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Cytological Evidence of Pseudo-arrhenotoky in Two Phytoseiid Mites, *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius womersleyi* Schicha

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ABSTRACT

Pseudo-arrhenotoky was investigated by chromosome observation in *Phytoseiulus persimilis* and *Amblyseius womersleyi*, both of which have the basic number of chromosomes. To confirm male diploidy at the early stage of embryogenesis, eggs were supplied for chromosome observation immediately after they were deposited. In addition, eggs were collected under two prey conditions, "ample" and "poor" and their chromosomes were observed in order to examine the relationship between ploidy and sex. Eggs deposited by the two species showed haploid-diploid sequences corresponding to those of male and female offspring under both "ample" and "poor" prey conditions. However, male diploidy and heterochromatinized chromosomes were not observed in the deposited eggs. Therefore, the first eggs expected to be males were extracted from the body cavities of female *P. persimilis* for chromosome observation. The observation confirmed male diploidy at an earlier stage of embryogenesis which takes place in the female body cavity. The coexistence of haploid and diploid cells in the same egg was also observed at the next stage. However, no heterochromatinized chromosomes were observed at any embryonic stages in the female body cavity, under the present experimental conditions. The present study suggests that pseudo-arrhenotoky occurs at least in *P. persimilis* with the common number of chromosomes, and that the timing of genome elimination is different from that of the species with an aberrant number of chromosomes.

Key words: phytoseiid mites, pseudo-arrhenotoky, reproduction, sex, chromosome

INTRODUCTION

The concept of pseudo-arrhenotoky was proposed as the mode of reproduction in phytoseiid mites (Schulten, 1985). According to the concept, male eggs need fertilization to begin their embryogenesis, and the paternal genome in the male eggs is eliminated through heterochromatinization to produce haploid adult males. Evidence to support the concept was derived not only from cytological but also from genetic and ecological investigations.

Karyotypes in many phytoseiid species have been investigated regardless of the potentiality of pseudo-arrhenotoky (Hansell et al., 1964; Wysoki and Swirski, 1968; Wysoki, 1973; Blommers-Schlosser and Blommers, 1975; Wysoki and McMurtry, 1977; Wysoki and Bolland, 1983). Almost all species investigated had a haploid number of 4 and a diploid number of 8 chromosomes, and 3 thelytokous species had 8 chromosomes (Wysoki, 1985). In addition, the ratio of haploid to diploid eggs appeared to be equal to that of males

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to females (Hansell et al., 1964). These karyotypes are commonly known as the genetic system "haplo-diploidy", and the reproductive mode in the haplo-diploid system is called "arrhenotoky" since haploid males in the system develop without copulation (White, 1973; Oliver, 1977; Bull, 1983). With most phytoseiid mites, however, females begin to produce eggs of both sexes only after copulation. Thus, this non-arrhenotokous reproduction in phytoseiid mites was initially called "para-haploidy" (Oliver, 1977).

Although karyotype analyses did not show male diploidy, other investigations contributed to the concept of pseudo-arrhenotoky. Amano and Chant (1978b) and Schulten et al. (1978) showed that adult females of phytoseiid mites increased egg production relative to the duration of copulation. The results suggested that the production of eggs required a signal of insemination. In addition, it was shown that the effect of x-ray irradiation on adult males was transmitted to the offspring of both sexes in *Amblyseius bibens, Phytoseiulus persimilis* (Helle et al., 1978) and *Metaseiulus occidentalis* (Hoy, 1979). They, therefore, suggested that sperms were required to begin the embryogenesis even in male eggs. Subsequently, a diploid number of chromosomes was observed in male eggs at an early embryonic stage in which eggs immediately after being deposited by *M. occidentalis* showed diploid chromosomes and a set of chromosomes was eliminated through heterochromatinization (Nelson-Rees et al., 1980). The concept of pseudo-arrhenotoky was proposed. Schulten (1985) applied this concept to all phytoseiid species, except for thelytokous species, based on the genetic evidence of three species in different genera.

However, there are several problems related to the cytological observations that constitute the central evidence for the concept. In spite of many karyotype analyses, the key evidence for pseudo-arrhenotoky was derived from the chromosome observation of only one species, *M. occidentalis* (Nelson-Rees et al., 1980). The observation did not target specifically male eggs and the number of eggs observed was not sufficient to confirm diploidy in the early embryonic stage of deposited eggs. Furthermore, *M. occidentalis* is not a common species in its chromosome number. Wysoki (1985) reported that 47 of 55 species examined had a basic number of n=4 and that *M. occidentalis* had n=3, which was aberrant and known only in 5 species (the other 3 species are thelytokous). Therefore, stronger cytological evidence in a species with the common number of chromosomes (n=4) is required in order to confirm the existence of pseudo-arrhenotoky in phytoseiid mites.

In the present study, two species, *P. persimilis* and *A. womersleyi*, karyotyped as having the common number of chromosomes were investigated. Male diploidy was expected at least in *P. persimilis* since pseudo-arrhenotoky in *P. persimilis* had been suggested genetically (Helle et al., 1978). In addition, Toyoshima and Amano (1998) reported that the females of *P. persimilis* and *A. womersleyi* increased the proportion of male offspring to female offspring under poor prey conditions. If both species are pseudo-arrhenotokous mites, two kinds of males, diploid phenotypic males and haploid males, are expected in the male offspring produced under the poor prey condition. In other words, some males under the poor prey condition may develop from diploid eggs by changing phenotype only. If this is so, pseudo-arrhenotokous females can also control sex ratios easily when they encounter a shortage of prey in the environment. Investigation of the chromosomes of eggs deposited under poor prey conditions will suggest the mechanism of sex determination in pseudo-

arrhenotokous mites.

MATERIALS AND METHODS

Phytoseiulus persimilis Athias-Henriot and *Amblyseius womersleyi* Schicha were used for chromosome observation. The population of *P. persimilis*, obtained from the laboratory of Hokkaido University in Japan, has been maintained in our laboratory for more than 10 years. The population of *A. womersleyi* was originally collected on our campus (Matsudo, Chiba-pref.) in September, 1995. Both populations were maintained in transparent plastic cups (12 cm in diameter and 6 cm in height). Pine resin was applied to the rims of the cups to prevent mites from escaping. The green-form of the two-spotted spider mite, *Tetranychus urticae* Koch, reared on kidney bean plants, *Phaseolus vulgaris* L., was used as prey. For experiments, predatory mites were reared separately on a bean leaflet (2×2 cm) with abundant prey (the "ample" prey condition). The leaflet was laid on wet cotton placed in a small transparent plastic cup (7 cm in diameter and 4 cm in height) to prevent mites from escaping and was kept at 25℃ with a relative humidity of 70-80% and a photoperiod of 16L:8D.

An unmated female and a male, 24-48 hours after the final ecdysis, were paired on the leaflet and were given sufficient time to copulate. The male was removed after copulation and the mated female was kept on the leaflet for oviposition. Once the female started ovipositing, the eggs were taken out of the leaflet within 30 minutes of being deposited, and used for chromosome observation. The eggs were placed on a clean slide glass, squashed and then stained with 1% aceto-orcein for 20 minutes. Stained specimens were examined using a phase contrast microscope.

Chromosomes of all eggs deposited by 10 females of *P. persimilis* under the "ample" prey condition were observed in sequential order. With *A. womersleyi*, chromosomes of the first 10 eggs of 10 females were observed in the same manner. In both species, the sequential order of ploidy was compared with that of sex previously reported (Toyoshima and Amano, 1998) to identify male diploidy.

To examine whether the males produced under poor prey conditions were diploid phenotypic or haploid males, gravid females were reared under the poor prey conditions. Five prey eggs per day for *P. persimilis* and 3 prey eggs per day for *A. womersleyi* were selected as the "poor" prey conditions. The conditions were settled by the use of acrylic plates, described in detail in a previous paper (Toyoshima and Amano, 1998). Chromosomes of the eggs deposited under the poor prey conditions were examined again in sequential order and the order of chromosomes was compared with that of sexes previously reported by Toyoshima and Amano (1998).

The eggs in the female body cavities were to be prepared for chromosome observation to examine the process of chromosome elimination. In order to achieve this, the timing for extracting the first eggs that developed into males (Amano and Chant, 1978a; Toyoshima and Amano, 1998) from the female body cavities of *P. persimilis* was first determined. Ten pairs of unmated females and males were coupled separately on leaflets with abundant prey and were observed under a microscope at intervals of 15 minutes until the females deposited

second eggs. At the same time, another 20 females, which had deposited first eggs, were mounted at 15, 30, 45 and 60 minutes, respectively, after oviposition to identify the timing for the shell of the second egg to form. Timing for dissecting the idiosoma and extracting eggs from the body cavities of female *P. persimilis* was then determined from these observations. After the eggs had been extracted from the female body cavities, the chromosomes of the eggs were observed in the manner described above.

RESULTS

Females of *P. persimilis* and *A. womersleyi* deposited two kinds of eggs, which had 4 or 8 chromosomes, with the same proportions of male and female offspring among their broods. Heterochromatinized chromosomes (usually stained dark and condensed), however, were not observed in these eggs. To compare ploidy with sex, the mean number of diploid eggs between adjacent haploid eggs was calculated in each species and under each prey condition. The mean sequences of ploidy compared with those of sex are shown in Table 1 for *P. persimilis* and in Table 2 for *A. womersleyi*. In both species, the sequence of ploidy also showed a close relationship with that of the sex of offspring under the ample and poor prey conditions. Male diploidy was not confirmed among the eggs selected 30 minutes after being deposited. Therefore, the first five eggs of *P. persimilis* selected within 15 minutes after being deposited were investigated to identify diploid male eggs and heterochromatinized chromosomes. The ratios of haploid eggs to diploid eggs in each group were compared with those of males to females (Table 3). It was reconfirmed that the first eggs tended to be male and haploid, and that no heterochromatinized chromosomes were observed.

Since male diploidy was not observed in the preceding experiments, the first eggs produced by *P. persimilis* at 14, 15, 16, 17 and 18 hours after the copulation started were

Table 1. Comparison of ploidy with sex in the eggs deposited, during the early oviposition period, by *P. persimilis* females under ample and poor prey conditions.

Prey condition for female	Type[2]	n[3]	Sequence[4]															
Ample	Ploidy	10	h	ddd	h	dddddd	h	dddddd	h	ddddddddd	h	dddd	h	ddddd	h			
				(3.3)[5]		(5.9)		(5.7)		(8.6)		(4.0)		(4.8)				
	Sex	20	♂	♀♀♀	♂	♀♀♀♀♀♀	♂	♀♀♀♀♀	♂	♀♀♀♀♀♀♀♀♀	♂	♀♀♀♀♀♀♀♀	♂					
				(3.1)		(6.3)		(4.9)		(9.6)		(8.0)						
Poor[1]	Ploidy	10	h	d	h	d	h	d	h	d	h	d	h	d	h			
				(1.3)		(1.0)		(1.1)		(1.0)		(1.3)		(1.0)				
	Sex	17	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂			
				(1.1)		(1.1)		(1.0)		(0.9)		(1.3)		(1.1)				

[1] The prey condition in which 5 prey eggs were supplied daily for ovipositing females.

[2] Genotype or phenotype.

[3] Replicates of sequences.

[4] The sequence of ploidy was shown by "h" for haploid and "d" for diploid. The sequence of sex was shown by "♂" for male and "♀" for female (after Toyoshima and Amano, 1998).

[5] The numbers shown in parentheses below "d" and "♀" indicate the mean numbers of diploid eggs between haploid ones and those of females between males, respectively.

Table 2. Comparison of ploidy with sex in the first ten eggs deposited by *A. womersleyi* females under ample and poor prey conditions.

Prey condition for female	Type [2]	n [3]	Sequence [4]									
Ample	Ploidy	10	h	dd (2.2)[5]	h	dddd (3.9)	h	d				
	Sex	20	♂	♀♀ (2.4)	♂	♀♀♀ (3.3)	♂	♀♀				
Poor [1]	Ploidy	10	h	d (1.3)	h	d (1.0)	h	d (1.1)	h	d (1.0)	h	d (1.3)
	Sex	17	♂	♀ (1.1)	♂	♀ (1.1)	♂	♀ (1.0)	♂	♀ (0.9)	♂	♀ (1.3)

[1] The prey condition in which 3 prey eggs were supplied daily for ovipositing females.
[2] Genotype or phenotype.
[3] Replicates of sequences.
[4] The sequence of ploidy was shown by "h" for haploid and "d" for diploid. The sequence of sex was shown by "♂" for male and "♀" for female (after Toyoshima and Amano (1998)).
[5] The numbers shown in parentheses below "d" and "♀" indicate the mean numbers of diploid eggs between haploid ones and those of females between males, respectively.

Table 3. Comparison of ploidy and sex of offspring in the first five eggs deposited by females of *P. persimilis* under the ample prey condition.

	Sequence of eggs deposited				
	First	Second	Third	Fourth	Fifth
Percentage of haploid eggs	86 (35)	0 (35)	10 (10)	20 (10)	10 (10)
Percentage of males	90 (101)	5 (100)	7 (30)	21 (29)	7 (15)

The number of eggs observed is shown in parentheses.

extracted from the body cavities (the timing of the extraction was determined by the observation using mated females; Fig. 1). The number of eggs successfully extracted from the cavities and the number of eggs, in which the haploid and diploid phases of chromosomes were observed, are shown in Table 4. Many eggs failed to give a clear picture of the chromosomes because of technical difficulty. Eggs extracted 14 hours after the start of copulation were all diploid and 10 out of 11 eggs after 16 hours were haploid. After 15 hours, 6 eggs were haploid, 3 diploid, and 4 had both haploid and diploid cells.

DISCUSSION

Male diploidy was observed in *P. persimilis* as evidence of pseudo-arrhenotoky, but the time taken to eliminate chromosomes was different from that of *M. occidentalis*, reported by Nelson-Rees et al. (1980). In the present study, the first-laid eggs, which had been proven to be male in our previous study (Toyoshima and Amano, 1998), were haploid, and next male eggs were also haploid in both *P. persimilis* and *A. womersleyi* (Tables 1-3). While male diploidy was not detected in the eggs after being deposited, we could confirm male diploidy at the early embryonic stages in *P. persimilis* by extracting male eggs from the female body cavities (Table 4). With *A. womersleyi*, we were unable to examine chromosomes of eggs

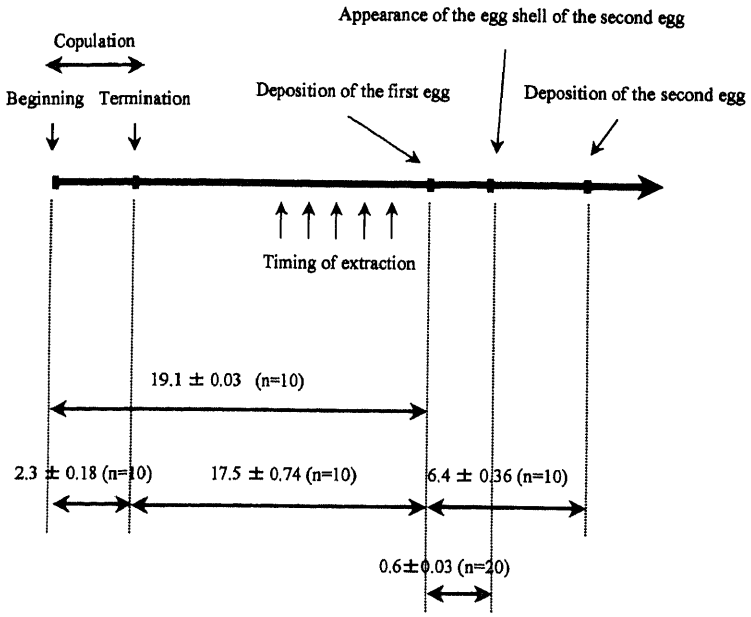

Fig. 1. The time schedule of reproductive behavior, from the start of copulation to the deposition of the second egg, in *P. persimilis*. Values are in hours (Mean ± SE). The time between the beginning of copulation and the deposition of the first egg was less variable than that between the termination of copulation and deposition. The eggshell of the second egg appeared 30 minutes after the first egg was deposited. The second eggs needed 6 hours for deposition. The reproductive schedule in *P. persimilis* indicates that the timing to extract the first eggs was better between 14 and 18 hours after the start of copulation.

Table 4. Number of the first eggs in *P. persimilis* with various chromosome phases.

	Time after beginning of copulation (hours)				
	14	15	16	17	18
No. of eggs extracted	31	48	32	8	10
No. of eggs with diploid phase	5	3	1	0	1
No. of eggs with both haploid and diploid phases	0	4	0	0	0
No. of eggs with haploid phase	0	6	10	0	2

The eggs were extracted from the female body cavities at different time points of embryogenesis, and stained with 1 % aceto-orcein just after extraction.

because of the difficulty in extracting eggs from females, which are smaller than *P. persimilis*. Further investigation with different techniques is required to confirm male diploidy in *A. womersleyi*.

We could not identify the heterochromatinized chromosomes and chromosome elimination in this study. However, the coexistence of haploid and diploid cells in the same egg was observed at an early stage of embryogenesis (Table 4). Thus, in *P. persimilis*, it is probable that, instead of heterochromatinization, an unknown system is responsible for the chromosome elimination.

The difference in timing for chromosome elimination between *M. occidentalis* and *P.*

persimilis may be due to the difference in the number of chromosomes: *M. occidentalis* has n=3 whereas *P. persimilis* has n=4. Since almost all species examined have n=4 (Wysoki, 1985), n=3 in *M. occidentalis* may have also originated from n=4. In other words, we speculate that one of 3 chromosomes in *M. occidentalis* is a resultant of 2 of 4 chromosomes combined. Therefore, the total DNA content in the haploid genome (the DNA C-value) appeared to be equal among all species. If this is so, chromosomes in *M. occidentalis* must be larger than those in other species with the basic number of chromosomes. Since it was reported that the DNA C-value affects cell cycles (Cavalier-Smith, 1978), the size of chromosomes may also affect cell cycles. Accordingly, the timing for chromosome elimination in male eggs may also be different. However, a literature review did not reveal any size difference in the chromosomes or a delay of cell cycles.

The present study has also proven that the males produced under poor prey conditions were genetic males; i.e. the male eggs were haploid. This result rules out the possibility that these males were originally females whose course of development became altered, resulting in phenotypic males with a diploid genome set. It is unfortunate that the present study did not clarify the exact time and the mechanism of sex determination in the eggs of phytoseiid mites. To understand pseudo-arrhenotoky and its evolutionary meaning in phytoseiid mites, further studies on fertilization and chromosome elimination in male eggs, as well as the mechanism of sex determination, are needed.

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摘　要

チリカブリダニとケナガカブリダニにおける偽産雄単為生殖の細胞学的証明　豊島真吾・天野　洋(千葉大学園芸学部応用動物昆虫学研究室)

チリカブリダニとケナガカブリダニが偽産雄単為生殖をするか，染色体観察によって調査した．まず，発生の早い段階で雄が2倍性であることを確認するために，産下直後の卵の染色体を観察した．さらに,「十分餌」および「少餌」という2餌条件において産下された卵の染色体を観察し，核相と性の関係を調査した．両種とも，産下された卵において半数体と倍数体が観察され，その頻度や産下される順番から，それぞれ雄と雌に対応すると推察された．そこで，胚発生のさらに早い段階の核相を調べるために，雌成虫の体内から第1卵(雄に発育する卵)を摘出し，その卵の染色体を観察した．すると，比較的早い段階の卵はすべて2倍数の染色体を持ち，発生の経過とともにその割合が低下することが観察された．また，2倍数の染色体を持つ卵の割合が減少する途中の段階で，2倍数の染色体を持つ細胞と1倍数の染色体をもつ細胞が混在する卵が観察された．ヘテロクロマチン化した染色体は観察されなかった．以上の結果から，少なくともチリカブリダニは偽産雄単為生殖をすることが確認された．そして，2倍数から染色体が減少していくタイミングは，すでに報告されている種のそれよりも早かった．