i>Jotus linea</i>	♂	1	▷	May '50	▷
	♂	1	juv.	Mar. '51	Iwakuni
	♂	6	immat.	Apr. '51	▷
<i>Aelurillus dimorphus</i> ...	♂	3	1 immat., 2 ad.	May '51	Mitaki
	♂	3	juv.	Apr. '49	Hiroshima Univ.
	♂	1	immat.	May '49	▷
	♂	3	▷	Apr. '50	Mitaki
	♂	2	▷	May '50	Iwakuni
	♂	2	▷	May '50	Mitaki
<i>Evophrys aninotatus</i>	♂	1	ad.	June '50	▷
	♂	6	3 immat., 3 ad.	Apr. '49	▷
<i>Icius magister</i>	♂	11	5 immat., 6 ad.	May '49	▷
	♂	7	ad.	June '49	▷
	♂	1	immat.	Mar. '50	▷
	♂	3	2 immat., 1 ad.	Apr. '50	▷
	♂	1	immat.	Mar. '51	▷
	♂	4	2 juv., 2 immat.	Apr. '49	▷
<i>I. elongatus</i>	♂	5	4 immat., 1 ad.	May '49	▷

Species	Sex	No. of indiv.	Stage	Month	Locality
<i>Icius elongatus</i>	♂	2	1 immat., 1 ad.	June '49	Mitaki
	♂	3	immat.	Apr. '50	"
	♂	1	"	Mar. '51	"
<i>Icidella vittata</i>	♂	2	"	Apr. '50	"
	♂	3	ad.	May '50	"
	♂	2	"	June '50	Iwakuni
	♂	2	immat.	Apr. '51	Mitaki
	♂	1	"	May '51	Fukuoji
<i>Rhene atrata</i>	♂	2	"	Apr. '49	Hiroshima Univ.
	♂	1	"	Mar. '50	" "
	♂	3	"	Apr. '50	" "
	♂	2	"	Apr. '50	Mitaki
	♂	1	ad.	Sept. '50	"
<i>Siticus littoralis</i>	♂	1	ad.?	June '46	Tvärminne:Finland
Lycosidae					
<i>Lycosa T-insignita</i>	♂	1	ad.	Feb. '49	Hiroshima Univ.
	♂	4	"	Mar. '49	" "
	♂	12	"	Apr. '49	" "
	♂	2	"	May '49	" "
	♂	6	"	Mar. '50	" "
	♂	1	"	Mar. '50	Iwakuni
	♂	1	"	Apr. '50	Hiroshima Univ.
<i>L. cinereofusca</i>	♂	1	immat.	Apr. '51	Miiri village
<i>L. coelestis</i>	♂	1	ad.	May '51	Nishihakushima
<i>L. pseudoannulatus</i>	♂	3	"	Sept. '49	Mitaki
<i>L. sp.</i>	♂	2	"	Mar. '51	Iwakuni
Pisauridae					
<i>Pisaura lama</i>	♂	3	immat.	Apr. '50	Mitaki
	♂	1	"	Apr. '50	Showa village
	♂	1	"	May '50	Mitaki
	♂	5	"	Mar. '51	Iwakuni
	♀	3	"	Mar. '51	"
<i>Dolomedes angustivirgatus</i>	♂	3	ad.	May '49	Mitaki
	♀	2	immat.	Mar. '50	"
	♀	2	"	Apr. '50	"
	♂	1	"	Apr. '50	"
	♂	2	"	May '50	"
<i>D. pallitarsis</i>	♂	2	juv.	Mar. '51	Iwakuni
	♂	1	immat.	Apr. '51	Mitaki
	♂	1	"	Apr. '51	Miiri village
	♂	1	"	May '51	Fukuoji

Species	Sex	No. of indiv.	Stage	Month	Locality
<i>D. saganus</i>	♂	1	ad.	July '50	Mukaijima Is.
<i>D. sulfureus</i>	♀	2	juv.	Mar. '50	Mitaki
	♀	1	♂	Apr. '50	♂
	♂	2	immat.	Apr. '50	♂
	♂	2	♂	May '50	♂
	♂	1	♂	May '50	Iwakuni
	♂	1	♂	June '50	Ujina
	♂	1	ad.	July '50	Iwakuni
	♀	1	immat.	Apr. '51	Ujina
	♀	1	♂	Apr. '51	Mitri village
Agelenidae					
<i>Agelena limbata</i>	♂	3	juv.	May '50	Iwakuni
	♂	8	immat.	June '50	Mitaki
	♂	5	3 immat., 2 ad.	June '50	Iwakuni
	♂	8	3 immat., 5 ad.	July '50	Mitaki
	♂	4	2 immat., 2 ad.	Sept. '51	Hiroshima Univ.
<i>A. opulenta</i>	♂	3	1 immat., 2 ad.	Aug. '49	Mitaki
	♂	2	1 immat., 1 ad.	Aug. '50	♂
<i>Tegenaria corasides</i>	♂	1	juv.	Apr. '50	Nishihakushima
	♂	3	2 juv., 1 immat.	May '50	Iwakuni
	♂	3	immat.	July '50	♂
	♂	1	juv.	Aug. '50	♂
<i>Cryphoea silvicola</i>	♂	1	ad.?	Aug. '49	Helsingfors:Finland
Urocteidae					
<i>Uroctea compactilis</i>	♂	9	4 juv., 5 immat.	May '50	Iwakuni
	♂	2	1 immat., 1 ad.	July '50	Funakoshi
	♂	6	3 immat., 3 ad.	July '50	Iwakuni
	♂	1	ad.	June '50	♂
	♂	3	immat.	Mar. '51	♂
	♂	1	ad.	June '51	♂
Theridiidae					
<i>Crustulina guttata</i>	♂	1	ad.?	May '48	Tvärminne:Finland
<i>Enoplognatha foliicola</i> ...	♂	1	immat.	Apr. '50	Mitaki
	♂	3	♂	Apr. '50	Showa village
	♂	1	♂	Apr. '50	Iwakuni
	♂	4	♂	May '50	Showa village
	♂	1	ad.	May '50	Iwakuni
<i>Lithyphantes dubius</i>	♂	1	immat.	May '50	Showa village
	♂	4	♂	May '50	Iwakuni
	♂	1	♂	Apr. '51	♂
	♂	2	♂	May '51	♂

Species	Sex	No. of indiv.	Stage	Month	Locality
<i>Ariamnes cylindrogaster</i>	♂	1	immat.	Aug. '49	Mitaki
	♂	1	juv.	Sept. '49	"
<i>Theridium tepidariorum</i>	♂	1	ad.	Oct. '49	Nishihakushima
	♂	5	3 immat., 2 ad.	Apr. '50	Iwakuni
	♂	4	1 immat., 3 ad.	May '50	"
<i>T. sterninotatum</i>	♂	1	immat.	May '50	"
	♂	1	"	May '50	Mitaki
<i>Nesticus</i> sp.	♂	2	ad.	Oct. '50	Showa village
Linyphiidae					
<i>Linyphia albolimbata</i> ...	♂	1	ad.	May '50	Iwakuni
	♂	6	"	Mar. '51	Hiroshima Univ.
	♂	4	"	Mar. '51	Iwakuni
<i>L.</i> sp.	♂	3	ad.	Mar. '51	"
	♂	3	"	Apr. '51	"
	♂	1	"	May '51	Fukuoji
<i>L. marginata</i>	♂	1	immat.	Aug. '50	Showa village
	♂	1	ad.	Sept. '50	" "
<i>L. montana</i>	♂	1	"	Oct. '50	" "
Tetragnathidae					
<i>Tetragnatha japonica</i> ...	♂	5	immat.	Apr. '48	Mitaki
	♂	4	"	May '48	"
<i>T. squamata</i>	♂	2	"	Apr. '49	Hiroshima Univ.
	♂	3	"	May '49	" "
	♂	2	"	Apr. '50	Mitaki
	♂	2	"	Mar. '51	Iwakuni
<i>Leucange blanda</i>	♂	4	juv.	Mar. '50	"
	♂	3	immat.	May '50	"
	♂	1	"	May '51	"
	♂	4	2 immat., 2 ad.	June '51	"
Argiopidae					
<i>Araneus fuscocoloratus</i> ...	♂	1	immat.	Mar. '51	Iwakuni
	♂	1	"	Apr. '51	"
<i>A. saganus</i>	♀	3	"	Apr. '51	"
<i>Cyclosa sedeculata</i>	♂	1	ad.	Mar. '51	"
	♂	5	"	Apr. '51	"
	♂	4	"	Apr. '51	Ujina
<i>Larinia punctifera</i>	♂	4	immat.	Apr. '51	Mitaki
<i>Meta yunohamensis</i>	♂	12	5 juv., 7 immat.	Apr. '51	"
	♂	1	ad.	May '51	"
Mimetidae					
<i>Ero japonica</i>	♂	4	ad.	May '50	Iwakuni

The three Finnish materials were fixed and embedded by HACKMAN and were sent to me. As the paraffin blocks were too small they were re-embedded and sectioned by me.

The observations were carried out largely by using the fixed preparations. In some species squash preparation by MAKINO and NISHIMURA's method (MAKINO and NISHIMURA, '50, '52) were used.

The mixtures used are mentioned in the descriptions of each species. As regards methods of preservation and staining see my previous papers (SUZUKI, '51a, '52a). In the present study the FEULGEN's reaction was also used with HEIDENHAIN's iron hematoxylin staining.

The drawings were made at table level with the aid of an objective 120, ocular 20 and ABBE's camera lucida.

OBSERVATIONS OF CHROMOSOMES

The families are generally arranged according to BRISTOWE's classification (BRISTOWE, '38). The first is Liphistiidae, second, Atypidae and the third, families of Arachnomorphae, that is, Uloboridae and Dictynidae of cribellate spiders, which are followed by the ecribellate spiders in the following order: Dysderidae, Pholcidae, Drassidae, Salticidae, Lycosidae, Pisauridae, Agelenidae, Urocteidae, Theridiidae, Linyphiidae, Tetragnathidae, Argiopidae and Mimetidae.

I. Suborder Liphistiomorphae

The present suborder contains only a single living family Liphistiidae, all members of the family are remarkably characteristic of morphology, which show very interesting particularities in many respects. It is well known that they are extremely old in origin and very primitive. Consequently the chromosomal survey of these ancient spiders is very intriguing, because some new, significant results may possibly be found. The liphistiids are restricted within the east Asia, and nothing has ever been known of their cytology.

1. Family Liphistiidae

(1) *Heptathela kimurai*

(Figs. 1-5)

Some of the materials used were gathered in Kumamoto city and its suburbs, others in Yunoura village (these two localities situate in Kumamoto Prefecture, the southernmost Japanese island named Kyushu). A number of individuals from juvenile to adult were fixed during the time between May and August. As fixatives the follow-

ings were tried: normal and modified NAVASHIN's solutions, ALLEN-BOUIN's solution, FLEMMING's weak and strong solutions, and BENDA's solution. The testes of this species are very slender, tube-like organs which are greatly crooked as intestinal canals of higher animals; in larger individuals they are easily dissected out. In fixation the dissected testes were immersed in the mixtures. The male becomes adult after the middle of August.

The spermatogonial division stage is considerably long, it lasts at least from May to July; in the testes from medium sized individuals or subadults fixed during such months are found numerous dividing spermatogonial cells. On the other hand the maturation divisions are carried out during rather short period. In my experience the suitable period observing meiotic divisions is during the middle of July and of August. In these periods the testes of subadults show numerous meiotic division figures. The male gonads from one individual just completed the last moulting on August 24th largely contain sperms, with only a few secondary spermatocytes or spermatids.

Although the present species shows remarkable characters in chromosomes as stated below, an unexpected difficulty was involved in the fixation of chromosomes. Namely, while mitotic chromosomes can be preserved in very good condition by any of the above mixtures, the meiotic spindles are unfixable by the same fixatives. Notwithstanding a number of freshly secured materials in suitable stage were fixed for some years no good preparations which permit close observation of meiotic chromosomes were obtained. The condition is worst among all materials studied by the author, though more or less similarities were found in *Atypus karschi* (Atypidae), *Uroctea compactilis* (Urocteidae) and some others. Accordingly the present observation was compelled to be limited mainly within the spermatogonial chromosomes.

Spermatogonia (Figs. 1-3): As stated above, in the testes from subadult males fixed from May to early July are present numerous spermatogonial metaphase plates. The cell plates are remarkably large in size, 20μ or more in diameter, which are the largest of all the species I studied. On this large metaphase plate there exist numerous small chromosomes, all of which are of acrocentric type, and are regarded to be of terminal or subterminal spindle attachment. They are always small rod-shaped, at most $1-2\mu$ in length; their proximal end being slightly sharpened; and most of them, especially larger ones are slightly curved. This chromosome size together with that of the following species *Atypus karschi* is the smallest of all so far investigated. When compared the present chromosomes with those of *Agelena*, *Tegenaria* (Agelenidae), *Plexippus* (Salticidae), *Heteropoda* and *Thelcticopis* (Sparassidae) belonging to modern spiders (Arachnomorphae), one would be

astonished at the remarkable size difference between them. In many species of these genera the largest chromosome is about $6\ \mu$ long, which is 3 times or more as large as that of *Heptathela kimurai*. Though they are small their number is remarkably large, it is unexpectedly numerous that accurate count of their elements becomes difficult. As already mentioned above, the gonial chromosomes are usually fixed in good condition, so that most of the elements can be clearly analysed, but in many cases some of the smaller pairs are often overlapped or fused into several blocks, which prevent exact counting. The diploid number was preliminarily reported as 80 (SUZUKI, '49), further investigation, however, reveals that the number is too small. It was found that in earlier observation some pairs of smaller elements were counted as single one due to more or less unsatisfactory differentiation of preparations. In many excellent metaphase plates at least ninety chromosomes can be counted. The metaphase spindle shown in Fig. 1 includes 96 elements, that in Fig. 2 95 and that in Fig. 3 94. In many other figures approximately 95 diploid number can be counted. Thus it is very difficult to decide the number accurately, but 96 seems to be the true diploid number. Each pair of the chromosome complements shows similar appearance, which is of a little and gradual size difference, no particular elements can be detected morphologically.

The gonial chromosomes of the present species show thus notable peculiarities in their size and number, in particular their number is remarkably larger than that of any other spiders belonging to the remaining two suborders. The second largest number ($2n$) is 46; we have no species at present that have between 46 and 96. It is quite interesting that the most primitive spider *Heptathela kimurai* differs greatly from other species in chromosome number. This fact may be of much importance in the interpretation of the chromosomal evolution in these animals.

First division (Figs. 4-5): In the testes fixed in mid-summer there found many first division figures, but as already mentioned good dividing figures in which chromosomes can be counted clearly are hardly obtainable. In most slides many chromosomes are fused into large irregular blocks or deformed greatly, exhibiting abnormal appearance. It is quite strange that in spite of good fixation of gonial metaphase no favourable result is obtained in the first division. The reason must be explained by further study in future. Among numerous preparations few metaphase plates were observed, in which about 47 to 48 elements were barely counted, though they are not of course so good spindles. The bivalents are strongly condensed; many of them are slightly deformed and assuming somewhat V- or dumb-bell-shape.

It is of great significance to demonstrate the occurrence of X-

elements in this species, so that careful observations were done. As described above in the gonial chromosome complements no special elements are distinguishable. Early prophase stages of growth period are observed, but later stages can hardly be traced due to the bad fixation. The metaphase spindle shown in Fig. 4 is very large, in which one pair of elements are seen on the eccentric position; they are slightly pointed spheres of about equal size. Their feature resembles very much that of X-elements in many other species studied. Accordingly such elements seem to represent 2 X's. In the metaphase of the present species, however, some abnormal univalents are often scattered on the spindle, so that to obtain more conclusive result further study is desired.

Second division: In the second metaphase the chromosomes are more condensed than previous division, here also many elements are fused to each other and no favourable figures were obtained.

II. Suborder Mygalomorphae

This is the second suborder of Araneae, which is considered to be old next to the foregoing suborder. In this group only a single species *Dugesiella hentzi* (Aviculariidae) has so far been studied cytologically by PAINTER ('14). According to him the species possesses comparatively high chromosome number, which is larger than that of most modern spiders. As the members of this group possess particular morphological characters and habits, their cytological survey seems to be very interesting. But the species are not so popular that the material is not so easily obtained. In the present paper only a single species *Atypus karschi* was investigated.

2. Family Atypidae

(2) *Atypus karschi*

(Figs. 6-12)

The species is a common Japanese purse-web spider encountered in gardens of any other outdoor places. Numerous specimens from juvenile to adult were captured during March and July in the botanical garden of our University. The testes from those spiders were dissected out and immersed into the following fixatives: normal and modified NAVASHIN's solutions, BENDA's fluid, etc. These solutions gave good results for the mitotic chromosomes, but they are found not suitable for meiotic chromosomes. Here also, excellent fixation of meiotic spindles is difficult, though in the present case it is less difficult.

The male of this species matures in July, and the maturation divi-

sions take place during May and July. Accordingly the testes of subadult males fixed in such season are suitable for the study of maturation spindles. On the other hand to obtain spermatogonial metaphase figures is extremely difficult. In this respect the present species is the most difficult of all species treated by me. In order to know when gonial division takes place many individuals of different growing stages were gathered from early spring to summer and their testes were investigated by preparations. As the result I succeeded to obtain some gonial metaphases in the slides of testes fixed at the end of March and on April 5th. In those days the spiders are still in hibernation and their bodies are rather small in size. It seems that the gonial division takes place during relatively limited period of growth.

Spermatogonia (Fig. 6): As mentioned just above the metaphase spindles are only rarely found in earlier season. The cell plates of spermatogonia are very large, in which very small but many chromosomes exist. Despite the smallness of chromosomes they are fixable in good condition. They assume small rods of smooth outline, at most 1μ or so in length, their proximal end are slightly pointed. In the complements exists only slight size difference, most are of nearly similar size and shape, no peculiar elements representing sex-chromosomes are recognizable. In metaphase plates some of the smallest elements are often overlapped each other, so that accurate count of chromosomes can not be done with ease. Some metaphase plates show 43 to 44 chromosomes. Probably 44 is the true diploid number.

This number is equal to that of *Dugesiella hentzi* reported by PAINTER ('14), though in the latter no gonial chromosomes could be obtained. Compared these numbers with those of modern spiders, the former are distinctly larger than most of the latter.

First division (Figs. 7-12): Numerous first division metaphases are obtained in slides, but it is more or less difficult to obtain excellent figures in which all chromosomes can be counted clearly. When the slides are differentiated normally, some dyads are fused or connected with their ends to each other, which do not reveal their true form or number. On the other hand if differentiated somewhat intensely, the chromosomes, especially smaller ones become obscure in form, which prevent a minute observation. At any rate the first metaphase spindle, in general, is very large in size. Most of the bivalents are of V-shape, but when slightly stained, such bivalents exhibit large V of long arms, which are of irregular contour, or slender rods which are furnished with spherical thickening at ends. Due to the failure of obtaining excellent spindles the determination of bivalent numbers is difficult. But careful observation reveals that at least 20 bivalents are present, in addition to them one or two more elements are found in many plates. Although

accurate number of autosomes can not be decided, 21 or 22 (more probably 21) is regarded as to be true.

In the first maturation division X-elements are comparatively clearly identified. At metaphase I two elements exist on the eccentric position of the spindle, mostly between the equatorial plane and one pole, the feature is similar to many others mentioned below. In profile view the X-elements are intensely condensed, which assuming spherical bodies of similar size. They are closely attached to each other, but their individuality is demonstrated by changing the focus. In some spindles where chromosomes are not so much condensed, the X's are clearly distinguishable as two small rod-shaped elements. At later stages of the first division, namely at anaphase and telophase also these 2 X's can be identified. Therefore it is fairly true that in the male of the present species are two multiple sex-chromosomes. Here also the two X's remove without being divided to the same pole in the first division.

The second division metaphase could not be clearly observed.

III. Suborder Arachnomorphae (=Araneomorphae)

This is the third suborder of Araneae, which includes very numerous modern spiders of various families. In this suborder many species have so far been investigated cytologically by different workers. The present author has also studied the chromosomes of 88 species belonging to 19 families, of which 55 species of 15 families are described in the following pages, the remainings were reported in the previous papers (SUZUKI, '51a, '52a; SUZUKI and OKADA, '50).

Cribellatae

Whether cribellate spiders should be separated from ecribellate spiders or not has long been a matter of controversy and different opinions have been proposed by various arachnologists. Among them SIMON (1892-1903), BERLAND ('32), KISHIDA ('30), BRISTOWE ('38) and some others have segregated these two groups; on the other hand F. O. P. CAMBRIDGE (1897-1905), DAHL ('04, '05), PETRUNKEVITCH ('23), and GILTAY ('26) are representatives who do not recognize their separation. Further description on this point is out of question of this paper. It is sufficient to describe that the conclusion whether they should be separated or not cannot be obtained merely by the comparative study of morphological characters. Here cytological investigation seems to be of much meaning.

In Cribellatae only two species, *Amaurobius sylvestris* (PAINTER, '14) and *Dictyna arundinacea* (HACKMAN, '48) have been studied cytologically. The former, in chromosome number, and the latter, in chromosome form

show considerable specialities, therefore chromosome study of further species is very interesting. In this article two-species of Uloboridae and one species of Dictynidae were studied. As fully stated below similar cytological results to HACKMAN's was obtained in Dictynidae, but in Uloboridae no particular result was discovered.

3. Family Uloboridae
(3) *Miagrammopes orientalis*
(Figs. 13-26)

The body of the present species is slender, showing distinct morphological characteristics. The materials used are individuals captured largely from March to May, and partly in October. The gonads from juvenile, subadult and adult specimens were dissected out and immediately immersed into modified NAVASHIN's solution. The spermatogonial metaphases were obtained in the testes fixed in March and October. The female gonads preserved in April showed some oogonial metaphases. The meiotic division figures were observed in the preparation of testes fixed in May.

Spermatogonia (Figs. 13-15): The chromosomes and cell plates are relatively small. The metaphase chromosomes are usually intensely stained, which assuming stout rods of smooth outline. Some are straight and others slightly bent. In some slides all chromosome elements are of typical rod-shape, the appearance of which varies greatly from that mentioned just above. All metaphase plates show the diploid chromosome number of 22. It is difficult to find out special elements in the spermatogonial complements.

First division (Figs. 18-24): In the first maturation division 2 X's are always traceable during entire stages. At diakinesis two condensed globular bodies are present attached closely to nuclear membrane, they are highly heteropycnotic and clearly distinguishable from autosomal bivalents, which are of one proximal chiasma, showing large V-shape. At metaphase I 2 X's situate on the intermediate position between one pole and equatorial plane; each of them connecting to the same pole with individual spindle fibre. Through the first division the 2 X's are closely arranged, parallel to each other and behave as if a single element. In spite of being few, it is somewhat difficult to count autosomal bivalents due to their smallness and irregular arrangement of some elements. Close observation, however, reveals that their number is ten. At metaphase I the bivalents are condensed, exhibiting rod or more or less dumb-bell-shape. In slides such abnormal feature as shown in Fig. 24 was obtained, in which 2 X's are likely to separate and each moves towards the opposite pole. A similar feature has already been reported in other spiders by some authors (PÄTAU, '48; HACKMAN, '48; SUZUKI, '51a,

'52a; BOLE-GOWDA, '52). Here also it is probable that two elements near one pole are true X's, while those near opposite pole are univalent autosomes which were failed to form the bivalent.

Second division (Figs. 25-26): At second metaphase the cell plate and chromosomes are so small that it is not easy to observe them. The chromosomes are minute rods or spherical bodies, in some plates they show double forms. Some contain 10 elements and others 12 ones.

Oogonia (Figs. 16-17): In one gonad from the young female fixed in the middle of April were found oogonial metaphase spindles. The cell plate is nearly equal to the spermatogonia. The chromosomes are slender rods, in Figs. 16-17 24 diploid number is accurately counted. This number is larger by 2 than that of the male, so that the diploid set of females should contain 4 X's. But in the oogonial metaphase also these X's are indistinguishable by their morphological character.

(4) *Uloborus varians*

(Figs. 27-30)

This species is small sized, so the dissection of testes is somewhat difficult. The testes from many individuals were preserved during March and May. As fixative modified NAVASHIN's solution was employed. In chromosomes similar condition can be found between the present species and foregoing one.

Spermatogonia (Fig. 27): A number of metaphase spindles were obtained, but due to the smallness of chromosomes the observation was exceedingly difficult. The metaphase plate shown in Fig. 27 contains 22 chromosomes in diploid set. All the elements are of acrocentric type, showing small rods with tapering end. Only a slight size difference can be found in the complements. In the complements 2 X's can not be identified.

First division (Fig. 28): The cell plate is very small. In the profile aspect of metaphase I X's are clearly recognizable as two rod elements on the eccentric position of the spindle. The number of autosome bivalents can not always be clearly counted, but in some figures ten bivalents are present. The form of bivalents at metaphase is approximately similar to that of *Miagrammopes orientalis*.

Second division (Figs. 29-30): In this species two kinds of second metaphase plates are observed. Some contain ten elements and others 12 ones, 2 of which are X-elements. The size and form of the chromosomes resemble the foregoing species.

4. Family Dictynidae

In this family only a single species *Dictyna arundinacea* was studied

cytologically by HACKMAN ('48). By his report the species shows very characteristic chromosome feature, namely the autosomes are entirely metacentric; regarding the 2 X's in his material there are some questions. Therefore the study of the Japanese *Dictyna* is very interesting. The present author investigated two Japanese species of *Dictyna foliicola* and an undetermined species of the same genus, and obtained nearly equal result to that of HACKMAN. As the chromosomal conditions of the two Japanese species are totally equal, only the chromosomes of *Dictyna foliicola* will be mentioned.

(5) *Dictyna foliicola*

(Figs. 31-36)

During March and May numerous specimens were collected; their testes were fixed by modified NAVASHIN's solution. The cell plates and chromosomes of the present species are very small, so that the material is not favourable for chromosome study.

Spermatogonia (Figs. 31-32): In the testes from adult individuals fixed in May exist many spermatogonial metaphase plates. But as stated above very small chromosomes are packed in small nuclear plates, which prevent minute observation. Among numerous slides only a few good dividing gonial spindles were obtained. The chromosomes are very thin and most of them are regarded as metacentric elements. The larger pairs are easily distinguished as metacentrics, but smaller pairs, particularly when they are overlapped, are often difficult to discriminate whether they are metacentrics or acrocentrics. In Figs. 31-32 24 elements are accurately counted, 22 of which are metacentrics and the rest two acrocentrics. It is probably true that in the diploid set are present twenty-two metacentrics and two acrocentrics. The two acrocentrics are considered to be sex-chromosomes.

First division (Figs. 33-36): Here also the chromosome elements are extremely small and it is considerably difficult to carry out accurate observation. In many metaphase plates, however, 11 autosome bivalents and two X's can be counted. Some of the bivalents are of V-shape, but many of them are much condensed, assuming more or less minute globular form. The 2 X's are clearly distinguishable without exception. As in many other spiders they stand on the intermediate position between the equatorial plate and one pole. In *Dictyna arundinacea*, HACKMAN ('48) could not obtain the detail whether X's are two separated elements or single (metacentric) one, but in the present material they are found to be double acrocentric elements. As stated by HACKMAN in the first division each of the X's lies close to each other, so that the X's seem often as a single element, but in some figures shown in Fig. 35 they are distinctly recognized as two rod-elements. At metaphase I the

autosomal bivalents form one terminal or subterminal chiasma, so in the profile view they look as if two elongated elements were attached at one end, while the remaining parts were stretching freely in the nucleus. As in *Dictyna arundinacea* no ring bivalents can be found.

The secondary spermatocyte metaphase could not be clearly observed.

It is very interesting that the two *Dictyna* species from Finland and Japan show similar remarkable chromosomal features. Further chromosomal study in this family is intriguing.

Ecribellatae

In the present group the chromosomes of 13 families including 52 species have been investigated.

5. Family Dysderidae

The members of this family are characteristic of taxonomy, which are regarded to be primitive within the Arachnomorphae. For example, in PETRUNKEVITCH's classification ('28) the present family belongs to the first Branch Tetrasticta in the Arachnomorphae, in BRISTOWE's system ('38) also the family falls on the first Group Dysderoidea in the Ecribellatae. As the species of this family are of such taxonomic interest their cytological study is particularly interesting. No species have so far been studied cytologically. In the present article *Ariadna lateralis* has been selected as a single representative. As fully stated below quite different chromosomal feature has been obtained in this species.

(6) *Ariadna lateralis*

(Figs. 37-46 and Figs. 325-330)

The material used in the present study is one adult male which was secured on June 22 in Iwakuni city. The fixative used is CAROTHER's solution. The testis of this species is one pair of somewhat stout, very long tubes, which resembles rather those of *Heptathela kimurai* or *Atypus karschi* than other modern spiders. So despite of a single material many preparations were obtained. In the slides no spermatogonial spindles were obtained, and as the result the following description is concerned only on the maturation division spindles.

First division (Figs. 37-42 and Figs. 325-330): In the testes vast number of spermatocytic cells, spermatids and sperms are found. The first metaphase spindles are extraordinarily characteristic, namely the shape, size and number of chromosomes entirely differ from those of other species so far studied. When stained by HEIDENHAIN's iron hematoxylin in normal degree, four giant chromosome blocks which are

intensely stained black are found in relatively small cell plate of the primary spermatocyte. They are so curious in appearance that at first glance I wondered whether such figures are true first metaphase or not. Close observation, however, reveals that they are true metaphase plates. In some cases, when viewed from lateral, all chromosome elements are arranged on the equatorial plane, which bear spindle fibres and regarded as metaphase chromosomes. Furthermore, distinct anaphase and telophase stages can be fully recognized. The haploid number of chromosomes is undoubtedly 4, 3 of which are autosomal bivalents and the remaining one is regarded as to be X-element.

In order to know such chromosomes in more detail, the slides stained in normal degree were differentiated again in 0.5 to 1 per cent iron alum solution. When immersed for 3 to 4 hours, the chromosomes are decolored in considerable degree, but the central portions remain unchanged, which showing still dark color. Here we can see the dark colored chromosome matrix is covered by thin hyaline sheath. By SCHRADER ('49) and some others the matrix of chromosome is covered by pellicle (or sheath), but the demonstration of such pellicle is difficult except in some cases. The hyaline sheath found in the present material may be direct evidence of its existence.

In profile view of metaphase I, three bivalents are arranged in orderly manner on the equatorial plane, the X-element situates usually on the outer side of the spindle. At anaphase the X does not divide, while other three bivalents divide and each half goes to the opposite poles. It is often observed that at telophase undivided single X goes to one of the poles with divided autosomes. Thus the presence of single X is fairly true.

Second division (Figs. 43-46): The cell plates and chromosomes of the second metaphase are smaller than those of the first metaphase. Some plates include 3, and others 4 elements (3 autosomes plus one X). The chromosomes are oval or ellipsoidal and deeply stained. Even in the cell plates with 4 elements the X cannot be clearly identified.

The chromosomes of this species are thus remarkably different in many respects from those of other spiders reported. The material used in the present study is an adult male and its spermatogonial chromosomes can not be observed, if they be clarified in future study, many interesting chromosomal conditions would be known. On the other hand, as already stated above in this family no species have been reported cytologically. But it seems to be of much interest to note HACKMAN's unpublished data. I was told by his private letter that he had investigated the chromosomes of *Segestria senoculata* belonging to the Dysderidae. By him the *Segestria* males show 14 diploid chromosome number, 10 of which are metacentrics and the rest 4 acrocentrics (X's

are two acrocentrics). This karyotype is equal to that of *Aranea dumetorum* (HACKMAN, '48) and *A. scylla* (SUZUKI, '51a) of the Argiopidae.

When the chromosomes of *Ariadna lateralis* (and *Segestria senoculata*) are compared with those of many others, we are obliged to presume that the karyotype of the former might have derived from some others. As it will be stated in the later section, the more primitive the spiders are the higher chromosome number they possess, in other words many modern spiders have rather small numbers. In this respect *Ariadna lateralis* is very interesting, for its chromosome number is the lowest of all materials so far studied. So far as the chromosomes are concerned the species seems to be most highly developed contrary to its systematic position. But in this case we may interpret it that the chromosomal evolution has been taken place without any relationship with speciation of external characters and habits.

6. Family Pholcidae

PAINTER ('14) studied the chromosome of *Spermaphora meridionalis*, one species of this family, but his description was concerning merely to the presence of 2 X's in it. In the present article *Pholcus crypticolens* has been studied as a representative of the family.

(7) *Pholcus crypticolens*

(Figs. 47-49)

The systematic position of the present family is somewhat confused. By some authors the family is placed close to Theridiidae and Linyphiidae, etc., but some others, for example BRISTOWE ('38) regards the family to be rather more closely related to Scytodidae and Ochyroceratidae. By him these three families constitute a special section in Scytodoidea Group, which is arranged next to the Dysderoidea. Therefore the chromosome study of this family is of much interest. Two species of *Spermaphora* and *Pholcus* were studied cytologically by PAINTER ('14), but he could not obtain satisfactory results; only an existence of XXO-mechanism was reported in *Spermaphora*. The reason was that the germ cells were very small and the chromosomes were unfixable.

The materials employed in this study are male gonads which were gathered in April and May. They were fixed in modified NAVASHIN's solution. By the author's experience this mixture gives usually good results in many spiders chromosomes, but in *Pholcus crypticolens* the result is contrary to expectation. As PAINTER states in his paper the germ cells of the present material are very small and it is very difficult to have a favourable fixation.

Spermatogonia (Figs. 47-48): There exist many gonial metaphase plates in the preparations, but good figures in which all chromosome elements are clearly traced are extremely rare. In many cases the chromosomes are very much shrunk or in some fused each other, which prevent clear examination. Only a few slightly good slides were obtained. In general the chromosomes are small, most of them are of metacentric type. As many irregular formed chromosomes are packed in the small nuclear plate the determination of their number is not easy. In one plate shown in Fig. 48 about 21 chromosomes are counted, most of which are metacentric elements, only one element situated at 6 o'clock of the dial in the figure is regarded as acrocentric. In the plate of Fig. 47 approximately 23 elements are counted, here also most of them showing V-forms. As stated below, in the first metaphase 11 bivalents and 2 X's are recognized, so that the diploid number is considered to be 24. In the gonial plates observed some smaller elements might be hidden by others. The two rods seen in the complements are likely to be sex-chromosomes and the remaining (probably 22) metacentric elements are autosomal.

First division (Fig. 49): In this division also the chromosomes are very much condensed and some of them are fused, assuming irregular mass. The presence of 2 X's, however, is always clearly distinguished through all stages of the first division. Here also a similar condition to many spiders with 2 X's can be observed. Namely at metaphase I the 2 X's show an eccentric situation on the spindle, in lateral aspect they are identified as two completely separated rods of almost equal size, which lie well apart from the autosomal group. In some metaphases eleven autosome bivalents are counted. They are extremely condensed and of somewhat spherical or oval shape.

No favourable secondary spermatocytes were obtained.

The chromosomes of *Pholcus crypticolens* thus show close similarity to *Dictyna* species of the Cribellatae. As the occurrence of metacentric chromosomes is exceptional in spiders, the chromosomes of the present species are precious.

Group Clubionoidea

The present group is composed of such families as Clubionidae, Anyphaenidae, Sparassidae, Thomisidae, Salticidae, Oxyopidae, Lycosidae, Pisauridae, Agelenidae, Argyronetidae, Urocteidae and some others (BRISTOWE, '38). In the present paper Drassidae, Salticidae, Lycosidae, Pisauridae, Agelenidae and Urocteidae are mentioned. The chromosomes of Clubionidae, Sparassidae, Thomisidae and Oxyopidae have already been reported in the previous paper (SUZUKI, '52a).

7. Family Drassidae

This family has been well known cytologically, the chromosomes of eight species of seven genera were reported by PAINTER ('14) and HACKMAN ('48). All species studied show similar chromosomal feature. Namely haploid number 12 and 2 X's are characteristic for all of them. In the following the chromosomes of one species is given.

(8) *Drassodes* sp.

(Fig. 50)

Only a single immature specimen captured at the end of April was fixed by modified NAVASHIN's mixture. As the individual was too young the species name could not be determined. In the slides of testis spermatogonial metaphases alone were obtained.

Spermatogonia (Fig. 50): The gonial chromosomes are very small, at longest 1.5μ long. Though they are small, they are arranged well apart each other and clearly observed. All elements are of acrocentric type, assuming small rods of slight size difference. Every plate contains 22 chromosomes without exception. The identification of X's is difficult in the gonial complements. Though in the present species no maturation divisions were observed, the comparison with the allied forms suggests that two out of the 22 diploid chromosomes are X's.

The gonial metaphase chromosomes of this family have so far been reported only in *Berlandina cinerea* by HACKMAN ('48), remarkable resemblance is found between the karyotypes of *Berlandina* and the present species.

8. Family Salticidae (= Attidae)

The present family is very large and contains a number of species. The chromosomes of eight species of eight genera were published by PAINTER ('14), HACKMAN ('48) and SHARMA ('50). It was known by these workers that except *Myrmarachne formicaria* which has an XO-system and 11 pairs of autosomes, all species show equal chromosomal condition, namely the chromosome number is 15 in haploid and with XXO-mechanism. In order to know further occurrence of a single X and to find out some relationship between taxonomy and cytology the following 14 species of 10 genera have been researched cytologically.

Division Pluridentati

Subfamily Myrmarachninae: *Myrmarachne japonica*

Division Fissidentati

Subfamily Hasariinae: *Hasarius albarius*; *Hasarius adansoni*Subfamily Plexippinae: *Plexippus paykulli*; *Plexippus setipes*;

Plexippus crassipes; *Jotus linea*

Subfamily Pelleninae: *Aelurillus dimorphus*

Subfamily Heliophaninae: *Evophrys aninotatus*

Subfamily Dendryphantinae: *Icius magister*; *Icius elongatus*; *Icidella vittata*; *Rehne atrata*

Subfamily Sitticinae: *Sitticus littoralis*

Of these *Hasarius*, *Jotus*, *Aelurillus*, *Evophrys*, *Icius*, *Icidella* and *Rehne* are quite new material for cytology. General feature of chromosomes is nearly similar all through the materials, that is to say, the 14 species males possess 13 pairs of autosomes and 2 X's. It is of much interest that the Japanese *Myrmarachne japonica* differs in chromosome number and sex-determining mechanism from Finnish *Myrmarachne formicaria*. It is also worthwhile noting that remarkable size difference can be found in different species, furthermore, tetraploid spermatocytes are discovered in *Hasarius adansoni* and *Icius magister*. No clear mitotic chromosomes have so far been reported by earlier workers, but in the present study they are obtained in most of the species researched, moreover in *Myrmarachne japonica*, *Hasarius albarius* the chromosomes of the females are obtained.

Subfamily Myrmarachninae

(9) *Myrmarachne japonica*

(Figs. 51-61)

As stated above HACKMAN ('48) studied Finnish *Myrmarachne formicaria*, in which he found a different chromosomal condition ($n=12$ and XO-system) from that typical to many others of the same family, so that the cytological investigation in Japanese *Myrmarachne* is of much interest. The materials used in this study are a number of gonads from juvenile, subadult and adult gonads captured during April and June. All the gonads were preserved by modified NAVASHIN's solution. In the present species the chromosomes of both female and male sexes were observed. Particularly the chromosomes of the female were obtained in the slides of ovaries from immature individuals fixed in late April and early May.

Spermatogonia (Fig.51): Numerous metaphase spindles were obtained, but in many cases the chromosomes are clumped each other and it is rather difficult to obtain good figures. The careful observation reveals that the diploid number of chromosomes are exactly 28. The nuclear plates and chromosomes are of medium size. The chromosome elements are of acrocentric type. Some pairs are very elongated rods which are of somewhat irregular contour, others are small in size, assuming short rod shape. Every presumed homologous pair lie parallel to each other

closely or with a short distance. One pair of larger elements in the complement are regarded to be two multiple sex-chromosomes.

Oogonia (Figs. 52-53): In the preparations of female gonads from immature specimens were found numerous oogonial metaphase plates, some of which are pictured in Figs. 52-53. The nuclear plates are about equal to the spermatogonial plates, but the oogonial chromosomes are more intensely condensed, most of which are short rods with slightly pointed end, some are straight and others slightly curved. In good spindles 30 chromosomes are clearly counted. The number is larger by two than that of the spermatogonia, so 4 out of 30 elements must be multiple X's. In spite of close examination, it is never possible to identify such 4 X's in the oogonial complements.

First division in males (Figs. 54-58): The nuclear plate of the metaphase I is relatively large in size, in which large bivalents are present. Most bivalents are of large V shape, sometimes some of V's possess very elongated limbs, in such cases the bivalents are regarded as to have a proximal chiasma. Rarely in some plates does a single ring bivalent which is of two chiasmata exist. In some plates the bivalents are more intensely condensed, which look like two spherical bodies or small segments attached at one point. In most metaphases the number of bivalents are easily counted. They are 13 without exception. Besides these every plate contains two univalent X's, therefore the haploid number of the first metaphase is always 15. It must be mentioned that chromosome number and sex-chromosomes are different between the present material and HACKMAN's *Myrmarachne formicaria*. The 2 X's are always recognizable in the first division. At metaphase I they lie on the outermost circle of the spindle and standing in discrete level from others. Usually both elements are stout rod of about similar size, which are completely separated, sometimes they are considerably separated. In many cases each of the X's is longitudinally split. In some metaphases they stay very near to one pole. Thus it is completely certain that the present *Myrmarachne japonica* possesses two X-elements, which undividedly go to one pole in the first division in quite normal way. Furthermore, the presence of such multiple X-elements is demonstrable by observing the meiotic division in the female.

First division in females (Fig. 59): Some primary oocytes were obtained, in one of which the haploid chromosome number was clearly counted. The metaphase plate shown in Fig. 59 possesses 15 elements, which are condensed, assuming dumb-bell-shape; all the 15 elements are regarded as bivalents, no univalent X's are distinguished. Therefore 13 bivalents out of 15 should be autosomal and the remaining two sex-chromosomal. Thus in the first division of the female 4 X's form two normal bivalents, and thus it is difficult to identify the X's in that

plate by their morphology and behaviour. The result accords with those published by MONTGOMERY ('08), PATÁU ('48) and HACKMAN ('48) in some other spiders.

Second division (Figs. 60-61): A number of good secondary spermatocytes were observed. As X's are pre-reductional, two classes of second metaphases are formed. One contains 13 elements and the other 15 ones (13 autosomes plus 2 X's). Though the univalents are small, their number is clearly counted. The univalents are of rod shape of varying size, in some plates the elements are more or less elongated and not smooth in outline.

As mentioned above the present species shows $2n=28$, $n=15$ in the male, and $2n=30$, $n=15$ in the female and the sex is determined by XX:XXXX mechanism. Through all stages of meiotic prophase in the male the two X's are recognizable as two heteropycnotic elements near the nuclear membrane. The results differ remarkably from those published by HACKMAN ('48) in *M. formicaria*. As his study is devoid of the observation of mitotic chromosomes and second division, close re-examination is necessary, I think.

Division Fissidentati

Subfamily Hasariinae

(10) *Hasarius albarius*

(Figs. 62-66)

The present genus has never been studied cytologically. The spermatogenesis was researched on the slides of testes fixed during April and May, the female mitotic chromosomes were obtained in the slides of ovaries from subadult specimens fixed in April. For fixation of material modified NAVASHIN's solution and modified BENSLEY's mixture were employed. All of them produced good results. The chromosomes of this species are large and very favourable for cytological study.

Spermatogonia (Fig. 62): Many spermatogonial spindles were obtained. The good metaphase plates show 28 diploid chromosome number without exception. All chromosomes are acrocentrics and assuming rod-shape of tapering end; most of which are slightly curved. Only slight size difference is present in the chromosome complements. In the complements 13 pairs are regarded as autosomes, the remaining two sex-chromosomes. But the latter can never be identified in the gonial division.

First division (Fig. 64): In the metaphase I exist 15 chromosome elements, 13 of which are autosome bivalents and the rest two sex-chromosome univalents. The bivalents are large condensed rods, many of which are of median constriction; V-formed bivalents are rarely found. The

two X's are distinguishable at any stage of the first meiotic division. At prometaphase I they lie parallel to each other on the outer circle of spindle, assuming stout rods, sometimes somewhere near median is slightly constricted. In the first division they go undividedly to one pole. No size difference can be detected between them.

Second division (Figs. 65-66): The cell plates of the secondary spermatocytes are relatively large and favourable for observation. The chromosomes are condensed, and showing spherical bodies. Two kinds of metaphase plates are recognized, one half contains 13 autosome univalents, and the remaining half 13 autosomes and 2 X-elements. In one figure with 15 elements pictured in Fig. 65, two of larger rod-shaped elements are distinguishable from others, which are regarded as to be X-elements

Oogonia (Fig. 63): In the preparations of female gonads preserved in April exist many oogonial metaphase plates, in some of which the chromosome number can be accurately counted. The number is always 30, which is two more than that of the male. The presence of 4 X's in the female is thus fairly demonstrated in this material. The cell plates and chromosomes of oogonial spindles are remarkably larger than those of spermatogonia. For example, the largest pair of chromosomes are 4.3μ long in the female, while 2.6μ in the male. In spite of size difference remarkable resemblance can be found between the chromosome forms of the both gonial plates. As shown in Fig. 63 oogonial chromosomes are of acrocentric type, assuming large, elongated rod-form, most of which with tapering end. Some are slightly and a few strongly bent. Although four out of 30 chromosomes are regarded as sex-chromosomes, they are never distinguishable in the gonial complements.

(11) *Hasarius adansonii*

(Figs. 67-78 and Figs. 331-332)

The materials used are testes of ten individuals secured during April and June. As soon as the testes are dissected out they are put into fixing solution. The fixatives used are normal and modified NAVASHIN's solutions, CAROTHER's solution and modified BENSLEY's fluid. All of them gave favourable results. In this species also the chromosomes are so large that they are suitable for cytological investigation. The general feature of chromosomes is similar to that of the previous material in accordance with their systematic relationship.

Spermatogonia (Figs. 67-68): The metaphase plates show invariably $2n=28$. The chromosomes are all acrocentric, the form of which resembles *Hasarius albarius* mentioned just above (compare Figs. 67-68 with Fig. 62). Some chromosomes are very elongated, some others medium

sized rods and still others very short; especially several pairs situated on the central portion of Fig. 67 seem to be somewhat spherical or short rods, which are not their true shape, they do not stand in orderly manner on the plane, showing sectioned surface. In these complements also 2 X's are indistinguishable.

First division (Figs. 69-75 and Figs. 331-332): In every metaphase I are present 2 univalents and 13 bivalents; the former represent complex sex-chromosomes and the latter autosomes. The shape of bivalents differs somewhat in different plates. The bivalents shown in Fig. 75 are condensed and of dumb-bell-shape or somewhat V shape, in the middle of which with a clear slit. In Fig. 74 exist some large V-formed bivalents and condensed rod or dumb-bell-shaped ones. The 2 X's are invariably identified at any stage of the division. At metaphase I they stand on the eccentric position of the spindle, usually approaching near one pole. In profile view the two elements show about equal length, often somewhere near median with distinct constriction. Through the first division they lie parallel to each other, but in one figure (Fig. 72) the two X's are attached at one end and the remaining parts being separated widely, resulting to assume V-form.

It is exceedingly interesting that some polyploid primary spermatocytes are found in one testis. Figs. 78 and Figs. 331-332 show such polyploid spermatocytes, in Figs. 331-332 two large cells, one of which is polar view and the other profile, are found surrounded by normal cells. These polyploid cells are remarkably larger in size than surrounding cells. But the metaphase chromosomes of such cells are nearly equal in size and shape to those of the normal spermatocytes (compare Fig. 78 with Figs. 73-75). Although their number can not be clearly determined, in one figure shown in Fig. 78 about 30 elements are counted, which is about twice the haploid number, so that the spermatocyte is regarded as to be tetraploid. In the Fig. 78 the X-elements are not clearly distinguishable.

Second division (Figs. 76-77): As in the previous division the two X-elements go undividedly to a single pole, and two kinds of secondary spermatocytes are formed, one of which contains 13 autosomes and two X's, namely with $n=15$, the other only 13 autosomes. In some plates the univalents are elongated rods, but in others the elements are condensed, short rods.

Subfamily Plexippinae

In this subfamily *Plexippus paykulli* has been studied cytologically by SHARMA ('50). His study has been concerned largely with the cytoplasmic inclusions but not on the chromosomes. The present author investigated three species of *Plexippus* (one of which is the same as that

reported by SHARMA) and one species of *Jotus*. The results obtained show that the general morphology of chromosomes in these species are nearly equal to those of other subfamilies. In *Plexippus* very large chromosomes and some particular chromosome structure were observed.

(12) *Plexippus paykulli*

(Figs. 79-86)

As stated above the Indian individual of this species was searched by SHARMA ('50). By him the species males possess two X-elements, which remove without being divided to one pole in the first meiotic division and divide equationally in the ensuing division. But on the chromosome number no description was given in his paper.

The present observation was carried out on the preparations of male gonad from one subadult captured in July. The material was fixed in FLEMMING's weak solution. All stages of spermatogenesis were traced in the same slide.

Spermatogonia (Figs. 79-81): The cell plates and chromosomes are extremely large, the largest of all materials studied by me. Some of gonial metaphase plates show such sizes as 23×15 ; 21×16 ; 18×17 (diameters in μ), which are approximately equal to those of *Heptathela kimurai* reported in the foregoing pages. When compared these largest plates with the smallest ones known in *Linyphia*, *Tetragnatha*, *Theridium* and *Dictyna*, etc. one would be astonished why such great size difference has been produced. In accordance with the size of cell plates the chromosomes are also notably large. The largest chromosome is 5.1μ long. Thus the present species is one of the most favourable material for chromosome study.

All metaphase plates contain 28 diploid chromosomes. The chromosomes are acrocentric, and of stout, elongated rod-shape; some of which are straight, but others are slightly or somewhat strongly bent, sometimes one or two pairs are bent very acutely. Some elements at their distal ends are of knob-like thickening, and exhibiting bizarre contour. The outline of most chromosomes is usually smooth, but some have slightly irregular contour. At metaphase some pairs of chromosomes exhibit longitudinal split. Several pairs situating in the central circle are more or less stout rods or spherical bodies, which are considered to be revealing their distal thickening. The chromosome complements of Fig. 80 are somewhat deeply stained and of deep bluish colour. When differentiated in considerable degree, such figures as shown in Fig. 79 are obtained, in which terminal (proximal) short regions of chromosomes are more faintly stained than the remaining parts. The feature is more clearly shown in the Fig. 81. In which some chromosomes possess very fine, short thread-like appendage at their proximal end, one element is

furnished at its terminal end with one globule. These appendages are stained faintly with hematoxylin and seem to be homologous to setae known in the chromosomes of some plant materials. As will be mentioned below such figures are more fully observed in *Plexippus setipes*.

Two of the diploid set chromosomes are regarded as sex-chromosomes, but they can not be clearly identified in the complements of spermatogonia.

First division (Fig. 82-85): The primary spermatocyte is also very large in size, it is of nearly equal diameter to spermatogonial metaphase. The chromosome shape varies in different figures, in some plates all elements are very elongated, in others highly condensed. In spite of the variation of shape, their number is constant, namely at metaphase I there exist invariably thirteen autosome bivalents and two univalent X's. The metaphase plates of Figs. 82 and 84, show very large elements, where the autosomal bivalents are very large; some have very elongated limbs; at apex of some V's a small globular body is found. In these figures the X's are recognized as elongated two rods. On the other hand, in Fig. 83 most of the bivalents assume dumb-bell-shape; V-formed elements are rarely found. As in other species the 2 X's stand in discrete level, standing parallel to each other. Of course in the first division they remove undividedly to a single pole.

Second division (Fig. 86): The nuclear plates of the secondary metaphases are remarkably large in size. The univalents are short rods; pointed at one end and thickened at another end. Many of them are slightly curved, exhibiting somewhat irregular appearance. In many cases every univalents show double structure. One half of metaphase plates contains 15 elements and the other 13 ones. But even in the former the X's are indistinguishable.

(13) *Plexippus setipes*

(Figs. 87-88)

The chromosomes of this species closely resemble those mentioned above in accordance with their taxonomic relationship. For the chromosome study a number of testes were fixed, most of which were preserved by normal and modified NAVASHIN's solutions, partly by FLEMMING's strong solution. The testes preserved in May showed all stages of spermatogenesis.

Spermatogenesis (Fig. 87): The metaphase plate is very large, which is nearly equal to that of the previous species. In every metaphase 28 chromosomes are clearly counted, two of which are considered to be sex-chromosomes. The chromosomes are very large rod-shaped. The two chromosomes situating at 10 and 11 o'clock of Fig. 87 are distinctly the largest, which are acutely bent and showing somewhat irregular

contour. It seems that they correspond to sex-chromosomes. The chromosomes of some plates show very interesting feature. As is shown in Fig. 87, almost all chromosomes are furnished with a chromatic filament of different length at their apices. The filament is stained very faintly by hematoxylin; some are very short and spine-like; some others of medium size; and still some others of considerable length. At terminal end of such filaments is found a minute globule. As already stated they are considered to correspond to setae. Concerning their fine structure I intend to study in the near future.

First division (Fig. 88): The haploid number of chromosomes counted at metaphase I is invariably 15, 13 of which are autosomal bivalents and the rest two sex-chromosomes. The bivalents are large and stout, some of which are of large V-shaped and others of short or large rod. The large rod elements are slightly constricted in median and show more or less dumb-bell-shape. The X-elements are always distinguishable as two rods of nearly equal size. Usually both elements stand close to each other.

(14) *Plexippus crassipes*
(Figs. 89-95)

The male specimens can not be easily collected, while females are often encountered. In the present study only a single subadult male was employed as material. The testis was fixed by FLEMMING's weak solution, which gave fairly good results. The general feature of chromosomes is equal to that found in the previous two species. Namely the present species shows 28 diploid chromosome number and with two multiple sex-chromosomes.

Spermatogonia (Fig. 89): Large acrocentric chromosomes are packed closely in the metaphase plate of spermatogonia. Some of them are overlapped each other, but due to their large size, the number can be definitely counted. In every plate exist 28 chromosomes, two of which are considered to be X-elements. The chromosomes are stout, long rods of different size. Some standing on the central portion of the plate are smaller. The size of chromosomes and cell plates are similar to that of just above mentioned two species. The two X's can not be identified in the gonial complements.

First division (Figs. 90-94): The nuclear plates and chromosomes of metaphase I are so large in size that the material is suitable for cytological study. In that plate haploid number of 15 is clearly counted, of course 13 of which are autosomal bivalents and the rest two X-elements. At early metaphase I the bivalents are of interstitial chiasma, most of which assuming large V-shape, during full and late metaphase I the chiasma is terminalized and the bivalents look as if two segments were

attached to each other. Throughout the first meiotic division the X-elements are recognized at particular situation of the spindle, the 2 X's may be closely attached or lie with some distance. Sometimes both elements stand very close to one pole during metaphase and anaphase. Apparently they are similar in size.

Second division (Fig. 95): Compared with the previous two species the nuclear plate and chromosome elements are smaller, but here also some plates bear 15 elements and others with 13 ones. The chromosomes are elongated or short rods.

(15) *Jotus linea*

(Figs. 96-99)

The materials used are subadult and adult males captured during March and May. The testes of these specimens were immersed into normal and modified NAVASHIN's fluids. The number of chromosomes and sex-determining mechanism are equal to those of other species of the same subfamily, but the present species differs in size of chromosome and cell plate, i. e. it is distinctly smaller than that of *Plexippus*, and of medium size.

Spermatogonia (Fig. 96): The diploid chromosome number is undoubtedly 28, here also two of which are regarded as sex-chromosomes. All chromosomes are of acrocentric type, showing rod shape. Except some pairs of smaller size situating on the inner circle, the elements show slight size difference. All the elements are smaller than those of *Plexippus*-species. For example, even the largest pair in the complements are at most 3μ long. At any stage of spermatogonial division the X's are indistinguishable.

First division (Figs. 97-99): At first metaphase 2 X-elements and 13 autosome bivalents are clearly recognized. The bivalents are highly condensed and most of them assuming short dumb-bell-shape, only a few large V-shaped bivalents are found in Fig. 97. The two X's stand at metaphase on the intermediate situation between the equatorial plate and one pole; in the profile view they usually show elongated rods, which lie parallel and very closely, so that sometimes they seem to be a single element, but even in such figures, when observed carefully from the pole by changing the focus, their individuality is demonstrated.

Second division: Two kinds of metaphase plates are formed. One contains 13 autosomes and two X's, but the other with only autosomes.

Subfamily Pelleninae

The present subfamily has never been studied cytologically. In this paper the following species was researched as a representative.

(16) *Aelurillus dimorphus*
(Figs. 100-105)

The testes from the specimens captured during April and May were preserved largely by modified NAVASHIN's solution and partly by ALLEN-BOUIN's solution and CAROTHER's solution. All stages of spermatogenesis were observed, the chromosome condition does not show any peculiarities from others of the same family.

Spermatogonia (Figs. 100-101): All elements show rod shape of different size. In many figures they are crowded on the plate, so that it is not always easy to count their number, but in some spindles diploid number of 28 is clearly counted. Here also the identification of X's is not possible.

First division (Figs. 102-104): The cell plate and chromosomes of metaphase I are of considerable size. On the metaphase plate exist 13 bivalents and two univalents, the former are autosomal and the latter sex-chromosomes. One half of bivalents are of V-shape and the remainings of rod-shape which is slightly constricted near the median. At metaphase the 2 X's are forming accessory plate, almost equal in size, stand close to each other, but sometimes they lie apart with considerable distance.

Second division (Fig. 105): Two sets of secondary metaphases are found. One possesses 15 elements and the other 13 ones. The univalents are short rods with somewhat pointed ends.

Subfamily Heliophaninae

No species has been published cytologically. I have been able to survey the chromosomes of the following two species.

(17) *Evophrys aninotatus*
(Figs. 106-108)

The present spider is very small in size and show very characteristic features. The testes from three specimens secured in May and June were fixed by modified NAVASHIN's solution and CAROTHER's solution. In the material fixed on May 21 were found all stages of spermatogenesis.

Spermatogonia (Fig. 106): Excellent dividing spindles are obtained, in which chromosome elements are arranged well apart and the diploid number of 28 is clearly counted. The shape and arrangement of gonial chromosomes resemble remarkably those of *Hasarius albarius* already described. As shown in Fig. 106 all elements are of acrocentric type, the largest pair is $3.1\ \mu$, the smallest one $1.7\ \mu$ long. Between each pairs of the complements only slight size difference can be found. In Fig. 106

two pairs of chromosomes situated at six to seven o'clock are larger than others and with some distinct contours; especially one pair of them are slightly but distinctly constricted near apex. These two elements are likely to be multiple sex-chromosomes, but it seems to be premature to give definite conclusion.

First division (Figs. 107-108): Metaphase I shows 15 chromosomes in haploid number, 13 of which are autosomal bivalents and the remaining two, univalents representing X-elements. The bivalents are not so much condensed, some assume V-shape, others rod-shape or dumb-bell-shape. At metaphase I the two X's are recognizable as two completely separated bodies. Sometimes they lie very near to one pole, while autosomes are staying on the equatorial plate. In profile view they are short rods of nearly similar length.

Second division: Here also two sorts of secondary metaphase spindles are obtained.

Subfamily Dendryphantinae

In this subfamily the chromosomes of two species of *Dendryphantes* and *Pseudicus* have so far been reported by HACKMAN ('48). These species males possess $n=15$ including 2 X's. In this paper *Icius magister*, *Icius elongatus*, *Icidella vittata* and *Rhene atrara* were employed as material. The results obtained accord in general with that published hitherto. In *Icius magister* tetraploid spermatocytes can be found again.

(18) *Icius magister*

(Figs. 109-122)

The spiders of the present species differ greatly in coloration by different sexes, formerly both sexes were regarded as different species. The cytological study reveals that they are never separated species, but a single species. Numerous individuals were collected during March and June and their gonads were preserved by normal and modified NAVASHIN's fluids and ALLEN-BOUIN's mixture, all these gave favourable results. The testes of subadult or adult specimens fixed in April and May are most favourable for chromosome study.

Spermatogonia (Figs. 109-112): A number of excellent metaphase spindles were obtained. All figures contain 28 chromosomes without exception. The shape of chromosomes differs in different plates, though they are invariably of acrocentric type. In some cases (Figs. 109-110) the chromosomes are stout, comparatively short rods of smooth outline. Except somewhat smaller pairs standing on the central portion, only a slight size difference exists in the chromosome complements. Some

elements exhibit longitudinal split. In Fig. 111 all elements are slightly longer than those of Figs. 109-110, and a few of larger elements are acutely bent, possessing more or less irregular contour. In Fig. 112 very stout elements are packed in the plate, they are stumpy rods; some of which assuming scale-form, some others, bizarre contour. The shape and arrangement of such chromosomes resemble very much those of *Philodromus roseus* (Philodrominae, Thomisidae) reported in my previous paper (SUZUKI, '52a: compare Fig. 112 with SUZUKI's '52a Fig. 16 A-D). In Fig. 111 one chromosome situating at 5 o'clock is distinguishable by its largest length and irregular form. As its homologous element can not be clearly identified, it is not conclusive that it corresponds to one of the sex-chromosomes. In general it is never easy to distinguish sex-chromosomes in the gonial complements.

First division (Figs. 113-118): The metaphase I always shows $n=15$, two of which are X-elements and the remaining 13 autosome bivalents. At metaphase most bivalents assume V-shape, some with short rod or dumb-bell-shape. The 2 X's are always distinguishable as two elongated rods lying parallel to each other. At full metaphase they stand usually on the intermediate situations between one pole and equatorial plane, sometimes they lie very near to one pole. In profile view of Fig. 117 the 2 X's lie parallel with a very short distance, which are nearly equal in size and shape; in Fig. 116 they are highly condensed and attached so closely that they look like a single rod. At anaphase the two X's stand on the outer circle of the spindle, which are almost immovable, while autosome bivalents divide and remove towards each pole.

Second division (Figs. 121-122): The secondary metaphase plates are relatively large in size and favourable for observation. As mentioned above the two X's go to a single pole and two kinds of metaphases are formed. One contains 13 elements and the other 15 ones. The chromosomes are curved rods of different size and of somewhat irregular outline. In many plates they show double structure.

Tetraploid spermatocytes (Figs. 119-120): In the present species also polyploid spermatocytes have been found in one individual. Such polyploid cells exist in normal testis, forming some particular cysts. The cell plates of these polyploid spermatocytes are notably larger than those of normal ones, on the other hand the chromosomes are about equal in size and shape. Fig. 119 shows one polyploid primary spermatocyte metaphase, in which about 28 bivalents are counted. Therefore it might be tetraploid. Fig. 120 is a giant secondary spermatocyte metaphase found in the same slide as Fig. 119, here also the size and shape of chromosomes are quite similar to those of the normal secondary spermatocytes. In that figure approximately 30 elements are counted, accordingly it is also regarded as tetraploid.

(19) *Icius elongatus*

(Figs. 123-125)

The present species occurs in the similar habitats with the foregoing one and these two species seem to be very closely related to each other. The materials used were captured during the season when *Icius magister* was captured, and fixed by the same solutions as the previous case. The general feature of chromosomes of this species resembles that of *Icius magister* in accordance with systematic relationship.

Spermatogonia (Fig. 123): The spermatogonial metaphases show the diploid chromosome number of 28, here also two of which are regarded as multiple X-elements. The chromosomes seem to be a little smaller in size than *Icius magister*, but other morphological features are approximately the same between them. In this case also the identification of sex-chromosomes is never possible in the gonial complements.

First division (Figs. 124-125): Thirteen bivalents and two sex-chromosome univalents are clearly distinguished in every metaphase I. Regarding the form of bivalents similarity is found between the present species and the *Icius magister*. In the present material also the 2 X's remove undividedly to a single pole at first anaphase as in many others, but some irregularities are found in the division. Fig. 125 is a profile view of metaphase I, in which two rod elements stand near to the both poles. They are symmetrical to the equatorial plane and nearly equal in size and shape. By changing the focus either of the both elements does not reveal double structure, and thus regarded as single element (univalent). Concerning the irregularities of some autosomes in the first maturation division some reports are published by SOKOLSKA ('25), PÄTAU ('48), HACKMAN ('48), SUZUKI ('51a, '52a) and BOLE-GOWDA ('52). By these workers it was shown that at first metaphase some autosomes which are failed to form bivalents remove precociously to either of the poles and they are coexisting with true X-elements near one pole. It seems that two univalents found in Fig. 125 are not such mis-divided autosomal univalents but abnormally divided X-elements. But the future fate of such abnormal spermatocyte can not be traced.

Second division: Two kinds of secondary spermatocytes were found. One bears $n=13$ and the other $n=15$.

(20) *Icidella vittata*

(Figs. 126-136)

The body of this species is smaller than that of *Icius* stated just above, and with a beautiful greenish blue colouration. The materials used are testes from subadult and adult individuals collected during April and June. They were fixed largely by modified NAVASHIN's solution

and partly by ALLEN-BOUIN's solution. So far as chromosomes are concerned close similarity can be found between the present material and *Icius magister*.

Spermatogonia (Fig. 126): Spermatogonial metaphase shows invariably the diploid number of 28. The size and shape of chromosomes resemble very much those of *Icius magister*. In this case also all the elements are stout rods of comparatively short length, only a little size difference is found in the complements. In Fig. 126 many chromosomes are longitudinally split and very stout, with only pointed proximal end. In the spermatogonial division two sex-chromosomes are not distinguishable.

First division (Figs. 127-134): As in many salticid-spiders 13 autosome bivalents and two X-elements are present in the first division metaphase. In the present material two kinds of metaphase plates are observed, in some plates the chromosome elements are not so much condensed but of considerable size, in others they are intensely condensed, which are of small size. In the former the bivalents are of large V-form or dumb-bell-shape, in which two short rods are connecting at their apices; short rod or spherical elements are rarely found. In the latter case the bivalents including 2 X's are very small, in the profile view such bivalents seem as if two small dots were attached to each other at one point, and are of typical dumb-bell-shape.

At any stage of this division the two X's are very clearly distinguished. At metaphase both elements lie closely on the eccentric position of the spindle, in other plates stand, with a considerable distance. Concerning their shape, some variation can be found. Through the first division they show typical rod shape in general, but in some cases, particularly when condensed intensely, they look like two small spherical bodies with pointed ends. At telophase I also the two X's are fairly recognizable by their situation. By this time they are found to lie on the outermost circle of the spindle, separated from the autosomal group in one half of the cell. In one figure they assume two elongated rods lying close to each other and show heteropycnotic condition, while autosomes remain still with unchanged smooth configuration.

Second division (Figs. 135-136): As in others some secondary metaphases contain two X's and 13 autosomes, but others, only 13 autosomes. The chromosomes in some plates are small rods or nearly spherical bodies of smooth outline, but in others they are rather slender rods of somewhat irregular contour. The identification of 2 X's in this division is difficult.

(21) *Rehne atrata*

(Figs. 137-139)

The testes from juvenile and subadult individuals captured in spring

were fixed by normal and modified NAVASHIN's solution. Both mitotic and meiotic chromosomes were clearly observed.

The chromosome number is $2n=28$, $n=18$ and the sex-determining mechanism of XXO-type is present. All the spermatogonial chromosomes are of acrocentric type, which are typical rod shaped, the shape and size of which resemble those of *Icius magister*. The autosome bivalents at metaphase I are V or dumb-bell-shaped. The two X's are rod elements of nearly similar length in the first division, which lie close to each other in eccentric position of the spindle.

Subfamily Sitticinae

In this subfamily the chromosomes of one Finnish spider *Sitticus terebratus* was reported by HACKMAN ('48). In the present study one more species of the *Sitticus* from Finland has been researched.

(22) *Sitticus littoralis*

(Figs. 140-141)

The present material is one male fixed by HACKMAN in Finland. The material was labelled by him as follows: Date: VI 1946; Locality: Tvärmine; fixed by NAVASHIN's solution. The paraffin block was re-embedded by me. The testis was too old to observe meiotic division, only some first meiotic division figures were found.

First division (Figs. 140-141): The haploid number of metaphase I is 15, 13 of which are autosomal bivalents and the rest two sex-chromosomes. The bivalents are highly condensed as in *Sitticus terebratus*, many of which are short rods of median constriction, some showing V-shape. The 2 X's stay at metaphase on the eccentric positions of the spindle, in Fig. 140 they are completely split longitudinally, showing more or less V-form, a slight size difference is recognizable between the both elements. But it can not be definitely concluded whether true size difference exists between the 2 X's or not, due to the absence of full materials.

9. Family Lycosidae

The earlier chromosomal studies of this family were carried out by MONTGOMERY ('05) and PAINTER ('14), following them HARD ('39) first reported on the chromosomes of *Schizocosa crassipes* using modern technique. Lately the chromosomes of 16 species have been published by HACKMAN ('48). Thus in Lycosidae the chromosomes are known in 19 species of 7 genera. All species studied show close resemblance in chromosomes, the two compound X's are always common in the members

and the haploid number of chromosomes ranges from 12 to 15.

The materials investigated in this report are five Japanese species, *Lycosa T-insignita*, *L. cinereofusca*, *L. coelestis*, *L. pseudoannulatus* and one undetermined species of *Lycosa*. The results obtained are generally similar to those so far reported by other workers. It is worthwhile noting that some clear size difference can be obtained between the both X's in certain species. The finding would be of much meaning in considering the evolution of multiple sex-chromosomes. Moreover, micro-chromosomes are demonstrated to occur in one species of the *Lycosa*.

Subfamily Lycosinae

All the materials so far studied and stated in the present article belong to the same subfamily Lycosinae.

(23) *Lycosa T-insignita*

(Figs. 142-148)

The spiders of this species are found in early spring. A number of spiders were secured between February and May. Most of the testes were fixed by NAVASHIN's solution, and partly by FLEMMING's weak solution, ALLEN-BOUIN's solution, CAROTHER's solution and modified NAVASHIN's solution. When preserved by FLEMMING's mixture the chromosomes show very fine, clear contour, on the other hand, when other fixatives are used they assume somewhat stout appearances. In the testes fixed on 1st of March showed very good spermatogonial metaphases. In general the materials preserved in March and April are favourable for the observation of maturation divisions. The cell plates and chromosomes of the present material are large and well fixed by any of usual mixtures, with the result that it is favourable for chromosome study.

Spermatogonia (Fig. 142): Though we have many chromosomal surveys in this family, very little is known about the spermatogonial chromosomes. The accurate counting of diploid chromosome set has so far been reported only in *Schizocosa crassipes* (HARD, '39) and *Lycosa saccata* (HACKMAN, '48). The gonial metaphase plates of this species always show 28 chromosomes. All chromosomes are of acrocentric type and assume rod shape. In the slides fixed by NAVASHIN's solution the rod elements are stout and some of them are of rather stumpy form. Between the largest and the smallest pairs exists some size difference. Some are slightly and others acutely bent, in particular, larger elements are of slightly irregular contours, showing bizarre appearance. One element situated at 12 o'clock of Fig. 142 is the largest one, which is acutely bent near apex and is distinguishable from others. As described below the present species male possesses two X-elements, which are not

equal in size. So that the chromosome of such a different form may be regarded as to be one of the X-elements. The remaining one X is not clearly distinguishable in the complements. In the slides fixed by FLEMMING's weak solution the chromosome complements are very fine and showing typical rod-shape.

First division (Figs. 143-146): Numerous excellent metaphase I were obtained, in which all elements are arranged on the equatorial plane well apart with equal distance. Thirteen autosome bivalents and 2 X-elements are clearly distinguished. The metaphase plate shown in Figs. 143-145 are taken from slides fixed by FLEMMING's weak solution, where the chromosomes show very clear contour. The autosome bivalents are typical V or horse-shoe-shaped, some others are of dumb-bell-shape, in the latter case they look as if two segments were connected with thin chromatic thread. The 2 X-elements are invariably distinguishable at any stage of the first maturation division. At metaphase I they lie on the peripheral position of the spindle, perpendicularly to the equatorial plane, they stand thus on the accessory plate of the spindle. Usually each of the 2 X's lies parallel to each other with a short distance, connecting with the same pole with an individual spindle fibre. It is of much interest that a slight but distinct size difference is always recognizable between the two X's. The feature is better noticed in the profile view. The difference seems to be nearly equal to that reported in *Lycosa fluviatilis* and *Trochosa ruficola* by HACKMAN ('48).

Second division (Figs. 147-148): The second metaphase plate is relatively large in size and favourable for observation. As stated above the two X's go undividedly to one pole in the previous division, two kinds of metaphase II are present. Only one half of the plates contains the X's. Fig. 147 possesses 15 elements and Fig. 148 13 ones. The chromosomes are condensed, assuming spherical bodies, some of which have slightly pointed ends. In Fig. 147 one is distinguishable in the complements, which is likely to be one of the 2 X's.

(24) *Lycosa cinereofusca*

(Figs. 149-158)

The following description is based on the observation of testis from a single subadult individual which was fixed by modified NAVASHIN's solution in April. In the same preparation all stages of spermatogenesis were observed.

Spermatogonia (Figs. 149-150): The cell plate of spermatogonia is as large as the previous species. The chromosome number is 28 in $2n$, which is equal to *Lycosa T-insignita*. The chromosomes are rod-shape of varying size, which are slightly or acutely curved. The chromosome

complements shown in Fig. 149 are relatively stout; showing more or less similarities to those of *Lycosa T-insignita*; in Fig. 150 all the elements are slender rods of somewhat irregular outline. Some elements possess a knob-like thickening at their distal end, and still some of them are irregularly bent and assuming a very bizarre appearance. In Fig. 150 one element may be distinguishable from others because of its different morphology. Namely the element is the longest one and near apex it is clearly constricted. Probably it is one of the X-elements. Concerning one more X no signs could be obtained.

First division (Figs. 151-155): The primary spermatocyte metaphase plate is very large in size, sometimes it is larger than that of the spermatogonia. On the rather limited area of plate are arranged the chromosome elements. Here also 15 chromosomes are very clearly counted, 13 of which are autosome bivalents and the remaining two are univalents corresponding to two compound sex-chromosomes. In spite of the employment of very common fixative the chromosomes are fixed in good condition, which exhibit very clear and fine appearance, and showing close resemblance to those of *Lycosa T-insignita* fixed by osmic acid. That is to say, the size and shape of bivalents strikingly resemble *L. T-insignita*.

The two X-elements of the present species are of much interest. Concerning their arrangement and behaviour similarities are found to others, however, it is worthwhile noting that a notable size difference exists between the two X's. As is shown in Figs. 151-155 one X is elongated rod, while the other one short rod. The difference is larger than in the previous species; in the present case one X is approximately 1.5 times as long as the other. The difference seems to be always constant through all stages of the first meiotic division. Consequently such a difference is regarded as to be characteristic of the present species. It recalls us a similar condition in Philodrominae, regarding this point further statement will be given in the later section of this paper.

Second division (Figs. 156-158): In this species also two sorts of secondary metaphase plates are distinguishable, one of which shows 15 elements and the other only 13 ones. The metaphase plates are relatively large, the chromosomes are rod-shape of different length.

(25) *Lycosa coelestis*

(Figs. 159-169)

The material used is one subadult male which was captured in May. The testis was fixed by modified NAVASHIN's fluid. In the same testis all important dividing figures were obtained. The cell plates and chromosomes are extremely large, the largest among the five lycosid spiders studied in this paper. The chromosome number differs from that of

other species.

Spermatogonia (Figs. 159-161): The close observation of spermatogonial metaphase reveals that the diploid number is invariably 26, which is fewer by two than that of the allied forms. In Figs. 159-160 all chromosomes are rod-shaped, most of them are very large rods of smooth outline; except some smaller elements situating in the central circle a slight size difference is found in the complements. The chromosome complements shown in Fig. 161 are more slender than those of Figs. 159-160, showing somewhat irregular outline. This kind of difference is probably due to the different ages of spermatogonia. Judging from the two X's observed in the meiotic division, they are regarded as to be the largest pair in the complements, but in the spermatogonial metaphase they are not always clearly distinguishable.

First division (Figs. 162-165): On the large sized first metaphase plate (about $19\mu \times 16\mu$ in diameters) are found big bivalents. The number of autosome bivalents is invariably 12, so that the haploid number is 14. The bivalents generally resemble in shape the previous two species of *Lycosa*, except the difference in size. In the present species also 2 X-elements are always recognizable in the first maturation division, almost always they stand on the eccentric position in the spindle, quite separated from autosomes, which are identified as two long rods lying parallel to each other. At any stage of the first division no size difference can be found between the X's, the condition is different from the above mentioned two species. Of course they remove undividedly towards one pole in the first division and divide equationally in the ensuing division. In one figure of the first telophase an abnormal condition is observed, in which one elongated chromatin bridge is present between the two autosomal groups. The fragments are not clearly observed. At this stage the divided autosomes are slightly decoloured, but in the upper group one pair of the longest elements still retain their deep colouration, which are probably representatives of the 2 X's (Fig. 165).

Second division (Figs. 166-168): The secondary metaphase chromosomes are large and of considerably characteristic forms. They are not so much condensed as in many other spiders but most of them exhibit elongated rods of more or less irregular outline. Some plates contain 12 element, but others 14 ones, 2 of the latter are sex-chromosomes. In some figures two larger elements are often distinguishable, in Fig. 166 the two largest rods lie parallel to each other, which are particularly heavily stained and of clear contour, in Fig. 167 they lie quite apart on the plate. Such distinct elements might be sex-chromosomes.