

XXXY Sex Chromosomes in Males of the Jumping Spider Genus *Pellenes* (Araneae: Salticidae)

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Abstract. Observations of male meiosis and female chromosome number indicate that eight species of *Pellenes* have the X_1X_2O male, $X_1X_1X_2X_2$ female sex chromosome system typical of salticids, four species have an $X_1X_2X_3Y$ male, $X_1X_1X_2X_2X_3X_3$ female system, and one species has both X_1X_2O and $X_1X_2X_3Y$ males. This is the first report of a Y chromosome in spiders. It is hypothesized that the $X_1X_2X_3Y$ system was derived from an X_1X_2O system by a tandem X-autosome fusion which yielded the X_2' and a centric autosome-autosome fusion which yielded the Y. Data on heteropycnosis, chiasmata, segregation, chromosome number and arm length support this hypothesis. The distribution of the $X_1X_2X_3Y$ system within the genus is phylogenetically confusing and suggests that the two sex chromosome systems have been maintained together as a polymorphism in some lineages for long periods of time or that there have been repeated derivations of the $X_1X_2X_3Y$ or X_1X_2O systems.

Introduction

Of approximately 300 spider species that have been cytologically described in the literature, all appear to be XO , X_1X_2O , or $X_1X_2X_3O$ in the male (White, 1973; Mittal, 1961, 1966; Diaz and Saez, 1965, 1966). In this paper I give the first report of a Y chromosome in spiders. Members of five of the thirteen species investigated of the jumping spider genus *Pellenes* Simon have an $X_1X_2X_3Y$ male, $X_1X_1X_2X_2X_3X_3$ female sex chromosome system.

In the family Salticidae the predominant male karyotype is 26 acrocentric autosomes plus two acrocentric X chromosomes. Twenty-nine species have been reported to have male $2n=26+X_1X_2O$ (Mittal, 1965; Das and Das, 1974), one species to have male $2n=24+X_1X_2O$ (Diaz and Saez, 1965), three species to have male $2n=20+X_1X_2O$ (Pinter and Walters, 1971), and four species to have male $2n=22+XO$ (Hackman, 1948; Bole-Gowda, 1959). Only acrocentrics have been reported from salticids. Typically, at first metaphase in meiosis of an X_1X_2O male, the X_1 and X_2 move precociously to one pole together,

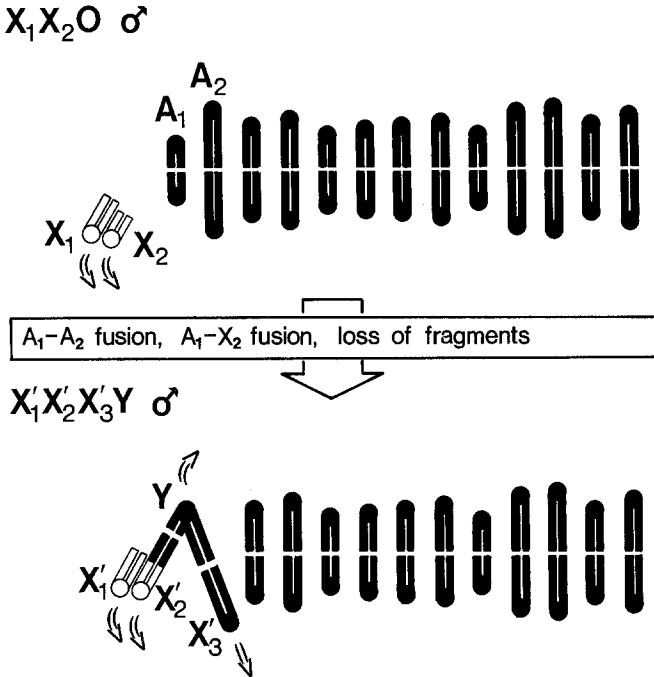


Fig. 1. Diagram of meiotic first metaphase in X_1X_2O males (above, with X_1X_2 plus 13 autosomal bivalents) and in $X_1'X_2'X_3'Y$ males (below, with $X_1'X_2'X_3'Y$ plus 11 autosomal bivalents). *Small arrows*: first anaphase movement of sex chromosomes. *Large arrow*: hypothesized evolutionary pathway from X_1X_2O to $X_1'X_2'X_3'Y$

so that half of the secondary spermatocytes contain the two X's and half contain none (Painter, 1914; Suzuki, 1954; Mittal, 1965). In species with X_1X_2O males, the females investigated show two chromosomes more than the males (Hackman, 1948; Suzuki, 1954; Sokolov, 1962, cited by Mittal, 1965) and hence are $X_1X_1X_2X_2$, producing all gametes with two X's. This X_1X_2O male, $X_1X_1X_2X_2$ female sex chromosome system is present also in some species of *Pellenes*. Evidence will be presented that the male $2n=22+X_1'X_2'X_3'Y$ karyotype found in other members of *Pellenes* was derived from a male $2n=26+X_1X_2O$ karyotype by an X-autosome and an autosome-autosome fusion (Fig. 1).

Materials and Methods

Examined were a total of 149 males and 9 females. These represent one species of the subgenus *Pellenes* (s. str.), eleven species of the subgenus *Habronattus* F.O. Pickard-Cambridge, and one species of the subgenus *Evarcha* Simon (see Table 1 for species examined and collecting localities). The classification of *Pellenes* into subgenera follows Lowrie and Gertsch (1955).

Most specimens used were adult males. Most of the subadult males and all of the subadult females used were allowed to drink 0.01 to 0.05% colchicine 2-4 h before fixation to accumulate meiotic and mitotic metaphases. All Figures except 16 and 17 are from specimens not treated with colchicine. All specimens were fixed by opening the abdomen in 3 parts absolute ethanol: 1 part glacial acetic acid. For most specimens the whole abdomen was Feulgen stained (7-11 min hydrolysis in 1 N HCl at 60°C) and the desired parts squashed in 50% or 75% acetic acid.

In males the testis was squashed; in females various abdominal tissues were squashed. The testes of some males of *P. cf. agilis* were fixed, squashed, then Giemsa stained. Slides were made permanent by the dry ice Euparal method. Remains of specimens used are deposited in my personal collection.

Data on segregation in the two kinds of males (X_1X_2O and $X'_1X'_2X'_3Y$) were obtained by scoring chromosome number and form in second division nuclei. Number and morphology of chromosomes in female mitotic metaphase nuclei, most from the digestive glands, were also scored. Quantitative data were obtained by scoring all confidently scorable nuclei seen in a systematic scanning of all or part of the slide.

Results

Table 1 lists the chromosome complements observed in male meiosis and female mitosis. Two sorts of males were seen. One sort showed in the first meiotic division the autosomal bivalents and two acrocentric univalents (the X_1 and X_2) (Figs. 6 and 8). These males are designated X_1X_2O males. Of the 71 X_1X_2O males examined, at least 67 had 13 acrocentric autosomal bivalents (see footnote a, Table 1). Hence $2n=26+X_1X_2O$ in most X_1X_2O males. That the two univalents are truly X chromosomes is indicated by observations presented below on segregation and female chromosome number. The other sort of male showed at first metaphase the autosomes, and acrocentric univalent ($X_{\equiv 1}$), and a trivalent consisting of an acrocentric (X'_2) paired with the short arm of a submetacentric or metacentric (Y) whose long arm is paired with another acrocentric (X'_3) (Figs. 9 and 15). These males are designated $X'_1X'_2X'_3Y$ males. (The superscript "" is added so that " X'_1 ", " X'_2 " can refer unambiguously to components of the XXX_Y system; it is not added to imply that the X'_1 is a derived form of the X_1 .) Of the 78 $X'_1X'_2X'_3Y$ males examined, at least 71 had 11 acrocentric autosomal bivalents (see footnote b to Table 1). Hence $2n=22+X'_1X'_2X'_3Y$ in most $X'_1X'_2X'_3Y$ males. That the three acrocentrics are X chromosomes and the submetacentric is a Y is indicated by observations presented below on segregation and female chromosome number.

Note in Table 1 that in 8 species, only X_1X_2O males were found and in 4 species, only $X'_1X'_2X'_3Y$ males were found. (Some species were represented by only few specimens, however.) In *P. hoyi*, 6 X_1X_2O males and 8 $X'_1X'_2X'_3Y$ males were found. *Pellenes (Evarcha) arcuata* and *P. (E.) falcata*, not studied here, have been reported to have male $2n=26+X_1X_2O$ (Sokolov, 1962, cited by Mittal, 1965; Hackman, 1948).

Observations of X_1X_2O Males

All chromosomes are acrocentrics of similar size, the longest being not more than about twice as long as the shortest (Figs. 4, 6, 8, and 10). The longer of the two X chromosomes is designated X_1 .

Segregation. The following observations confirm that half of the spermatids receive the X_1 and X_2 and half do not. At first metaphase the X_1 and X_2 typically lie off the plate, both toward the same pole (Fig. 8). The few first anaphase nuclei seen showed a 13:15 segregation (Fig. 10). One male of *P. viridipes* from Baysville was scored for chromosome number in second division

Table 1. Number of specimens examined from the given North American collecting localities and with the given chromosome constitutions (from meiosis in males and mitosis in females)

Species and collecting locality	Number of males		Number of females ^d		
	X ₁ X ₂ O ^a	X ₁ X ₂ X ₃ Y ^b	2n=30	2n=28	
Subgenus <i>Pellenes</i>					
<i>P. peninsularis</i> Emerton					
ONT: Dwight	79.0° W 45.3° N	2 ^c	0	—	—
ONT: Baysville	79.1° W 45.1° N	2	0	—	—
Subgenus <i>Habronattus</i>					
<i>P. agilis</i> Banks					
MASS: Castle Neck	70.8° W 42.7° N	7	0	—	—
<i>P. cf. agilis</i> Banks					
ONT: Long Point	80.1° W 42.5° N	27	0	3?	0
ONT: Rondeau Park	81.9° W 42.3° N	2	0	—	—
<i>P. americanus</i> Keyserling					
BC: Williams Lake	122.3° W 52.0° N	1	0	—	—
<i>P. hirsutus</i> G. et E. Peckham					
BC: Hedley	120.2° W 49.4° N	1	0	—	—
<i>P. oregonensis</i> G. et E. Peckham					
BC: Britannia Beach	123.2° W 49.6° N	2	0	—	—
CALIF: Point Reyes	123° W 38° N	2	0	—	—
<i>P. tarsalis</i> Banks					
CALIF: Point Reyes	123° W 38° N	3	0	—	—
<i>P. viridipes</i> Hentz					
ONT: Dwight	79.0° W 45.3° N	2	0	1	0
ONT: Baysville	79.1° W 45.1° N	6	0	—	—
ONT: Barrie	79.6° W 44.5° N	1	0	—	—
ONT: Long Point	80.1° W 42.5° N	5	0	—	—
ONT: Rondeau Park	81.9° W 42.5° N	2	0	—	—
<i>P. borealis</i> Banks					
ONT: Hamilton	79.8° W 43.3° N	0	32	0	2
ONT: Long Point	80.1° W 42.5° N	0	5 ^c	—	—
<i>P. cf. brumeus</i> G. et E. Peckham					
ALTA: Furman	114.0° W 50.0° N	0	2	—	—
ALTA: Gull Lake	114.0° W 52.5° N	0	1	—	—
<i>P. cf. calcaratus</i> Banks					
ONT: Baysville	79.1° W 45.1° N	0	10	0	2
MASS: Castle Neck	70.8° W 42.7° N	0	3	—	—
<i>P. decorus</i> Blackwall					
ONT: Port Cunnington	79.0° W 45.3° N	0	4	—	—
ONT: Dwight	79.0° W 45.7° N	0	1	—	—
ONT: Toronto	79.4° W 43.7° N	0	1	—	—
ONT: Toronto	79.3° W 43.7° N	0	2	0	1
ONT: Long Point	80.1° W 42.5° N	0	5	—	—
BC: Chasm	121.5° W 51.3° N	0	2	—	—
MASS: Ipswich	70.8° W 42.7° N	0	2	—	—

Table 1 (continued)

Species and collecting locality	Number of males		Number of females ^d	
	X ₁ X ₂ O ^a	X' ₁ X' ₂ X' ₃ Y ^b	2n=30	2n=28
Subgenus <i>Evarcha</i>				
<i>P. hoyi</i> G. et E. Peckham				
ONT: Port Cunnington	79.0° W 45.3° N	1	0	—
ONT: Bracebridge	79.3° W 45.1° N	1	0	—
ONT: Gravenhurst	79.5° W 44.9° N	1	0	—
ALTA: Fox Creek	117.0° W 54.6° N	1	0	—
ALTA: Gull Lake	114.0° W 52.5° N	2 ^c	0	—
ONT: Long Point	80.1° W 42.5° N	0	2	—
ALTA: Furman	114.1° W 50.0° N	0	1	—
BC: Williams Lake	122.3° W 52.0° N	0	1	—
BC: Hedley	120.2° W 49.4° N	0	1	—
BC: Christina	118.2° W 49.0° N	0	1	—
MASS: Mt. Toby	72.5° W 42.5° N	0	1	—
MINN: Arden Hills	93° W 45° N	0	1 ^c	—

^a All these males observed to have 13 autosomal bivalents except as noted under footnote c and except one male of *P. viridipes* from Dwight with 14, and one male of *P. oregonensis* from Britannia Beach with 14

^b All these males observed to have 11 autosomal bivalents except as noted under footnote c and except one male of *P. hoyi* from Mt. Toby with 9 bivalents plus one acrocentric-metacentric-acrocentric autosomal trivalent

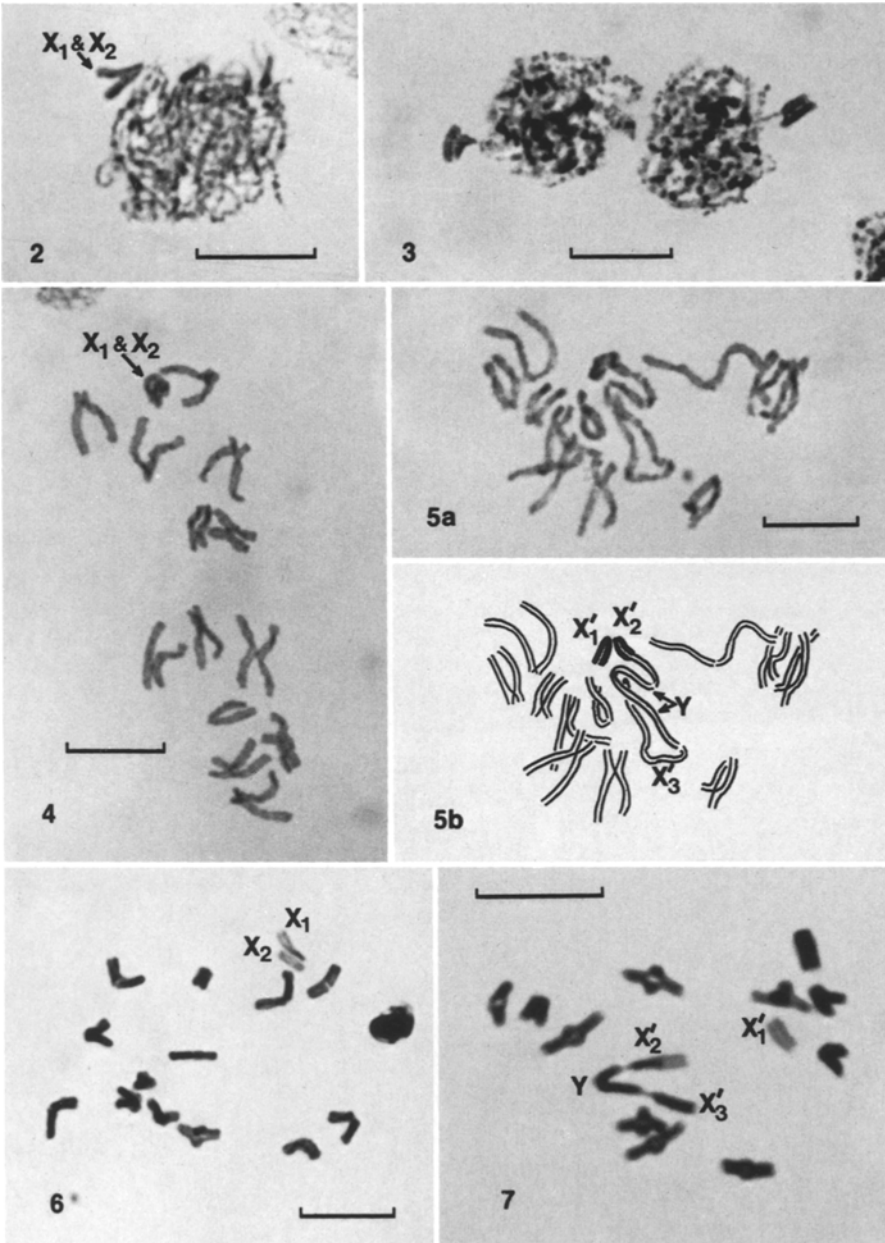
^c One male from each of these localities had an undetermined number of autosomal bivalents. Also, four males of *P. borealis* from Hamilton had an undetermined number

^d See "Observations of subadult females" for qualifications of these results

nuclei. Of thirteen second prophase and metaphase nuclei scored, ten had 13 acrocentrics and three had 15 acrocentrics. Of twenty second anaphase nuclei scored, eleven had 13 acrocentrics moving to each pole and nine had 15.

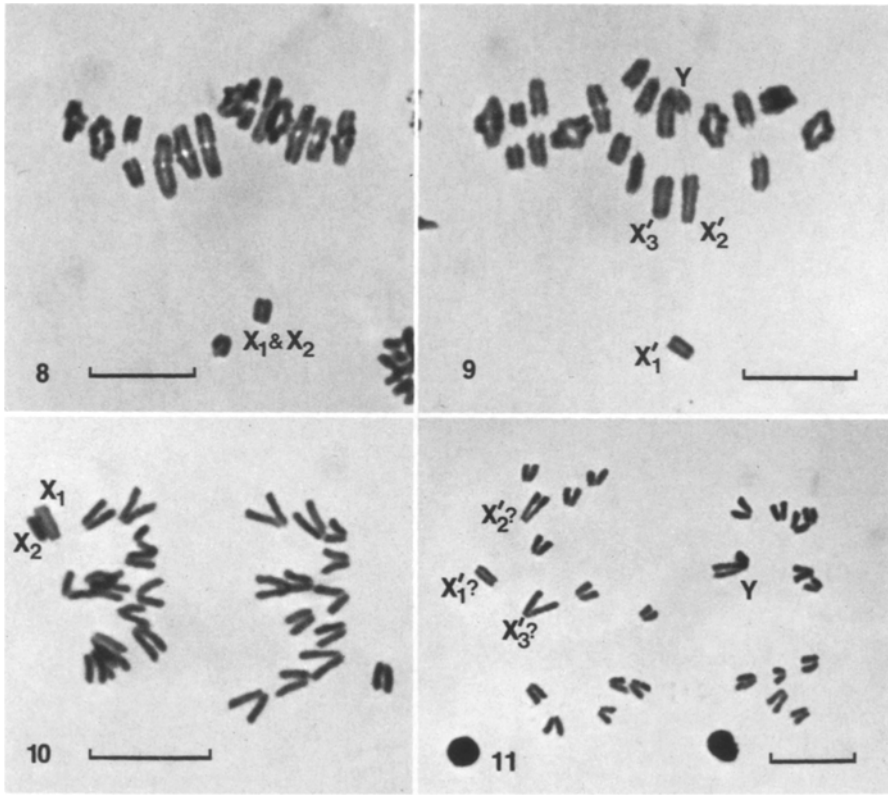
Chiasmata. At diplotene through first metaphase the X₁ and X₂ lie side by side with no visible chiasmata (Fig. 6), or they are separated (Fig. 8). There is usually a single chiasma in autosomal bivalents; occasionally there are two. At metaphase the chiasma localization varies with the species: *P. hoyi* and *P. viridipes* have some degree of terminal chiasma localization (Fig. 8), while in *P. agilis*, *P. cf. agilis* and *P. peninsularis* most chiasmata are proximal.

Heteropycnosis. A condensed body is visible among the pachytene threads. In some nuclei this body is seen to be made of two distinct elements (Fig. 2). These elements are interpreted to be the X₁ and X₂, following Painter (1914), Mittal (1965), and others. At diplotene, positive heteropycnosis of the X₁ and X₂ is sometimes evident (Fig. 4). Negative heteropycnosis of the X₁ and X₂ is



Figs. 2-7.¹ Meiotic prophase in X_1X_2O males (Figs. 2, 4, 6) and in $X'_1X'_2X'_3Y$ males (Figs. 3, 5, 7). Fig. 2. Pachytene nucleus, *Pellenes viridipes* (from Dwight). Fig. 3. Two pachytene nuclei, *P. borealis* (Hamilton). Fig. 4. Diplotene, *P. viridipes* (Baysville). Fig. 5a and b. a Diplotene, *P. decorus* (Chasm). b Chromatid diagram for a. Fig. 6. Diakinesis, *P. oregonensis* (Britannia Beach). Fig. 7. Diakinesis, *P. cf. brunneus* (Furman).

¹ Scale bars in Figs. 2-17 represent 10 μ m



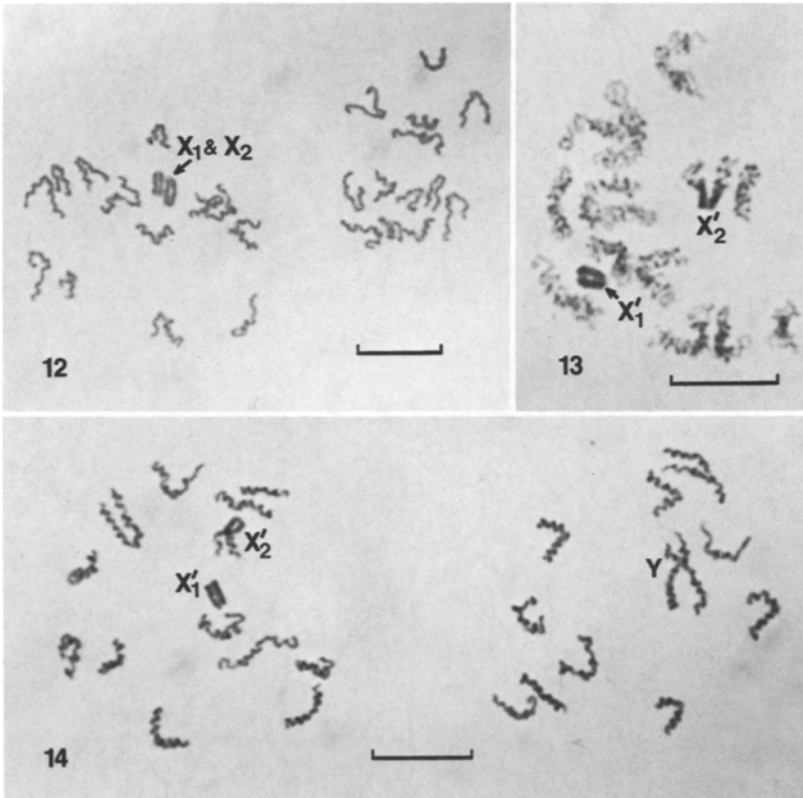
Figs. 8-11. First metaphase and anaphase in X_1X_2O males (Figs. 8 and 10) and in $X'_1X'_2X'_3Y$ males (Figs. 9 and 11). **Fig. 8.** Metaphase, *P. hoyi* (Fox Creek). **Fig. 9.** Metaphase, *P. decorus* (Chasm). **Fig. 10.** Anaphase, *P. viridipes* (Baysville). **Fig. 11.** Anaphase, *P. decorus* (Toronto)

occasionally evident at diakinesis, first metaphase and anaphase (Fig. 6). In second prophase nuclei with 15 chromosomes, heteropycnosis of two of the chromosomes is sometimes evident, while in nuclei with 13 chromosomes no heteropycnosis is seen (Fig. 12). The two heteropycnotic chromosomes are interpreted to be the X_1 and X_2 . At second metaphase and anaphase the X chromosomes were not distinguished from autosomes.

Mitosis. Twenty-eight acrocentric chromosomes were observed in a mitotic metaphase in a male *P. hirsutus*.

Observations of $X'_1X'_2X'_3Y$ Males

The autosomes are acrocentrics of similar size, the longest not more than about twice as long as the shortest (Figs. 5, 7, 9 and 11). The X'_1 , X'_2 , and X'_3 are all acrocentrics, the X'_2 being the longest and the X'_1 the shortest of the three. The Y is a submetacentric with an arm ratio of about 2:1 in members of *Habronattus* and a metacentric with subequal arms in members of *Evarcha*



Figs. 12–14. Second prophase in X_1X_2O male (Fig. 12) and in $X_1X_2X_3Y$ males (Figs. 13 and 14). Fig. 12. Two nuclei (one with X_1X_2 , one without), *P. viridipes* (Baysville). Fig. 13. One nucleus with $X_1X_2X_3$, *P. decorus* (Toronto). Fig. 14. Two nuclei (one with $X_1X_2X_3$, one with Y), *P. decorus* (Chasm)

(compare Figs. 9 and 15b). The morphology of the sex chromosomes is more or less constant in *Habronattus*, except that the Y of *P. decorus* appears to have a slightly more extreme arm ratio than does that of the other species, and *P. cf. calcaratus* appears to have a relatively longer X'_1 and X'_2 .

Segregation. The following observations confirm that half of the secondary spermatocytes receive the Y and half the X'_1 , X'_2 and X'_3 (Fig. 1). At first metaphase the X'_1 usually lies toward one pole [either separate from the X'_2 (Fig. 9) or less commonly lying beside the X'_2 (Fig. 15b)], and the X'_2 , Y and X'_3 form a V-shaped trivalent with the centromere of the Y pointing to the other pole (Fig. 9; this arrangement was seen in 70 of 93 nuclei scored in six males of *P. borealis*). Fairly frequently, however, the X'_2 , Y, and X'_3 are arranged linearly in the axis of the spindle (as in Fig. 15a), while the X'_1 lies either beside the X'_2 (in 10 of 93 nuclei) or near the pole to which the X'_2 is pointing (in 12 of 93 nuclei). If this linear orientation is actually a malorientation (as opposed to a squashing artifact), then judging by the following observa-

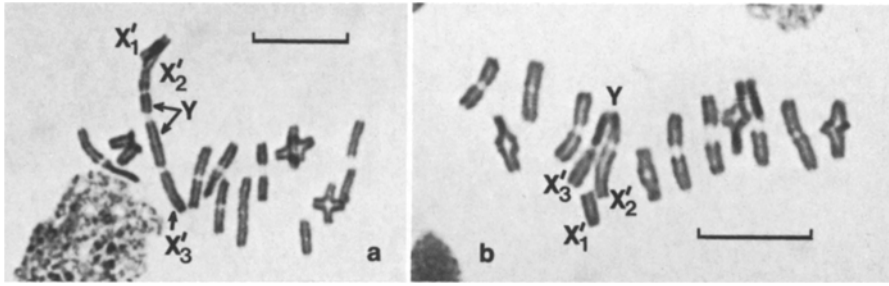


Fig. 15a and b. First metaphase in $X_1X_2X_3Y$ males. a *P. decorus* (Chasm). b *P. hoyi* (Furman)

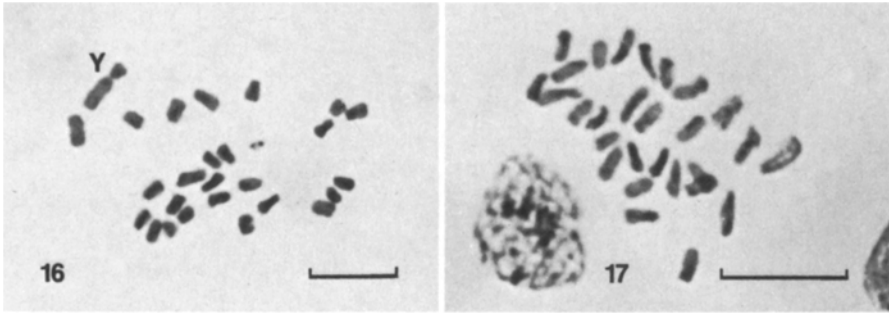
Table 2. Segregation in $X_1X_2X_3Y$ males: chromosome counts in second prophase and metaphase of meiosis in 7 males of *P. borealis*

Number of acrocentrics in nucleus	Number of nuclei	
	with submetacentric	without submetacentric
10	2	0
11	80	0
12	0	1
13	0	4
13.5	0	3
14	0	78
15	0	2

tions many such nuclei are eventually corrected or their division products eliminated. The several first anaphase nuclei seen showed a segregation of 14 acrocentrics to one side and 11 acrocentrics plus a submetacentric to the other (Fig. 11). In seven males of *P. borealis* from Hamilton, of 170 second prophase and metaphase nuclei scored, 78 had 14 acrocentrics, 80 had 11 acrocentrics plus a submetacentric, and 12 had other chromosome counts (Table 2).

Chiasmata. At diplotene through first metaphase the short arm of the Y has a single chiasma with the X_2 , which is almost always terminal (Figs. 5, 7, 9, and 15), but which is rarely interstitial or proximal (at the Y's centromere). The long arm of the Y has a single chiasma with the X_3 which at diplotene is usually terminal (Fig. 5), occasionally interstitial and rarely proximal. At metaphase the chiasma is almost always terminal (Figs. 9 and 15). The X_1 often rests beside the proximal portion of the X_2 , without chiasmata, in diplotene, diakinesis and sometimes first metaphase (Figs. 5 and 15). In the autosomes, most chiasmata are terminal or subterminal at metaphase (Figs. 9 and 15).

Heteropycnosis. A condensed body is visible among the pachytene threads. In some nuclei this body is seen to be made of two distinct elements (Fig. 3). Because of the terminal chiasmata, the sex chromosomes at diplotene usually look like a rope (Fig. 5a). At one end of the "rope" is a sometimes-positively-



Figs. 16 and 17. Mitosis in species with $X_1X_2X_3Y$ males. **Fig. 16.** Colchicined subadult male *P. decorus* (Toronto), nucleus probably from testis. **Fig. 17.** Colchicined subadult female *P. borealis* (Hamilton), nucleus probably from digestive glands

heteropycnotic “knot”, which represents the X_1 paired with X_2 . In diakinesis and first metaphase the X_1 and proximal portion of the X_2 occasionally appear negatively heteropycnotic (Fig. 7). In second prophase nuclei with 14 acrocentrics, heteropycnosis of one whole chromosome and the proximal portion of another is sometimes evident (Figs. 13 and 14), while in nuclei with 11 acrocentrics and a submetacentric, no heteropycnosis is seen (Fig. 14). Because the two heteropycnotic chromosomes are not visible in nuclei with the Y chromosome, these are interpreted to be two of the three X chromosomes.

Mitosis. Mitoses with 25 acrocentrics and one submetacentric were seen in males of *P. decorus* (Fig. 16).

Observations of Subadult Females

In each female, the chromosome number varied from nucleus to nucleus, most probably because of scattering of chromosomes during squashing.

Females of X_1X_20 Males. For one female of *P. viridipes* 11 nuclei were scored; 7 had 30 acrocentrics and 4 had fewer than 30. For three females of *P. cf. agilis* the preparations were poor, but the one or two countable nuclei in each specimen showed at least 30 chromosome arms. A count of 30 acrocentrics is consistent with the females having $2n = 26 + X_1X_1X_2X_2$. Sokolov (1962, cited by Mittal, 1965) reports 30 chromosomes from females of *Pellenes (Evarcha) falcata*.

Females of $X_1X_2X_3Y$ Males. The highest and most commonly scored mitotic chromosome count was 28 acrocentrics in one female of each of *P. cf. calcaratus*, *P. decorus* and *P. borealis* (Table 3 and Fig. 17). For one female of *P. cf. calcaratus* and two females of *P. borealis* the most commonly scored count was 28 chromosome arms, this also being the highest count (except for one nucleus with 30 arms in a *P. borealis* female; see Table 3). For all of the above females the somatic complement is considered to be 28 acrocentric chromosomes (Table 1). A count of 28 acrocentrics is consistent with the females having $2n = 22 + X_1X_1X_2X_2X_3X_3$.

Table 3. Mitotic chromosome counts in females of $\text{X}'_1\text{X}'_2\text{X}'_3\text{Y}$ males

Specimen	Number of nuclei scored	Number of nuclei with given number of acrocentrics		
		<28	28	>28
<i>P. cf. calcaratus</i>				
#1	46	27	19	0
#2	10	5	5 ^a	0
<i>P. borealis</i>				
#1	21	9	12	0
#2	17	8	8 ^a	1
#3	15	11	4 ^a	0
<i>P. decorus</i>				
#1	17	11	6	0

^a Insufficient spreading permits me to say only that these nuclei had 28 chromosome arms; it is possible that they contained some metacentrics

Discussion

My data indicate an $\text{X}_1\text{X}_2\text{O}$ male, $\text{X}_1\text{X}_1\text{X}_2\text{X}_2$ female sex chromosome system and usually 26 autosomes in *P. peninsularis*, *P. agilis*, *P. cf. agilis*, *P. americanus*, *P. hirsutus*, *P. oregonensis*, *P. tarsalis*, *P. viridipes*, and some populations of *P. hoyi* (possibly the more northern ones). In all of these, two acrocentric chromosomes are oriented to one pole in first metaphase of male meiosis; chromosome counts in second division nuclei in *P. viridipes* confirm that these two sex chromosomes segregate together. That females are $\text{X}_1\text{X}_1\text{X}_2\text{X}_2$ is supported by a count of 30 acrocentrics in a female of *P. viridipes*. Figure 1 depicts the chromosome complement and sex chromosome segregation in an $\text{X}_1\text{X}_2\text{O}$ male.

My data indicate an $\text{X}'_1\text{X}'_2\text{X}'_3\text{Y}$ male, $\text{X}'_1\text{X}'_1\text{X}'_2\text{X}'_2\text{X}'_3\text{X}'_3$ female sex chromosome system and usually 22 autosomes in *P. borealis*, *P. decorus*, *P. cf. calcaratus*, *P. cf. brunneus* and some populations of *P. hoyi*. In all of these, 3 acrocentric chromosomes are oriented toward one pole and one submetacentric toward the other pole in first metaphase of male meiosis; chromosome counts in second division nuclei confirm that the three acrocentrics segregate together and apart from the submetacentric. That females are $\text{X}'_1\text{X}'_1\text{X}'_2\text{X}'_2\text{X}'_3\text{X}'_3$ is supported by counts of 28 acrocentrics in females of *P. cf. calcaratus*, *P. decorus* and *P. borealis*. Figure 1 depicts the chromosome complement and sex chromosome segregation in an $\text{X}'_1\text{X}'_2\text{X}'_3\text{Y}$ male.

The chromosomal material homologous to the X_1 and X_2 of $\text{X}_1\text{X}_2\text{O}$ males is apparently reasonably intact in $\text{X}'_1\text{X}'_2\text{X}'_3\text{Y}$ males, as suggested by the presence of two heteropycnotic elements in the pachytene of $\text{X}'_1\text{X}'_2\text{X}'_3\text{Y}$ males (Fig. 3). In $\text{X}'_1\text{X}'_2\text{X}'_3\text{Y}$ males the early movement of the X'_1 to the pole (Fig. 9), the achiasmate pairing of the X'_1 with the proximal portion of the X'_2 (Fig. 15), and the positive (Fig. 5a) then negative (Fig. 7) heteropycnosis of the X'_1 and the proximal portion of the X'_2 all suggest that the X'_1 and the proximal portion of the X'_2 are homologous to the X_1 and the X_2 of $\text{X}_1\text{X}_2\text{O}$ males (indicated

on Figure 1 by leaving these portions white). This is further supported by observations of second prophase in $X_1'X_2'X_3'Y$ males (Fig. 13) in which one of the three X chromosomes (presumably the X_1') is entirely heteropycnotic (thus behaving like an X of an X_1X_2O male), another of the three (presumably the X_2') is heteropycnotic proximally (thus the proximal portion behaves like an X of an X_1X_2O male). The X_3' , the Y, and the distal portion of the X_2' behave like autosomes in their chiasmate pairing and lack of heteropycnosis.

That the $2n=26+X_1X_2O$ male karyotype was ancestral for the genus *Pellenes* appears most likely from the fact that this male karyotype is the only one known both from *Pellenes* and from other spiders (out-group comparison, Watrous and Wheeler, 1981). The X_1' , X_2' , X_3' and Y may have been derived from the X_1 , X_2 and two autosome pairs as follows (see Fig. 1): one sex chromosome has remained untouched (X_1'), while the other sex chromosome (the proximal portion of the X_2') has fused with an autosome (the distal portion of the X_2') whose homologue (the short arm of the Y) has fused with another autosome (the long arm of the Y) whose own homologue (the X_3') has remained untouched. This accounts for the reduction in autosome pairs from 13 to 11, and (in *Habronattus* at least) for the approximately equal lengths of the X_3' and the long arm of the Y, and the approximately equal lengths of the short arm of the Y and the distal non-heteropycnotic portion of the X_2' . Because the long arm of the Y is shorter than the X_3' in $X_1'X_2'X_3'Y$ males of *P. hoyi*, it is not as easy to argue for a direct origin of *P. hoyi*'s $X_1'X_2'X_3'Y$ system. Perhaps the metacentric Y of *P. hoyi* was derived from a submetacentric Y by a pericentric inversion or other rearrangement.

The autosome-autosome fusion (yielding the Y) was presumably centric. The X-autosome fusion (yielding the X_2') may have been tandem, or centric followed by a pericentric inversion or a centromere shift. White (1973, pp. 670, 225) apparently holds centric fusion – pericentric inversion explanations to be more plausible than tandem fusion explanations. If the fusion were tandem, then heterozygotes for the fusion (which would be females) would produce 50% aneuploid ootids whenever single cross-overs occurred in the ancestral X region (see White, 1973, p. 225). Hackman (1948) and Patau (1948) found that X_1-X_1 and X_2-X_2 bivalents are chiasmate in female spiders of several families, and single chiasmata are apparently usual. However, if the fusion were centric, then heterozygotes for the pericentric inversion or the centromere shift following the centric fusion would also produce aneuploid ootids. Thus the X_2' chromosome appears at least as difficult to establish by a centric fusion – pericentric inversion as by a tandem fusion. However, because a tandem fusion does not require a subsequent rearrangement, it is more parsimonious to assume that the X_2' arose by tandem fusion.

The stability of the X_1X_2O system in spiders against X-autosome fusions may be due in part to strong proximal chiasma localization in the male leading to improper disjunction in interchange heterozygotes (White, 1973). In at least some X_1X_2O species of *Pellenes*, however, there is some terminal localization in the autosomes. The strong terminal localization of the $X_2'-Y$ and $X_3'-Y$ chiasmata probably encourages proper disjunction of the sex chromosomes in extant $X_1'X_2'X_3'Y$ males, and may have aided the establishment of the $X_1'X_2'X_3'Y$ system.

Terminal localization in the sex chromosomes may also avoid infertility in $X_1X_2X_3Y$ *P. hoyi* males whose Y may have had a pericentric inversion or other rearrangement.

It is puzzling that both the X_1X_2O and $X_1X_2X_3Y$ systems occur in both subgenera *Habronattus* and *Evarcha*. *Pellenes* (*Habronattus*) *viridipes* and *P. (H.) cf. calcaratus* have many morphological similarities, yet *P. viridipes* is X_1X_2O and *P. cf. calcaratus* is $X_1X_2X_3Y$. In *P. (Evarcha) hoyi*, which is morphologically quite distinct from *P. viridipes* and *P. cf. calcaratus*, I have been unable to find consistent morphological differences between X_1X_2O and $X_1X_2X_3Y$ males. Thus, the phylogenetic relationships suggested by the morphology are different from those suggested by the chromosomes. We could suppose either that:

(a) the $X_1X_2X_3Y$ system has arisen more than once independently, or

(b) the $X_1X_2X_3Y$ system has reverted to the ancestral X_1X_2O at least once,

or

(c) the X_1X_2O and $X_1X_2X_3Y$ systems have been maintained together in the same lineage (as a polymorphism) for long periods of time (eg., from the most recent common ancestor of *P. cf. calcaratus*, *P. viridipes* and *P. hoyi* until the most recent common ancestor of *P. cf. calcaratus* and *P. viridipes*, and until the most recent common ancestor of northern and southern populations of *P. hoyi*), with eventual fixation for X_1X_2O in *P. viridipes* and for $X_1X_2X_3Y$ in *P. cf. calcaratus*, or

(d) reticulate evolution has reshuffled the distribution of the X_1X_2O and $X_1X_2X_3Y$ systems (eg., occasional hybridization between *P. cf. calcaratus* and *P. hoyi* has introduced the $X_1X_2X_3Y$ system into southern populations of *P. hoyi*), or

(e) the $X_1X_2X_3Y$ system arose only once, has not reverted to X_1X_2O , has not coexisted with X_1X_2O in the same lineage for long periods of time, and reticulate evolution has not lead to introgression of sex chromosome systems between long separated lineages.

Assumption (e) states that the sex chromosome systems are reliable indicators of phylogenetic relationships. Accepting (e) would require that *P. cf. calcaratus* and $X_1X_2X_3Y$ males of *P. hoyi* share an ($X_1X_2X_3Y$) ancestor not shared by either *P. viridipes* or X_1X_2O males of *P. hoyi*. This in turn requires that the similarities between the two *Habronattus* species or between the two sorts of *P. hoyi* are convergent, which I consider untenable. Assumption (a) appears unlikely as it would seem too fortuitous to have the only derivations of a Y chromosome known from spiders to occur in the same genus and with end products so similar in morphology and meiotic behaviour, although the sex chromosome systems of *Habronattus* and *Evarcha* are not identical, and factors such as lack of proximal chiasma localization may predispose this genus to such changes. Studies of C-bands or pachytene bands would provide evidence for assumption (a), if they could show that different autosomes were involved in the fusions in different species. Assumption (b) seems unlikely in that the two autosomal pairs involved in the fusion and one of the Xs would have to be reconstituted to look just as they do in primitively X_1X_2O species, and also a centromere would have to be provided for the autosome fused to the

X. Assumption (c) at first seems reasonable because both X_1X_2O and $X'_1X'_2X'_3Y$ males have been found in *P. hoyi*. However, the polymorphism would have to have been maintained for probably very many generations, despite possible heterozygote inferiority. That is, hybridization between males of one sort and females of the other would produce females heterozygous for the X-autosome fusion. These would behave like tandem fusion heterozygotes (see above) and would produce aneuploid gametes unless there is meiotic drive or suppression of chiasmata. [Indeed, northern and southern populations of *P. hoyi* may be distinct (reproductively isolated) sibling species.] Assumption (d) seems improbable, since introgression between morphologically very different species is required. Thus although it would seem that at least one of the assumptions (a) – (e) must be true, each appears unlikely for some reason.

While my data not supply an answer to this dilemma, they do indicate that one must be cautious in using chromosome data to reconstruct phylogeny (see also Farris, 1978 and Atchley, 1972). A cladogram constructed for members of *Pellenes* solely from the present data on chromosome morphology [using assumption (e)] would strongly conflict with what appear to me incontrovertible conclusions from morphological evidence. Because of the morphological differences between *P. hoyi* on the one hand and *P. viridipes* and *P. cf. calcaratus* on the other hand, I favour assumption (a), (b) or (c). However, a satisfactory understanding of sex chromosome evolution within the genus will have to wait until an independently well-corroborated hypothesis of phylogenetic relationships within the genus is available. With such a phylogenetic hypothesis, the possible pathways of sex chromosome evolution can be seen and assessed. The necessity of a prior phylogenetic hypothesis is not unique to studies of this genus – our estimates of the number of independent derivations of multiple sex chromosome systems in bats and mantids (White, 1973, pp. 640, 644), for example, would benefit from detailed cladistic analyses of these groups.

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