Presumed Paternal Genome Loss During Embryogenesis of Predatory Phytoseiid Mites

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1. Introduction

Predatory phytoseiid mites are classified into the family Phytoseiidae (Acari: Mesostigmata), the most diverse group of mesostigmatic mites (Kranz & Walter, 2009). More than 2000 species of phytoseiid mites have been described (Chant & McMurtry, 2007), almost all of which are small (0.3 mm–0.4 mm; Fig. 1a) and eat other mites, insects, pollen, and fungi. Since they also prey upon pest insects and mites in agricultural fields, they are considered to be a key agent in an integrated pest management system (Gerson et al., 2003). To understand their role in agriculture, their morphology (external and internal), life history characteristics, and behavioral traits have been studied for more than 50 years (e.g., Helle & Sabelis, 1985). Several species of phytoseiid mites, mentioned below, are useful agents and are the most studied species (the recent name is given in parentheses). Because of their small size, they are not considered as an experimental animal in anatomical analyses examining their life cycle characteristics.
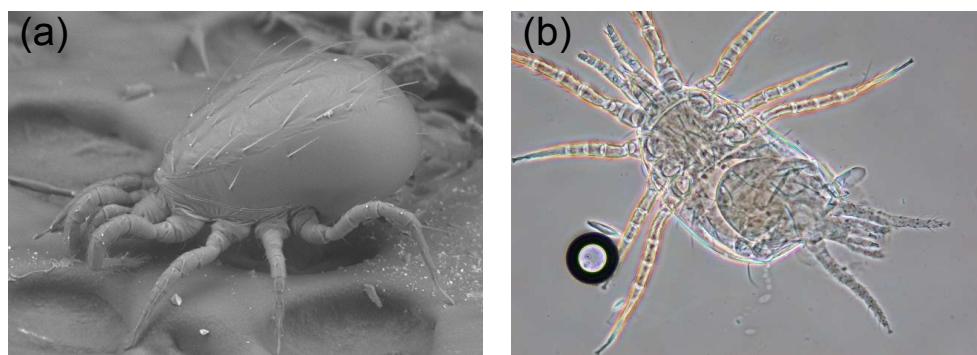

Fig. 1. External and internal appearance of phytoseiid mites. (a) *Neoseiulus womersleyi*. Body length is ca. 400 μm (scanning electron microscope image). (b) An example of delayed oviposition in *N. womersleyi*. Embryonic development proceeds regardless of the nutritional condition of the mother, and the larvae hatch in the mother's body (phase contrast microscope image).

The reproductive system of predatory phytoseiid mites has interested scientists for many years. While adult females start oviposition only after copulation, males and females were found to be haploids and diploids, respectively, by the karyotyping of eggs (Wysoki, 1985). Heterochromatinization in the part of the chromosomes was observed in eggs just after deposition (Nelson-Rees et al., 1980). Although cytological evidence is insufficient, this has been presented as pseudo-arrhenotoky: male-destined oocytes need to be fertilized to begin embryogenesis, and the heterochromatinization of the paternal genomes occurs in male-destined eggs, inducing the elimination of paternal genomes during embryogenesis, resulting in functionally haploid males (Schulten, 1985). This system was also described as "parahaploidy" in previous studies (e.g., Hoy, 1979) and was recently investigated as a form of "paternal genome loss" presented in several insects (Burt & Trivers, 2006). An explanation of several terms in relation to the reproductive system of phytoseiid mites is presented below.

"Pseudo-arrhenotoky" is described as a mode of reproduction, differing from true "arrhenotoky" in which haploid males in the arrhenotokous insects and mites are emerged parthenogenetically from unfertilized eggs. "Deuterotoky" and "thelytoky" are also known as parthenogenetic reproductive modes whereby mothers produce offspring of both sexes from unfertilized eggs (deuterotoky) and only female offspring from unfertilized eggs (thelytoky) (Bell, 1982; Norton et al., 1993). "Thelytoky" is also known in a few species of phytoseiid mites. "Parahaploidy" is described as a ploidy of the chromosomes, differing from true "haploidy" in males, which the haploid males are derived from haploid eggs (Hartl & Brown, 1970). "Paternal genome loss (PGL)" is a phenomenon of chromosome behavior and is divided into two classes, embryonic PGL and germ-line PGL (Ross et al., 2010b). In embryonic PGL, the paternal genome is eliminated during early embryonic development of males and is found in some armored scale insects. In germ-line PGL, the paternal genome is deactivated during male embryogenesis and is eliminated from the germline during or just before spermatogenesis. Germ-line PGL is found in most scale insects, sciarid flies, and in the coffee borer beetle. Since the prefixes "pseudo-" and "para-" are ambiguous, one may better describe the reproductive system of phytoseiid mites in terms of "PGL in diploid arrhenotoky," as in the case of scale insects (Ross et al., 2000a). Although "paternal genome elimination (PGE)" is a more accurate description of the same phenomenon (Herrick & Seger, 1999; Ross et al., 2010a, b), PGL is used in this article. Other terms should be referred to Bell (1982).

PGL in mites is known in three families: Anoetidae (phoretic with insects), Dermanyssidae (blood-feeding ectoparasites), and Phytoseiidae (Oliver, 1983). PGL in Anoetidae and Dermanyssidae has not been well studied and additional information is not available. In phytoseiid mites, although cytological evidence for PGL is insufficient to understand it thoroughly, elaborate hypotheses were developed to investigate their evolutionary significance (e.g., Nagerkerke & Sabelis, 1998). PGL in mites is believed to be an intermediate step from diplodiploid bisexual reproduction to haplodiploid arrhenotoky (Bull, 1983), as noted previously for PGL in scale insects (Schrader & Hugher-Schrader, 1931). Also, the evolutionary constraint of PGL in phytoseiid mites was examined from the viewpoint of sex ratio control (Sabelis et al., 2002). In recent studies, the evolution of PGL in scale insects was considered a consequence of genetic conflict between males and females, as well as between parents and offspring (Ross, 2010a). More genetic and cytological

evidence of PGL in mites is needed to incorporate it into the general framework of genetic conflict in scale insects to better understand the mechanisms of sex determination and evolutionary significance of PGL in mites.

In this article, previous researches and additional evidence in relation to PGL in phytoseiid mites are briefly reviewed with a plenty of literatures, in order to interest scientists working on the different research fields. Authors hope that these scientists will contribute new ideas toward the completion of the reproductive biology of phytoseiid mites. Further understanding of the PGL in phytoseiid mites will make a contribution, hopefully, to the comprehensive researches on the reproductive biology and embryogenesis of various creatures.

2. Experimental evidence for the notion, pseudo-arrhenotoky

Recent knowledge of PGL in phytoseiid mites, referred to by many scientists working on reproductive biology, is based upon experimental evidence published in the 1970s and reviewed in the 1980s. Almost all data are well documented and reliable. For instance, behavioral observation established some basic information as follows: (1) Phytoseiid females need to copulate with males to produce female and male offspring. (2) After copulation, they drastically change their prey-search activity and prey consumption. (3) Around 24–36 h after copulation, they start oviposition when fed abundant prey under comfortable conditions (Fig. 2). Karyotyping revealed that males are haploid and females are diploid. However, these data and experimental evidence are insufficient to support the mechanism of pseudo-arrhenotoky. In this section, the experimental evidence to support pseudo-arrhenotoky is summarized, and problems are pointed out.

2.1 Indirect evidence for insemination in male eggs

Based upon the idea that sperm only serve to activate the ovary to start egg production, a belief was held that all eggs are deposited with a male-biased sex ratio even when a few sperms are accepted by females during copulation. However, Amano and Chant (1978) and Schulten et al. (1978) showed that the amount of egg production is correlated with the amount of sperm accepted by females during copulation in *Phytoseiulus persimilis*, *Amblyseius andersoni*, and *Amblyseius* (= *Neoseiulus*) *bibens*. For instance, in a study of *P. persimilis*, in which copulation was artificially interrupted (Amano & Chant, 1978), the average number of eggs deposited gradually increased from 2.7 to 66.3 when the duration of copulation increased from 30 min to 130 min. It was experimentally confirmed in advance that the duration of copulation was nearly correlated with the amount of sperms accepted by females.

These experimental results suggest not only that insemination activates the ovary for egg production, but also that each egg requires fertilization for its embryonic development. However, the presumption is made that the role of sperm is similar to what is known as "pseudogamy" or "gynogenesis." In pseudogamy or gynogenesis, eggs only develop after penetration by sperm, but the sperm nucleus degenerates without fusing with the egg nucleus so that it makes no genetic contribution to the developing embryo (White, 1973). It may be partially true that such "borrowed sperms" help to produce male offspring in phytoseiid mites, because male offspring were produced by crossbreeding between closely related species, and these individuals (sons) had only maternal characters (Congdon & McMurtry, 1988; Ho et al., 1995).
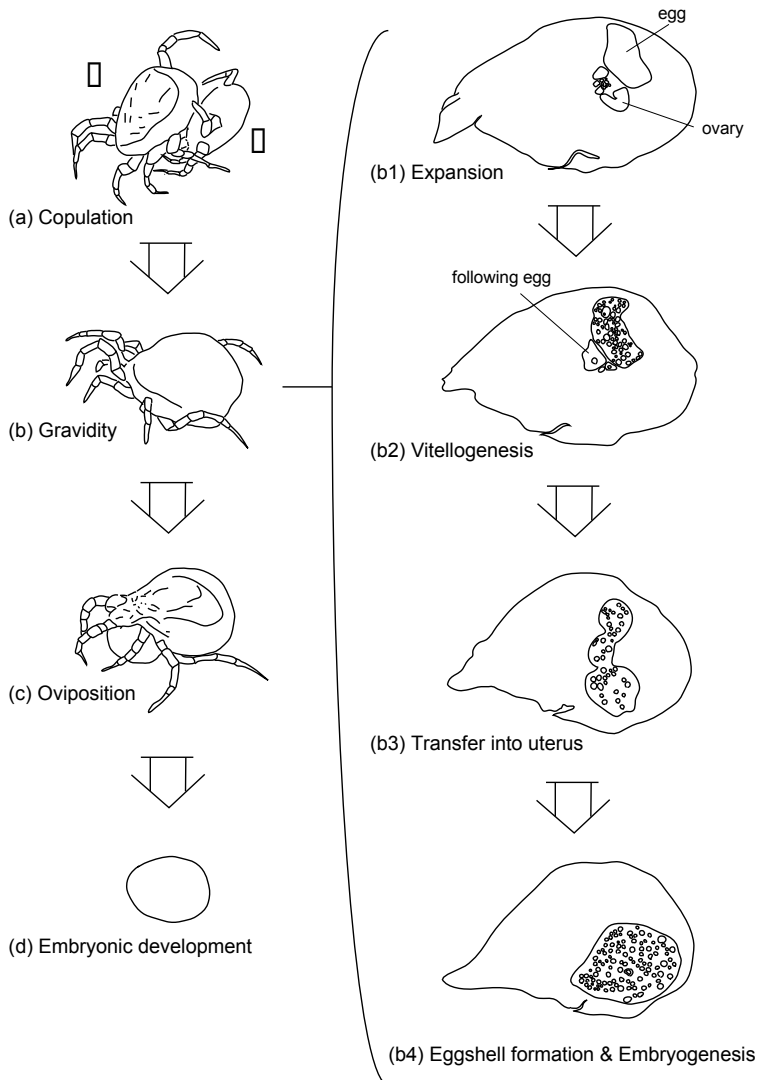
Fig. 2. Schematic illustration of reproductive events and the egg forming process during the gravid period. **Schematic flow on the left (a–d)**: A virgin female copulates for 2–3 h with a male. Mated females consume more prey than virgin females and become fat with a roundish body. Gravid females lay an egg 24–36 h after copulation and continue deposition of eggs, one by one, at intervals of ca. 8 h during which they consume large amounts of prey under ideal conditions. The egg is laid in the middle of embryogenesis. **Schematic flow on the right (b1–b4)**: After copulation, an oocyte expands toward the dorsal region from an ovary located in the center of the body; it is fertilized there and begins vitellogenesis. After expansion of other eggs, the first egg moves into the uterus in the ventral region and forms an eggshell there.

It was also shown at the almost same time that X-ray irradiation on adult males (fathers) induced sterility in male offspring (sons) in *P. persimilis*, *A. bibens* (Helle et al., 1978), and *Metaseiulus* (= *Galendromus*) *occidentalis* (Hoy, 1979). No daughters emerged, and total developing offspring was low in these experiments. A significant reduction in the expected number of sons was also seen. Many died in the early embryonic stages, while the survivors were sterile, even though mortality is not expected in the case of arrhenotoky. Thus, the irradiated paternal genomes are presumed to affect insemination and/or embryogenesis of daughters and sons, also suggesting that male offspring (sons) possess paternal genomes in their germ plasma, since the effect of radiation on the paternal genomes was seen in the male offspring as sterility.

2.2 Chromosome observations

According to studies on the karyotyping of many phytoseiid species (Hansell et al., 1964; Wysoki & Swirski, 1968; Wysoki, 1973; Blommers-Schlosser & Blommers, 1975; Wysoki & McMurtry, 1977; Wysoki & Bolland, 1983), most have a haploid number of 4 and a diploid number of 8 chromosomes, and 3 thelytokous species have 8 chromosomes, except for 3 (haploid) and 6 (diploid) chromosomes in 5 species (Wysoki, 1985). The chromosomes are generally acrocentric except for some metacentric one in a few species, and differ from each other only in size (1–4 µm). Since the ratio of haploid to diploid eggs was equal to that of males to females (Hansell et al., 1964), a haplodiploid genetic system was presumed in phytoseiid mites except for the thelytokous species. Although male diploidy was not confirmed in the karyotype investigations, heterochromatinized (heteropicnotic) chromosomes were observed in eggs immediately after deposition in *M. occidentalis* (Nelson-Rees et al., 1980).

Based on chromosome observation, pseudo-arrhenotoky was proposed (Schulten, 1985). According to this proposal, all eggs start their development from syngamy (2n = 6). The process of heterochromatinization starts within 24 h after egg deposition by arrangement of the 6 chromosomes into 2 groups: 3 heterochromatic chromosomes and 3 euchromatic chromosomes. The following stage strongly resembles the formation of bivalents, which is normally found at the diplotene stage in meiotic division. The heterochromatic chromosomes (n = 3) are eliminated from some or all cells, but almost certainly from the germ line. Thereafter, the male germ line and most somatic cells are haploid. Spermatogenesis in deutonymphs (immature stage) starts with a single equatorial mitotic division, and 2 sperm are produced.

Unfortunately, several events in the proposal have not been elucidated cytologically. In addition, the observation of heterochromatic chromosomes was not conducted in male eggs (the sexes of eggs were not specified), and the number of eggs observed was not sufficient to confirm diploidy in the early embryonic stages of deposited eggs. Furthermore, *M. occidentalis* is not a common species with regard to its chromosome number. Wysoki (1985) reported that 47 of 55 species examined have a basic number of n = 4 and that *M. occidentalis* has n = 3, which is exceptional and known in only 5 species. Therefore, further cytological evidence in a species with the common number of chromosomes (n = 4) is required to confirm the existence of pseudo-arrhenotoky or PGL in phytoseiid mites. In addition, no observations were made of the internal process of egg formation (Fig. 2b1–4) and embryogenesis in the proposal.

3. Presumed Paternal Genome Loss (PGL) in phytoseiid mites

In this section, the unrecognized and unpublished evidence for PGL in phytoseiid mites is briefly reviewed. The fusion of pronuclei and the elimination of the paternal genome during the early stages of embryogenesis in eggs destined to be male are shown as histological evidence for PGL. Inheritance of genetic markers from father to son is also explained as evidence for PGL. To clarify the evidence, the internal morphology, process of egg formation, and embryogenesis in phytoseiid mites are summarized. Hereafter, the eggs destined to be males and females are referred to as "male eggs" and "female eggs," respectively.

3.1 Internal morphology, process of egg formation and embryogenesis

The female genital system consists of a pair of spermathecae, a lyrate organ, an ovary, an oviduct, a uterus, a vagina, and a genital opening (see details in Di Palma & Alberti, 2001), which was determined by morphological comparisons between female and male specimens, as well as previous findings (Michael, 1892; Alberti & Hänel, 1986; Alberti, 1988). The spermathecae are a temporal storage of sperms just after copulation. The transfer of sperm from spermathecae into the ovary and the shape of sperm in the ovary have not yet been clearly elucidated (Di Palma & Alberti, 2001). It is hard to believe that the spermatozoa can find their opening and pass through the duct, since a lumen is hard to detect (G. Alberti, personal communication).

The morphology of the large lyrate organ consists of paired, distinct flattened arms separated indistinctly into several segments. The function of the lyrate organ is as a trophic (nutrimentary) tissue to support the rapid growth of oocytes and vitellogenesis. A nucleus and many mitochondria are distinguished in each segment. The lyrate organ is distinct in dermanyssid mites (Alberti & Hänel, 1986) and also in *P. persimilis* (Alberti, 1988). The ovary is located at the center of the two arms of the lyrate organ. The oocytes in the ovary are connected via nutritive cords (fusomes) to the nutritive tissue of the lyrate organ (Alberti & Hänel, 1986), although such connections are difficult to detect.

One of among several oocytes in the ovary expands toward the dorsal region of the body just after copulation (Fig. 2b) and starts vitellogenesis in the dorsal region (also see details in Toyoshima et al., 2000). Although the exact timing has not been confirmed, the penetration of sperm into the oocyte seems to occur when the oocyte occupies the dorsal region (Toyoshima et al., 2009). Following the completion of vitellogenesis (Fig. 2b2), the egg (an inseminated oocyte) passes via the short oviduct into the uterus (Fig. 2b3) and remains there, forming an eggshell and starting embryonic development (Fig. 2b4). Subsequently, the next oocyte expands and enters vitellogenesis in the dorsal region.

Superficial cleavage occurs in the centrolecithal egg cell in the early stages of ontogenesis. However, 2-, 4-, 8-, 16-, 32-cell stages are not well discriminated in the following steps because the egg in the uterus forms chorion of poor permeability, preventing the penetration of fixative solution for histological observation. After several nuclear divisions, spindle-shaped blastodermal cells are spherically distributed to form a continuous layer. The process of germinal band formation begins on the ventral surface of the periblastula. Entodermal cells have a rather loose distribution. Details of subsequent stages of embryogenesis are given in Yastrebtsov (1992).

The eggs, in the blastula stage, are deposited one by one (Fig. 3). Young females deposit an average of one to five eggs daily at 25°C (Sabelis, 1985a). The degree of embryonic development in deposited eggs depends upon internal and external factors (Sanderson & McMurtry, 1984). As an extreme case, a hatched larva was seen in a female body (Fig. 1b).
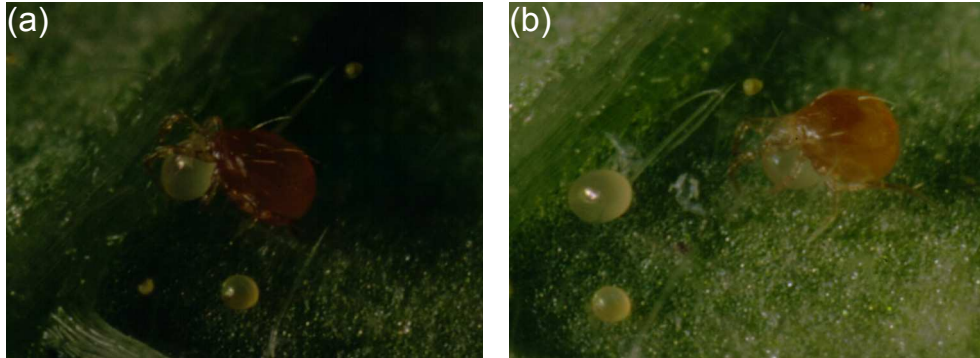

Fig. 3. Ovipositional behavior of *Phytoseiulus persimilis*. The first (a) and the second (b) eggs are laid, one by one, at 6-h intervals. As with all small phytoseiid mites, the egg is huge relative to the size of the gravid female. The rapid growth of large eggs may be supported by the lyrate organ, which is also large relative to the body.

3.2 Fusion of two pronuclei

As a first step in observing the insemination of male eggs, male eggs should be selected from the continuum of egg production during rearing experiments. The first egg should be the focus as a male egg candidate because over 90% of the first eggs develop into males (Toyoshima & Amano, 1998). According to the process of egg formation described above, the first egg in females just after copulation is determined easily and precisely by internal observation of the female. In contrast, unambiguously discriminating sperm in the ovary and recognizing their penetration into the oocyte (the first egg) are difficult (Di Palma & Alberti, 2001), because the sperms in the ovary have not yet been detected with full evidence.

Therefore, eggs with two pronuclei were sought thoroughly (Toyoshima et al., 2000). Then, two pronuclei were detected in the first egg before it had completed its expansion (at ca. 8 h after the end of copulatory behavior). The pronuclei were different in size, which was confirmed in a series of sliced specimens, and joined at the center of the egg. While yolk granules were developing and accumulating gradually around the joining pronuclei, the pronuclei were discriminated precisely by the double-membrane structure. When yolk granules filled the egg (at ca. 10.7 h after the end of copulatory behavior), the joining pronuclei began to change shape and finally fused. This observation was indirect evidence for insemination of male eggs, but histologically elucidated that the paternal genome fuses with the maternal genome in male eggs before embryogenesis.

After a period of time, the first egg moves into the uterus in the ventral region of the body (Fig. 2). A nucleus appears at the center of the egg and it later divides at the same position. The process of nuclear division following embryogenesis was not observed

histologically because the eggshell prevented the fixation of the interior of the egg. Therefore, eggs were extracted from the female body for observation of chromosomal behavior, as described below.

3.3 Elimination of paternal genome during early embryogenesis

While male diploidy was confirmed in the first egg during vitellogenesis in *P. persimilis*, male diploidy has not been detected in eggs after deposition. By karyotyping just deposited eggs, the first and the following (presumed) male eggs were haploid in *P. persimilis* and in *Amblyseius* (=*Neoseiulus*) *womersleyi* determined from a previous study (Toyoshima & Amano, 1999). The time needed for the elimination of chromosomes differed from that of *M. occidentalis*, as reported by Nelson-Rees et al. (1980). Therefore, male diploidy in the early embryonic stages must be confirmed by extracting the first egg (male egg) from the female body cavity. In the first egg extracted from females of *P. persimilis*, diploid cells were observed in an early stage of embryogenesis (the exact stage was not confirmed), and the coexistence of haploid and diploid cells in the same egg was also seen at a later stage of embryogenesis (Toyoshima & Amano, 1999). Finally, only haploid cells were observed in eggs just before deposition. Although heterochromatinized chromosomes were not identified in this experiment, paternal genomes in male eggs were confirmed to be eliminated during the early stages of embryogenesis just before deposition. The stage of embryogenesis was not determined for the extracted eggs.

The difference in timing for chromosome elimination between *M. occidentalis* and *P. persimilis* seems to be due to the difference in the number of chromosomes: *M. occidentalis* has three whereas *P. persimilis* has four. In other words, one *speculates* that one of the three chromosomes in *M. occidentalis* is a result of the combination of two of the four chromosomes. If the total DNA content in the haploid genome appears to be equal among all species, one of the three chromosomes in *M. occidentalis* must be larger than those in other species with the basic number. Since the DNA C-value affects cell cycles (Cavalier-Smith, 1978), the size of chromosomes may also affect cell cycles. As a result, the chromosome elimination in male eggs of *M. occidentalis* seems to occur at a later stage of embryogenesis, which is observed at the time the egg is deposited. This speculation should be confirmed experimentally in the future.

3.4 Inheritance of genetic markers from father to sons

Inheritance of paternal genetic elements from father to son was partially elucidated in *Typhlodromus pyri* by the random amplified polymorphic DNA (RAPD) markers (Perrot-Minnot & Navajas, 1995) and in *Neoseiulus californicus* by the direct amplification of length polymorphism (DALP) markers (Perrot-Minnot et al., 2000). Of two RAPD markers, one marker (330 base pairs: bp) was paternally transmitted to male and female offspring, and the other (990 bp) was paternally transmitted to all females and to some male offspring. Although RAPD markers sometimes showed ambiguous inheritance, the conclusion was made that obligate fertilization could account for the inheritance of nuclear genetic material from father to son, and the *speculation* was made that the paternal genomes were partially retained in some tissues. On the other hand, the inheritance of codominant genetic markers, which is detected by DALP, provided evidence for selective elimination of the paternal

genome among male tissues (Perrot-Minnot et al., 2000), also suggesting that sperm contained exclusively maternal genes whereas some male somatic tissues retained most paternal chromosomes.

The inheritance of a genetic marker from father to son was also confirmed in *N. womersleyi* (Toyoshima & Hinomoto, unpublished data). The sequence characterized amplified region (SCAR)-PCR marker was designed from a fragment amplified by using arbitrary primers (ca. 20 bases long) and used to confirm the inheritance of this marker. When a genetic marker was amplified from the extracted DNA of two populations of *N. womersleyi* with a primer set (Awa18A1&Awa18B3) by PCR and was digested by a restriction enzyme (*EcoR*I), the inheritance of paternal genetic material by sons was confirmed (Fig. 4). Since the paternal band was relatively weak in sons, only a small amount of template DNA was available for the primers in the extracted DNA solution. Whether the paternal genetic material is retained in a specific tissue or if the heterochromatinized fragments of paternal genomes are randomly dispersed in the entire body has not yet been clarified. According to the X-ray experiments mentioned above, as well as the weak presence of genetic markers, entire paternal genomes are probably retained only in the germ plasma of male offspring. Further investigation is necessary to confirm the maintenance of the effective paternal genomes in spermatocytes of male offspring.
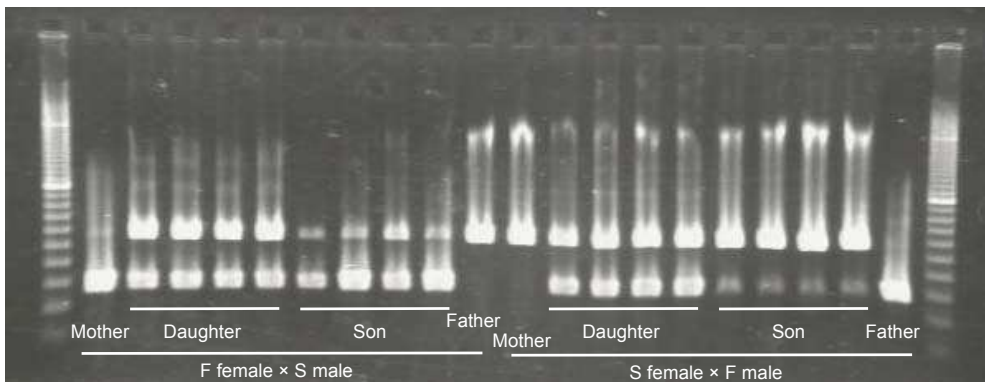
Fig. 4. An electropherogram of a SCAR-PCR marker in an agarose gel (Toyoshima & Hinomoto, unpublished data). A female of a strain of *Neoseiulus womersleyi* with a marker that ran fast on the gel (F) was crossed with a male with a slow marker (S), and vice versa. Daughters and sons have markers from both parents, but the paternal marker in the sons of both crosses is thin.

4. Presumed mechanisms of sex determination in phytoseiid mites

The sex of an individual is *determined* by an initial set of factors (genotypic or environmental). Then, sex *develops* (or *differentiates*) during an integrated series of genetic and physiological steps (Bull, 1983). The sex becomes *fixed* at a certain step during development. The initial set of factors (or "primary sex-determining signal") should be the focus in understanding the relationship between sex determination and PGL in phytoseiid mites.

The sex determination of phytoseiid mites was initially investigated by inbreeding experiments. While Poe and Enns (1970) indicated strong depression by inbreeding in two phytoseiid species, Hoy (1977) indicated weak or scant depression in *M. occidentalis*, and suggested that sex determination in *M. occidentalis* was not based on the multiple allele mechanism (complementary sex determination: CSD) that has been demonstrated for parasitoid wasps and the sawfly (e.g., Cook & Crozier, 1995). Although the sex of an individual is determined and fixed at fertilization in hymenopteran insects, sex may not be determined at fertilization in phytoseiid mites. Therefore, the elimination of the paternal genome in male eggs is not caused by the combination of alleles from the parents. Sex may be controlled by the mother and determined by the accumulation of certain substances in male eggs. In this section, a resultant phenomenon of maternal control is shown with a presumed mechanism of sex control.

4.1 Maternal control of offspring sex

Maternal control of offspring sex was demonstrated by sex ratio control under certain conditions. While the mother deposits eggs with female-biased sex ratios when prey is abundant, she deposits eggs with an even sex ratio ($♀:♂$=1:1) when prey abundance is insufficient (Friese & Gilstrap, 1982; Momen, 1996). Even in a prey patch with abundant prey, mothers also adjust the sex ratio of offspring in a manner that fits the sex ratio to the prediction of sex allocation theories (Hamilton, 1967). While the mother deposits eggs with a female-biased sex ratio when in isolation in a prey patch, she deposits eggs with an even sex ratio when in a crowd with abundant prey (Dinh et al., 1988; Nagerkerke & Sabelis, 1991). To estimate the mechanism to control the sex of the offspring by mothers, the nutritional condition of mothers should be considered as a proximate mechanism rather than the mating structure as a relatively ultimate (evolutionary) mechanism.

Female and male eggs deposited by gravid females of phytoseiid mites can be lined up in a sequence because the females deposit eggs one by one (Fig. 5). Several female eggs were shown to exist between male eggs in a sequence when gravid females deposit eggs with a female-biased sex ratio under abundant prey conditions. The number of female eggs between male eggs in a sequence decreased when gravid females adjusted the sex ratio of offspring in relation to the number of prey items available. Finally, only one female egg was present between male eggs in a sequence when gravid females deposited eggs with an even sex ratio under insufficient prey conditions (Toyoshima & Amano, 1998). It should now be presumed how to determine the sex of eggs.

The female eggs produced under abundant prey conditions may be changed into phenotypic males in response to the nutritional condition when produced. Paternal genomes would remain in the phenotypic males if PGL were genetically controlled but flexible. However, phenotypic sex is consistent with genetic sex (karyotype) in eggs produced with an even sex ratio under minimal prey conditions (Fig. 5; see also Toyoshima & Amano, 1999). Actually, the number of male eggs is similar among different prey conditions, although the total number of eggs decreased gradually in relation to the number of prey items available to gravid females (Toyoshima & Amano, 1998). The number of female eggs may have decreased rather than having changed into phenotypic males.

Abundant food

Sex	♂	♀	♀	♀	♂	♀	♀	♀	♀
Chromosomes	4	8	8	8	4	8	8	8	8

Minimum food

Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂
Chromosomes	4	8	4	8	4	8	4	8	4

Fig. 5. Relationship between karyotypes of eggs and sex of offspring produced by mothers under abundant and minimum food condition.

Mothers may be able to choose sperm with a gene for maleness at a certain locus to control sex ratios under poor nutritional conditions. In this case, the paternal genomes are eliminated after the maleness of the egg is determined genetically. On the basis of genomic conflict between mother and father (e.g., Ross et al., 2010a), however, the utilization of sperm for maleness under poor prey conditions is a great disadvantage to the father because the genetic material in the sperm is eliminated during embryogenesis, and as a result does not contribute to the son's characteristics. Therefore, fathers probably do not produce sexually dimorphic sperm. Unfortunately, it is completely difficult to elucidate, anatomically, the sexual dimorphism of sperms in the ovary or to discriminate, genetically, the sperm with a gene for maleness. Detecting sperm for maleness modified by the mother in her ovary also presents a problem.

The possibility exists that the control of oocytes or eggs by the mother (without paternal contribution) is a simple process for maternal control of offspring sex. Mothers may be able to absorb female eggs under poor prey conditions, known as oosorption (resorption of oocytes), which is an effective mechanism to save resources on eggs in various insect species (Bell & Bohm, 1975). This is based on the idea that the sex of oocytes is already determined in the ovary upon emergence. According to this idea, mothers do not choose a male-destined oocyte for fertilization to develop into a male egg but absorbs female eggs when encountering a poor prey patch.

Gravid mothers under starvation were observed at 12-h intervals to confirm the absorption of female oocytes and/or eggs in the mother's body (Toyoshima et al., 2009). When gravid mothers just after depositing the first (male) egg were restricted to no prey items, the mothers did not hold an egg in the uterus but held 1–2 eggs in the dorsal region (refer to Fig. 2). Since mothers laid an average of 1.8 eggs during starvation, the 1–2 eggs in the dorsal region were transferred into the uterus, one by one, to form an eggshell and were deposited regardless of the nutritional condition of the mothers. An oocyte expanded during the deposition of the eggs and was maintained for at least 72 h in the dorsal region after vitellogenesis (Fig. 6). Two pronuclei were conjugated at first but later fused when the egg was in the dorsal region. The egg was deposited as a female egg when abundant prey items were provided for the starving

mothers. According to this starvation experiment, female eggs may not be absorbed to control the sex ratio during food limitation. The confirmation was also made that oocyte expansion and vitellogenesis, as well as embryogenesis in the eggshell, advances until the nutrients in the starving mother are depleted. In this case, fertilization occurred after the sex of oocytes was determined, and the sex of the oocytes (and eggs) in the body was not influenced by the nutritional condition of the mothers. Paternal genomes in the egg may be eliminated during early embryonic development if the sex of the egg has been determined as male.
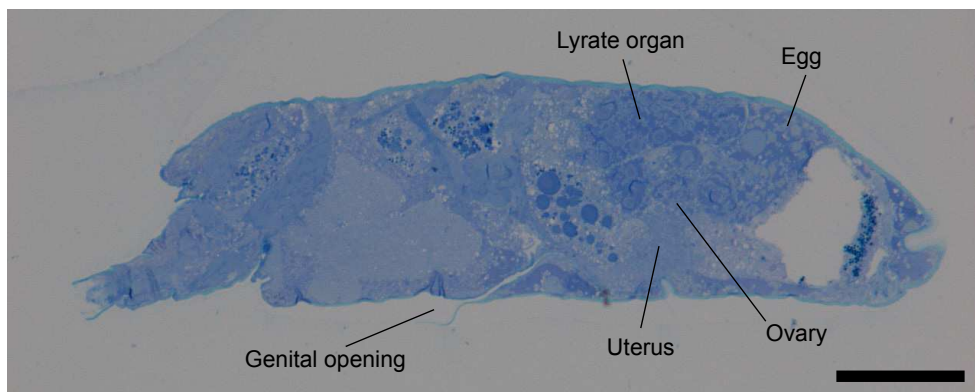

Fig. 6. Sagittal section of an adult female *Neoseiulus californicus* starved for 36 h. The starved female holds an egg in the dorsal region even after depositing an average of 1.8 eggs. The female is facing left (light microscope image). Bar = 50 μm.

4.2 Female-biased nutritional allocation in eggs

Mothers change their investment of nutrients into eggs in relation to the sex of eggs, as well as the prey consumption rate and their own age (Toyoshima & Amano, 1998). They produce larger eggs when consuming abundant prey, and gradually decrease egg size when aged and when prey consumption is restricted. In addition, they produce larger female eggs and smaller male eggs, and maintain the difference in size between sexes even when prey consumption is restricted. Although the evolutionary significance of sexual size dimorphism was discussed in a previous study, the sex determining mechanism of eggs was not yet determined from the size difference between sexes because of insufficient evidence.

The difference in egg size between sexes may be determined before fusion of the paternal and maternal pronuclei in the egg. According to an internal observation of starving females (Toyoshima et al., 2009), the vitelline membrane forms around the egg in the body of a starving female (Fig. 7c). The envelope was still not complete but interrupted. The adjacent, but not fused, pronuclei were visible in the egg enveloped with the vitelline membrane (Fig. 7a & 7b). The membrane is usually formed in oogenesis at the end of vitellogenesis. Since the size of the egg is determined when the vitelline membrane is formed, the combination of maternal and paternal genetic material did not influence size determination. In turn, the size of eggs was determined only by maternal control. More precisely, the size of eggs was influenced by sex, which was determined in response to the nutritional condition of the mothers.
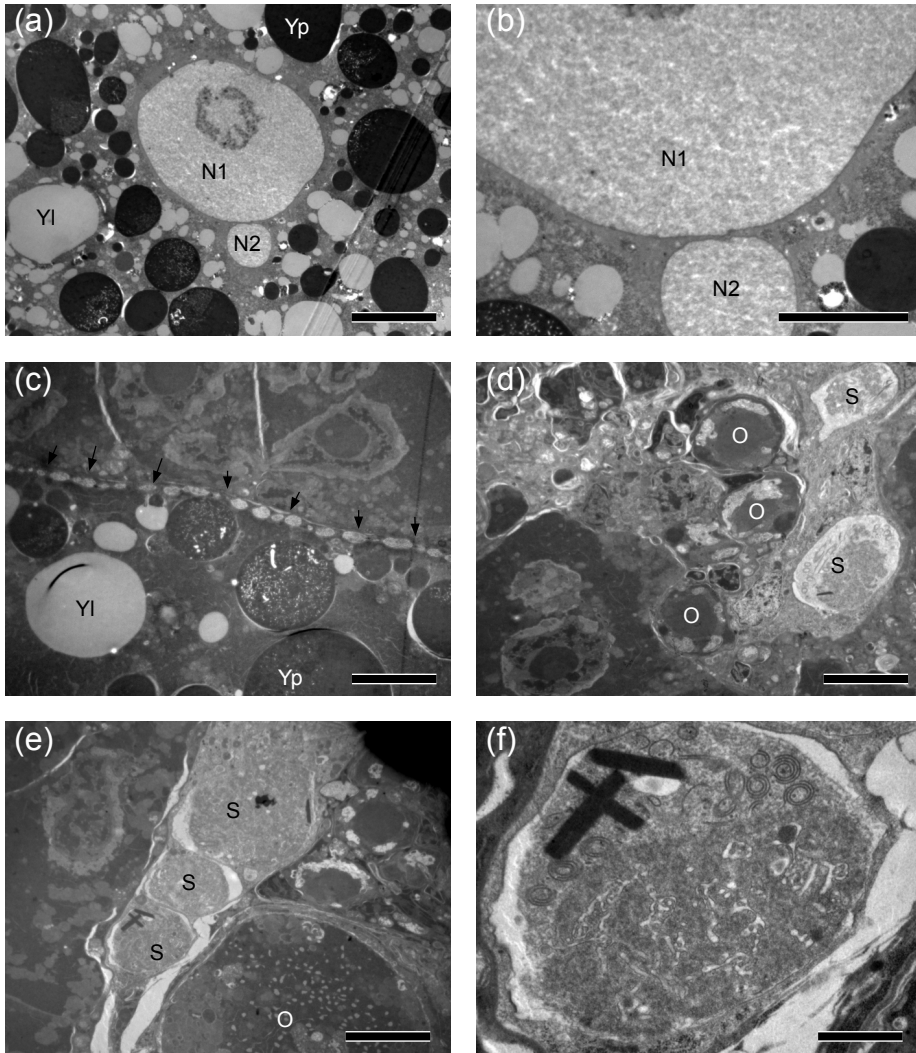
Fig. 7. Fine structures in the egg and around the ovary revealed by transmission electron microscopy. (a) Adjacent pronuclei in an egg filled with yolk granules. Bar = 10 µm. (b) Adjacent part of the pronuclei in (a). Bar = 5 µm. (c) The vitelline membrane (indicated by seven arrows). The envelope is still not completed, but interrupted. The vitelline membrane separates the egg with yolk granules (below) from the lyrate organ (above). Bar = 5 µm. (d) Ovary with several oocytes. Presumed sperms are visible around the ovary. Bar = 5 µm. (e) Presumed sperms between an oocyte and lyrate organ. Bar = 5 µm. (f) Detailed structure of a presumed sperm of (e). The detailed structure in the sperm cell is different from that shown in Di Palma & Alberti (2001). Black bars are not a staining artifact but an unknown structure. Bar = 1 µm. Abbreviations: N1, N2, nuclei; O, oocyte; S, presumed sperm; Yl, lipid-yolk granule; Yp, protein-yolk granule (unpublished micrographs by Toyoshima & Alberti).

How nutrition is invested into the eggs of each sex is still unclear. When female eggs are significantly different in size from male eggs, the size of eggs should be influenced only by the sex of the offspring. Thus, the sex of the eggs should be converted from male to female at a certain level of nutritional accumulation in the eggs. However, the size of the eggs is influenced not only by the sex but also the age and the nutritional condition of the mothers (Toyoshima & Amano, 1998). Female eggs deposited by mothers under poor prey conditions are smaller than male eggs deposited under abundant prey conditions, although the difference of egg size between the sexes (female eggs are larger than male eggs) is maintained at each prey condition. The switch from one sex to the other occurs at a relative criterion rather than an absolute criterion. The accumulation of a certain nutritional substance in addition to the minimum requirement of nutrition may lead to conversion of eggs from male to female. However, how the ratio of female to male is controlled in the ovary also remains unclear.

Sexual size dimorphism of eggs is also observed in an arrhenotokous phytophagous mite, *Tetranychus urticae* (Mache et al., 2010; Toyoshima, 2010), in which virgin mothers of this species lay only haploid male eggs, and fertilized mothers lay female (diploid) and male (haploid) eggs with female-biased sex ratios under good conditions on host plants. Male eggs produced by fertilized mothers are smaller not only than female eggs but also than male eggs produced by virgin females. If the sex of eggs produced by fertilized mothers is ignored, the eggs of fertilized females are not different from that of virgin females. From this comparison between eggs of fertilized and virgin females, virgin females are suspected to also produce concealable cytologically female eggs, which would be fundamentally destined as females but developed to males when not fertilized. This idea is not yet supported by data, but, if this is true, the evolutionary position of PGL in phytoseiid mites and arrhenotokous gynogenesis in certain animals may be understood in the course of the evolutional succession of sexual reproduction.

5. Future perspective

PGL in phytoseiid mites is still wrapped in mystery, although cytological and genetic evidence has accumulated since the 1980s. To understand the *evolutionary process* and *significance* of PGL in phytoseiid mites, we should clarify several events during oogenesis and embryogenesis (Fig. 8), as well as similar reproductive systems in closely related groups. The sex determining mechanism in eggs during oogenesis, sperm behavior in the ovary, and sperm penetration into oocytes are important events in the early reproductive process, which may lead to insights when exploring the signals for PGL. According to Di Palma & Alberti (2001), putative sperm cells extend a thick projection for insertion into an oocyte, and projections are visible around oocytes in the ovary (Fig. 7d–f). However, more investigation is necessary to determine when the sperm inserts into the oocyte and how the sperm nucleus behaves in the oocyte. Sperm behavior in the ovary and oocytes may also be influenced by the qualitative and/or quantitative differences between female-destined and male-destined oocytes. The difference in oocytes at the starting point in the ovary should be investigated histologically and genetically.

Thelytokous species are also known in phytoseiid mites. The thelytokous reproductive system is presumed to be derived from PGL because the number of thelytokous species is small compared to those with PGL. Comparative morphology of oogenesis and oocyte expansion in the ovary between thelytokous and PGL species will shed new light on the *evolutionary flexibility* and *constraints* of PGL in phytoseiid mites.

Penetration of sperm into oocyte
has not yet been detected in the ovary.

(a) Fertilization

Fusion of two pronuclei was detected in the egg
at the dorsal region of the body.

(b) Fusion of pronuclei

Nuclear division and superficial cleavage
have not been observed in the section.

Embryogenesis in the female body
has not been confirmed in the section.

Heterochromatinized chromosomes have
not yet been detected in the section.

Elimination of paternal chromosomes
probably occurr during early embryogenesis
in the female body, but has not been detected
in the section.

It has not been observed in the section
whether the **paternal chromosomes retain**
in a certain tissue nor the **fragmented**
chromosomes disperse in the entire body.

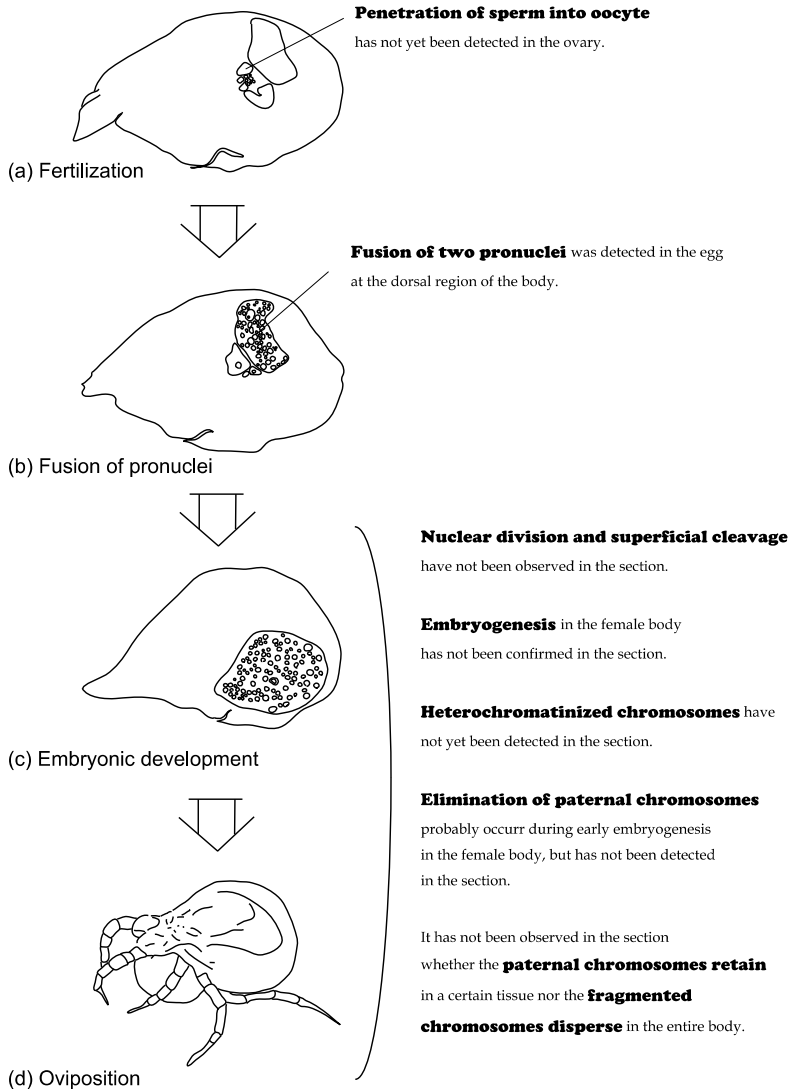(c) Embryonic development

(d) Oviposition

Fig. 8. Unknown events during oogenesis and embryogenesis of phytoseiid mites.

The process of the elimination of paternal genomes during embryonic development should be visualized to better understand the events of PGL. It is difficult, but not impossible, to penetrate the fixatives into the egg when wrapped in an eggshell in the female body. It is most important that the chromosome behavior in each cell during the blastula stage be observed to follow the heterochromatinized chromosomes, as a central point of the study of PGL. It may be able to reveal when, where, and how genomic material is eliminated during the embryonic development of males. Finally, we can start to understand why the paternal genome is eliminated and why this reproductive system is maintained in phytoseiid mites.

6. Acknowledgment

7. References

Alberti, G. (1988) Genital system of gamasida and its bearing on phylogeny. In: *Progress in Acarology,* Volume I, Basavanna, G. P. C. & Viraktmath, C. A. (Eds.), 479-490, Ellis Horwood, Chichester

Alberti, G. & Hänel, H. (1986) Fine structure of the genital system inthe bee parasite, Varroajacobsoni (Gamasida: Dermanyssina) with remarks on spermiogenesis, spermatozoa and capacitation. *Experimental & Applied Acarology*, Vol.2, pp. 63-104

Amano, H. & Chant, D. A. (1978) Mating behaviour and reproductive mechanisms of two species of predacious mites, *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius andersoni* (Chant) (Acarina: Phytoseiidae). *Acarologia*, Vol.20 pp. 196-213

Bell, G. (1982) *Masterpiece of Nature: The Evolution and Genetics of Sexuality*, 635p., Croom Helm, London

Bell, W. J. & Bohm, M. K. (1975) Oosorption in insects. *Biological Reviews*, Vol.50, pp. 373-396

Blommers-Schlosser, R. & Blommers, L. (1975) Karyotypes of eight species of Phytoseiid mites (Acarina: Mesostigmata) from Madagascar. *Genetica*, Vol.45, pp. 145-148

Bull, J. J. (1983) *The Evolution of Sex-Determining Mechanisms*, 316p., Menlo Park, Benjamin Cummings

Burt, A. & Trivers, R. (2006) *Genes in Conflict: The Biology of Selfish Genetic Elements*, 602p., Belknap Press of Harvard Univ. Press, Cambridge.

Cavalier-Smith, T. (1978) Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. *Journal of Cell Science*, Vol.34, 247-278

Chant, D. A. & McMurtry, J. A. (2007) *Illustrated Keys and Diagnoses for the Genera and Subgenera of the Phytoseiidae of the World*, 220p., Indira Publishing House, Michigan

Congdon, B. D. & McMurtry, J. A. (1988) Morphological evidence establishing the loss of paternal chromosomes in males of predatory phytoseiid mites, genus *Euseius. Entomologia Experimentalis et Applicata*, Vol.48, pp. 95-96

Cook, J. M. & Crozier, R. H. (1995) Sex determination and population biology in the Hymenoptera. *Trends in Ecology & Evolution*, Vol.10, pp. 281-286

Di Palma, A. & Alberti, G. (2001) Fine structure of the female genital system in phytoseiid mites with remarks on egg nutrimentary development, sperm access system, sperm transfer, and capacitation (Acari, Gamasida, Phytoseiidae). *Experimental & Applied Acarology*, Vol.25, pp. 525-591

Dinh, N. V., Janssen, A. & Sabelis, M. W. (1988) Reproductive success of *Amblyseius idaeus* and *A. anonymus* on a diet of two-spotted spider mites. *Experimental & Applied Acarology*, Vol.4, pp. 41-51

Gerson, U., Smiley, R. L. & Ochoa, R. (2003) *Mites (Acari) for Pest Control*. 539p., Blackwell Science Ltd., Oxford

Hamilton, W. D. (1967) Extraordinary sex ratios. *Science*, Vol.156, pp.477-488

Hansell, R. I. C., Mollison, M. M. & Putman, W. L. (1964) A cytological demonstration of arrhenotoky in three mites of the family Phytoseiidae. *Chromosoma (Berl.)*, Vol.15, pp. 562-567

Hartl, D. L. & Brown, S. W. (1970) The origin of male haploid genetic systems and their expected sex ratio. *Theoretical & Population Biology*, Vol.1, pp. 165-190

Helle, W., Bolland, H. R., van Arendonk, R., De Boer, R. & Schulten, G. G. M. (1978) Genetic evidence for biparental males in haplo-diploid predator mites (Acarina: Phytoseiidae). *Genetica*, Vol.49, pp. 165-171

Helle, W. & Sabelis, M. W. (1985) *Spider Mites Their Biology, Natural Enemies and Control, Volume 1B*. 458p., Elsevier, Amsterdam

Herrick, G. & Seger, J. (1999) Imprinting and paternal genome elimination in insects. In: *Genomic Imprinting*, R. Ohlsson, (Ed.), 41-71, Springer Verlag, Amsterdam

Ho, C.-C., Lo, K.-C. & Chen, W.-H. (1995) Comparative biology, reproductive compatibility, and geographical distribution of *Amblyseius longispinosus* and *A. womersleyi* (Acari: Phytoseiidae). *Environmental Entomology*, Vol.24, pp. 601-607

Hoy, M. A. (1979) Parahaploidy of the "arrhenotokous" predator, *Metaseiulus occidentalis* (Acarina: Phyotoseiidae) demonstrated by X-irradiation of males. *Entomologia Experimentalis et Applicata*, Vol.26, pp. 97-104

Krantz, G. W. & Walter, D. E. (2009) *A Manual of Acarology (3rd. ed.)*. 807p., Texas Tech University Press, Texas

Macke, E., Magalhaes, S., Khan, H. D.-T., Luciano, A., Frantz, A., Facon, B. & Olivieri, L. (2010) Sex allocation in haplodiploid is mediated by egg size: evidence in the spider mite *Tetranychus urticae* Koch. *Proceedings of the Royal Society B*, Vol.278, No.1708, pp. 1054-1063

Michael, A. D. (1892). On the variations in the internal anatomy of the gamasinae, especially in that of the genital organs, and on their mode of coition. *Transactions of the Linnean Society of London. 2nd Series: Zoology*, Vol.5, No.9, pp. 281-317

Nagerkerke, C. J. & Sabelis, M. W. (1991) Precise sex-ratio control in the pseudo-arrhenotokous phytoseiid mite *Typhlodromus occidentalis* Nesbitt. In: *The Acari, Reproduction, Development and Life-History Strategies*. Schuster, R. & Murphy, P. W. (Eds.), 193-207, Chapman and Hall, London

Nagerkerke, C. J. & Sabelis, M. W. (1998) Precise control of sex allocation in pseuso-arrhenotokous phytoseiid mites. *Journal of Evolutionary Biology*, Vol.11, pp. 649-684

Nelson-Rees, W. A., Hoy, M. A. & Roush, R. T. (1980) Heterochromatinization, chromatin elimination and haploidization in the parahaploid mite *Metaseiulus occidentalis* (Nesbitt) (Acarina: Phytoseiidae). *Chromosoma (Berl.)*, Vol.77, pp. 263-276

Norton, R. A., Kethley, J. B., Johnston, D. E. & OConnor, B. M. (1993) Phylogenetic perspectives on genetic systems and reproductive modes of mites. In: *Evolution and Diversity of Sex Ratio in Insects and Mites*, Wrensch. D. L. & Ebbert, M. A. (Eds.), 8-99, Chapman & Hall, New York

Oliver, J. H. (1983) Chromosomes, genetic variance and reproductive strategies among mites and ticks. Bulletin of the ESA, Vol.29, No.2, pp.8-17

Perrot-Minoot, M.-J., Lagnel, J., Migeon, J. & Navajas, M. (2000) Tracking paternal genes with DALP markers in a pseudoarrhenotokoys reproductive system: biparental transmission but haplodiploid-like inheritance in the mite *Neoseiulus californicus*. *Heredity*, Vol.84, pp. 702-709

Perro-Minnot, M.-J. & Najavas, M. (1995) Biparental inheritance of RAPD markers in males of the pseudoarrhenotokous mite *Typhlodromus pyri*. *Genome*, Vol.38, pp. 838-844

Ross, L., Pen, I. & Shuker, D. M. (2010a) Genomic conflicts in scale insects: the causes and consequences of bizarre genetic systems. *Biological Reviews*, Vol.85, pp. 807-828

Ross, L., Shuker, D. M. & Pen, I. (2010b) The evolution and suppression of male suicide under paternal genome elimination. *Evolution*, Vol.65, pp. 554-563

Sabelis, M. W. (1985) Development. In : *Spider Mites, Their Biology, Natural Enemies and Control, Volume 1B*, Helle, W. & Sabelis, M. W. (Eds.), 43-53, Elsevier, Amsterdam

Sabelis, M. W., Nagerkerke, C. J. & Breeuwer, J. A. J. (2002) Sex ratio control in arrhenotokous and pseudo-arrhenotokous mites. In: *Sex Ratios, Concepts and Research Methods*, Hardy, I. C. W. (Ed.), 235-253, Cambridge University Press, Cambridge.

Sanderson, J. P. & McMurtry, J. A. (1984) Life history studies of the predaceous mite *Phytoseius hawaiiesis*. *Entomologia Experimentalis et Applicata*, Vol.35, pp. 27-234

Schrader, F. & Hughes-Schrader, S. (1931) Haploidy of metazoan. *Quarterly Review of Biology*, Vol.6, pp. 411-438

Schulten, G. G. M. (1985) Pseudo-arrhenotoky. In: *Spider Mites, Their Biology, Natural Enemies and Control, Volume 1B*, Helle, W. & Sabelis, M. W. (Eds.), 67-71, Elsevier, Amsterdam

Schulten, G. G. M., van Arendonk, R. C. M., Russell, V. M., & Roorda, F. A. (1978) Copulation, egg production and sex-ratio in *Phytoseiulus persimilis* and *Amblyseius bibens* (Acari: Phytoseiidae). *Entomologia Experimentalis et Applicata*, Vol.24, pp. 145-153

Schultz, R. J. (1969) Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. *The American Naturalist*, Vol.103, pp. 605-619

Toyoshima, S. (2010) Effects of fertilization status and age of gravid females on the egg size in *Tetranychus urticae* Koch. *Journal of the Acarological Society of Japan*, Vol.19, pp. 107-112

Toyoshima, S. & Amano, H. (1998) Effect of prey density on sex ratio of two predacious mites, *Phytoseiulus persimilis* and *Amblyseius womersleyi* (Acari: Phytoseiidae). *Experimental & Applied Acarology*, Vol.22, pp. 709-723

Toyoshima, S. & Amano, H. (1999) Cytological evidence of pseudo-arrhenotoky in two phytoseiid mites, *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius womersleyi* Schicha. *Journal of the Acarological Society of Japan*, Vol.8, pp. 135-142

Toyoshima, S., Michalik, P., Talarico, G., Klann, A. E. & Alberti, G. (2009) Effects of starvation on reproduction of the predacious mite *Neoseiulus californicus* (Acari: Phytoseiidae). *Experimental & Applied Acarology*, 47: 235-247.

Toyoshima, S., Nakamura, M., Nagahama, Y. & Amano, H. (2000) Process of egg formation in the female body cavity and fertilization in male eggs of *Phytoseiulus persimilis* (Acari: Phytoseiidae). *Experimental & Applied Acarology*, Vol.24, pp. 441-451

White, M. J. D. (1973) *Animal Cytology and Evolution (3rd ed.)*. 961p., University Press, Cambridge

Wysoki, M. (1973) Further studies on karyotypes an sex determination of phytoseiid mites (Acarina: Mesostigmata). Genetica, 44: 139-145.

Wysoki, M. (1985) Karyotyping. In: *Spider Mites, Their Biology, Natural Enemies and Control, Volume 1B*, Helle, W. & Sabelis, M. W. (Eds.), 191-196, Elsevier, Amsterdam

Wysoki, M. & Bolland, R. (1983) Chromosome studies of phytoseiid mites (Acari: Gamasida). *International Journal of Acarology*, Vol.9, pp. 91-99

Wysoki, M. & McMurtry, J. A. (1977) Karyotypes of eight species of phytoseiid mites of the genus *Amblyseius* Berlese (Acarina: Mesostigmata). *Genetica*, Vol.47, pp. 237-239

Wysoki, M. & Swirski, E. (1968) Karyotypes and sex determination of ten species of phytoseiid mites (Acarina: Mesostigmata). *Genetica*, Vol.39, pp. 220-228

Yastrebtsov, A. (1992) Embryonic development of gamadis mites (Parasitiformes: Gamasida). *International Journal of Acarology*, Vol.18, pp. 121-141
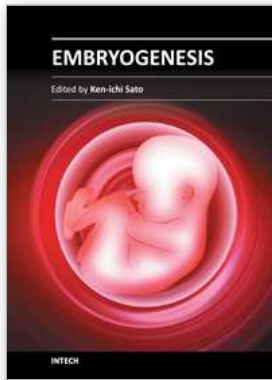
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