6

How Does the Alteration of Meiosis Evolve to Parthenogenesis? - Case Study in a Water Flea, *Daphnia pulex* -

Chizue Hiruta[1] and Shin Tochinai[1,2]
[1]*Department of Natural History Sciences, Graduate School of Science, Hokkaido University,*
[2]*Department of Natural History Sciences, Faculty of Science, Hokkaido University, Japan*

1. Introduction

This chapter reviews progress in our understanding of two different reproductive modes in *Daphnia pulex* and discusses the interesting modifications found in meiosis during parthenogenesis.

Most daphnid species reproduce parthenogenetically as well as sexually, resulting in the production of diploid progenies in both cases. In natural populations, parthenogenesis is the common mode of reproduction, and parthenogenetic offspring are all female. However, in response to certain environmental conditions, such as crowding or seasonal change, male offspring are also produced parthenogenetically, and sexual reproduction occurs (Hebert, 1978). Although they switch between parthenogenesis and sexual reproduction in response to environmental conditions, little is known about the molecular and cytological mechanisms switching and governing each reproductive mode. It can be interpreted that *D. pulex* develops a reproductive strategy utilizing 'parthenogenesis', which has high reproductive power, and 'sexual reproduction', which generates genetic diversity, in response to different environments. These theoretical studies have been made on evolutionary mechanism of reproductive modes (Decaestecker *et al.*, 2009); however, practically no study analyzing the evolutionary mechanism while taking the developmental constraints into consideration has so far been conducted. Our understanding of the evolution of reproductive strategy would increase once we precisely clarify the developmental gene programs operating there. We have recently started to develop *D. pulex* as an experimental model for studying oogenesis and developmental mechanisms during evolution.

1.1 Chapter contents

- At first, we explain why we are interested in the comparative research of two reproductive modes: parthenogenesis and sexual reproduction, and why we chose *D. pulex* as an experimental animal.

- From our experiments, it was concluded that diploid progeny are produced by non-reductional division in parthenogenetic *D. pulex*. We found that, when a parthenogenetic egg entered the first meiosis, division was arrested in the early first anaphase. Then, two half-bivalents, which were dismembered from each bivalent, moved back to the equatorial plate and assembled to form a diploid equatorial plate. Finally, the sister chromatids were separated and moved to opposite poles in the same manner as the second meiotic division.
- We hypothesized that *D. pulex* switches reproductive mode by controlling the maturation division of oocytes (arrest or progress), depending on whether the egg is fertilized or not.
- We use *D. pulex* as an example of the evolutionary process of parthenogenesis which arose from sexual reproduction. Suggestions for future research are also presented.

2. A water flea, *Daphnia pulex*

Water fleas of the genus *Daphnia* are members of the order Cladocera, which are small crustaceans that live in various aquatic environments varying from temporary ponds to large lakes as a cosmopolitan species. They occupy a key position (food chain) in aquatic communities, both as important herbivores eating algae and bacteria and as major prey items of fish, aquatic insects and other predators. Moreover, they have a value as environmental indicator organisms because of their high sensitivity to water quality. Thus, many previous studies have concentrated on ecology, taxonomy and toxicology. Recently however, the spotlight has been on the phenotypic plasticity of *D. pulex*. One example of this phenomenon is that they express morphological, life history, and behavioral defenses in response to chemical cues released by predators (Tollrian & Dodson, 1999).

2.1 General feature

2.1.1 Anatomical characteristics

The carapace is transparent and encloses the whole trunk, except the head and the apical spine. The trunk appendages are flattened, leaf-like structures that serve for suspension feeding and for locomotion. There is a single central compound eye in the head. The large, paired appendages used for swimming are second antennae.

Female: The body length of the female is about 1-3 mm. The first antennae are small and short, not extending beyond the rostrum. The ovaries are a pair of elongated organs lying on either side of the gut. In the posterior part of the each ovary, the oviduct opens into the brood chamber. As shown in Fig. 1C, oogonia and smaller oocytes are located in the most-posterior part of the ovary, and move anteriorly as development progresses. The number of eggs spawned in a clutch depends on the nutritional state and the size of the female. The space between the body and the carapace is used as a brood chamber.

Male: The males are smaller than the females. The rostrum is generally indistinct and the first antennae are large and long. The male has a copulatory hook, which is used for holding on to the female during mating, on the first thoracic leg. The testes are a pair of elongated organs lying on either side of the gut. The two gonopores open near the anus. As shown in Fig. 1F, the mature testis has spermatozoa in the lumen. Spermatogenesis begins at the walls and proceeds into the innermost part (lumen).

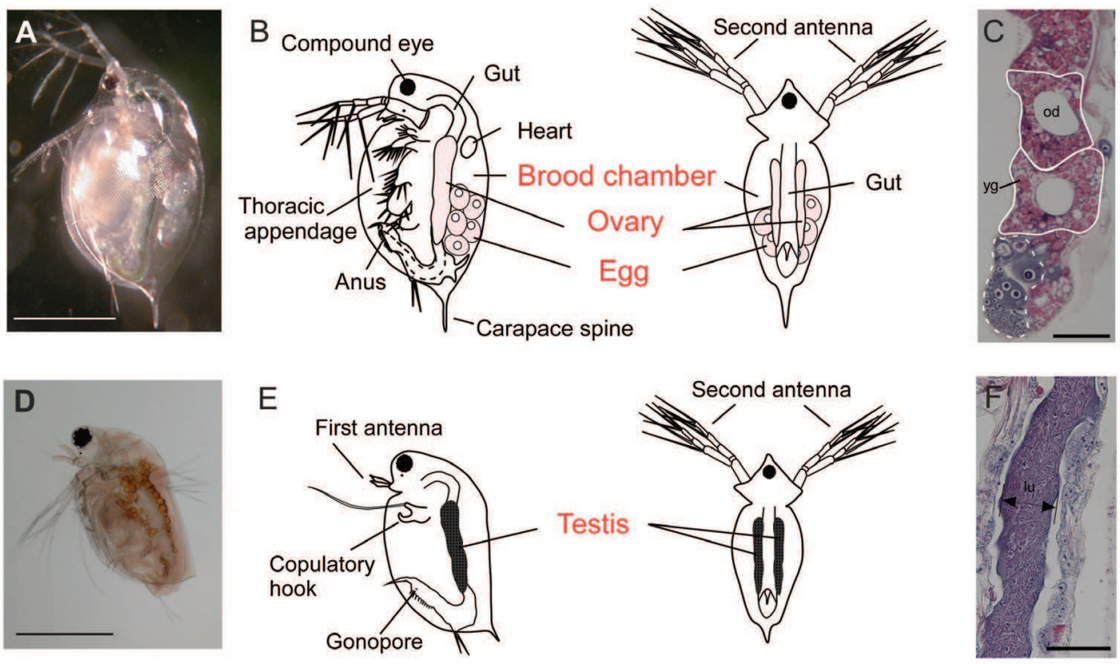


Fig. 1. *Daphnia pulex*. Anatomical characteristics of an adult female (top row) and male (bottom row). (A,D) Photo. Scale bars = 1 mm in A; 0.5 mm in D. (B,E) Lateral (left) and frontal (right) view of anatomy. (C) Sagittal section of the ovary. The largest eggs (solid white line) contain a large amount of yolk granules and oil droplets. Oogonia and smaller oocytes are located in the most-posterior part of the ovary (dashed white line). Scale bar = 50 μm. yg, yolk granule; od, oil droplet. (F) Sagittal section of the testis. Sperms are located in the lumen. Scale bar = 100 μm. lu, lumen.

2.1.2 Life cycle

Daphnia pulex reproduce either by parthenogenesis or sexual reproduction and populations are almost exclusively female. Under favorable conditions, eggs are produced in clutches of one to several dozen, and one female may produce several clutches, which is linked with the molting process. The eggs are laid in the brood chamber shortly after molting. Embryonic development occurs in the brood chamber and the larvae are miniature versions of the adults. The neonates are released from the brood chamber just before the mother molts. In this way, the parthenogenetic individual repeats the cycle of molting, egg laying and releasing during her life (Fig. 2, Non-resting cycle).

Unfavorable conditions, such as changes in water temperature or food deprivation as a result of population increase, may induce the production of males. In other words, a single parthenogenetic female can produce either parthenogenetic female offspring and/or males for sexual reproduction. The male clasps the female and display copulatory behavior. Then the resting eggs are produced (Fig. 2, Resting cycle). Only two large eggs produced in a single clutch (one from each ovary) are enclosed in an ephippium which used to be a part of the dorsal exoskeleton and is darkly pigmented with melanin. The resting eggs are resistant to desiccation and freezing during winter, playing an important role in colonizing new habitats or in the re-establishment of an extinguished population after unfavorable conditions.

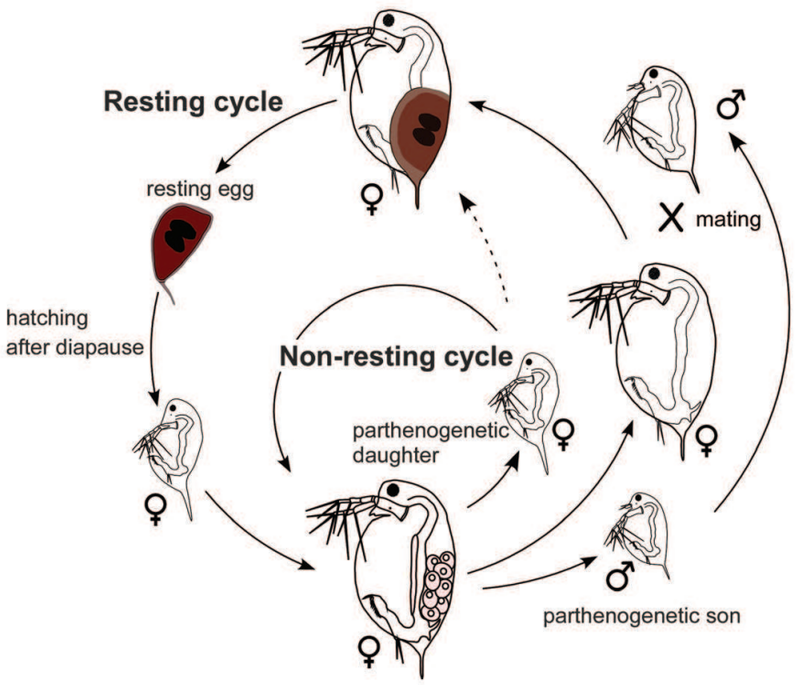


Fig. 2. Life cycle of *D. pulex*. Life cycle can be divided into 'resting cycle' and 'non-resting cycle'. In natural populations, parthenogenesis is the common mode of reproduction, and parthenogenetic offspring are normally female (parthenogenetic non-resting cycle). However, in response to unfavorable conditions, such as crowding or seasonal change, male offspring are also produced parthenogenetically, and then sexual reproduction occurs (sexual resting cycle). Although a female usually produces resting eggs requiring fertilization by sperm, it sometimes happens that a female produces parthenogenetic resting eggs (parthenogenetic resting cycle, dashed arrow).

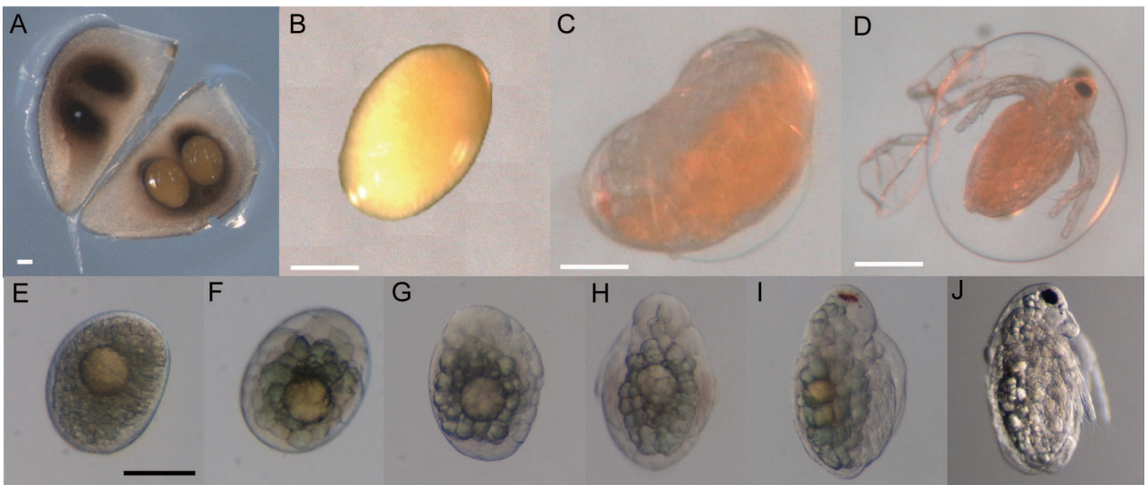


Fig. 3. Embryogenesis of resting egg (top row) and non-resting egg (bottom row). (A-D) The resting egg was produced by parthenogenesis. There was no difference in the manner of development between a parthenogenetic resting egg and a sexual resting egg. Scale bars = 100 µm. (E-J) Embryonic development was completed in about 3 days at 18°C. Scale bar = 100 µm.

There are two types of eggs, i) **resting egg**, also termed 'winter egg', 'diapause egg', 'dormant egg' or 'ephippial egg', ii) **non-resting egg**, also known as 'summer egg' or 'subitaneous egg', as mentioned above (Figs. 2 and 3). In any maturation stage they are easily distinguishable from each other in the ovary of the living animal. Although the non-resting eggs produce both female and male neonates, the resting eggs produce female offspring without exception.

Examination of the literature on reproduction of this species reveals a great deal of confusion regarding the relationship between the types of egg and reproductive modes. Interestingly, Hobæk & Larsson (1990) reported that the formation of male offspring and resting eggs are independently controlled, possibly by distinct sets of environmental cues. We have also observed parthenogenetic resting eggs in *D. pulex* (Fig. 2, dashed arrow) and confirmed that the eggs have the potential to develop normally (Fig. 3A-D). Most daphnids are believed to switch to sexual reproduction resulting in the production of resting eggs; however, several daphnid species, not forgetting *D. pulex*, can produce them without fertilization. Moreover, the switch to sexual reproduction involves a commitment by the mother to produce a resting egg case (ephippium) and to produce eggs capable of diapause. Thus, the decision to produce a non-resting or a resting egg must be made long before the point at which the egg is fertilized. So far, it is clear that 1) both non-resting and resting eggs are produced by parthenogenesis, 2) resting eggs are also produced by sexual reproduction. Whether non-resting eggs can be produced by sexual reproduction or not has never been studied.

2.2 *Daphnia pulex* as an evolutionary developmental biology (evo-devo) model

Daphnia pulex is an ideally suited laboratory animal for workers in the fields of development and genetic research: It is easy to raise under laboratory conditions, propagates quickly because of its short reproductive cycle, and can induce male offspring by a juvenile hormone, methyl farnesoate (Olmstead & Leblanc, 2002). In addition, new experimental techniques (e.g., *in situ* hybridization, immunofluorescence, microinjection, RNAi) were established in daphnid species over the past several years (Sagawa *et al*., 2005; Tsuchiya *et al*., 2009; Kato *et al*., 2011). Moreover, a recent description of the complete genome sequence for *Daphnia pulex* (Colbourne *et al*., 2011) and genetic linkage map (Cristescu *et al*., 2006) will provide us with a powerful tool for analyzing the molecular mechanism of any aspect in this species, including enigmatic meiotic processes. *Daphnia pulex* has only a 200-megabase genome and as many as about 31,000 genes. Thirty-six percent of *Daphnia pulex* genes have no detectable homologs with other animal species and about 13,000 genes have been identified as paralogs. There will be a good chance to find novel genes which enable the evolution of a unique reproductive strategy in this species. For these reasons, *D. pulex* started to garner attention as an evo-devo model animal (Jenner & Wills, 2007).

3. Reproductive mode of *D. pulex*

Daphnia pulex adopt parthenogenesis and sexual reproduction differentially in response to varied environmental cues as mentioned above. The production of diploid progenies is a common finding in both reproductive modes.

3.1 Parthenogenesis

The reproductive modes of *D. pulex* were studied over the past century. Previous studies suggested that *D. pulex* produces parthenogenetic eggs via apomixis; the nuclear division of mature oocytes should be an equational division equal to somatic mitosis (Kühn, 1908; Ojima, 1954, 1958; Zaffagnini & Sabelli, 1972). However, due to the presence of a large amount of yolk and the minute size of chromosomes, it was not easy to observe the nucleus during the process of oogenesis and therefore the behavior of chromosomes in the ovarian egg remained undescribed. In spite of previous reports that suggested the occurrence of mitosis in parthenogenetic oocytes, we found "abortive meiosis" instead during the oogenesis of parthenogenetic *D. pulex*, as mentioned below (see section 5). This finding suggests that parthenogenetic *D. pulex* may switch to sexual reproduction by progressing the maturation division of oocytes from arrest. Moreover, it would give parthenogenetic *D. pulex* a mechanism making recombination possible even under parthenogenesis. In other words, it can lead to offspring with genetic variability because chromosomal recombination can take place between homologous chromosomes, while there is no introduction of new genes from another individual.

3.2 Sexual reproduction

At present very little is known about the process of meiosis and fertilization during sexual reproduction in this species. Although it still is a matter of debate, Ojima (1958) reported that sperm seemed to penetrate into the ovarian egg. The structure of the spermatozoa is very atypical in daphnid species. The mature sperm of *D. pulex* lacks a flagellum and is therefore not actively mobile. Indeed, during mating, the male deposits sperm near the openings of the female gonopore. Studies are needed to clarify the timing of fertilization and the process of meiosis. It will provide us not only with insight into the switching mechanism of reproductive modes, but also with the differences between normal meiosis and abortive meiosis.

4. Oogenesis of parthenogenetic *D. pulex*

In this section, we offer a close overview of the growth and maturation of the parthenogenetic eggs. Mature and spawned eggs in the same brood were the same size and mostly synchronized in the maturation stage (cell cycle). It takes approximately 60 h for oocytes to grow in the ovary (from 0 to 60 h, Fig. 4). Inclusions such as yolk granules and oil droplets increased in size and number until the fully-grown egg is formed. The nuclear division apparatus appears just after molting (at 0 min after molting (0 AM): Fig. 4). We observed the precise states of nuclei in the eggs with the following timing during the course of parthenogenesis (from egg maturation to early development): 1) the time of molting of the female (Fig. 4, 0 AM), 2) the interval between molting and 13 min after molting. The parthenogenetic eggs began to migrate from the ovary to the brood chamber and this process was completed within about 3 min, 3) the time when oviposition was completed (Fig. 4, 0 min post oviposition (0 PO)), and 4) the time during which the parthenogenetic eggs in the brood chamber began to develop.

At the stage of 0 h of egg maturation, the oocyte was at first morphologically indistinguishable from the nurse cell (Fig. 5A). In early stages, the development of both

oocytes and nurse cells proceeds in a similar manner, but only the egg cells developed to form yolk granules and oil droplets during maturation, whereas the nurse cells became smaller in size and finally degenerated (Fig. 5B-D). With the passage of time, the yolk granules and oil droplets increased in number in the oocytes. Soon after grown juveniles were discharged from the brood chamber, the nuclear membrane of the fully-matured ovarian egg to be spawned gradually disappeared, and finally the breakdown of the germinal vesicle took place just before molting of the mother (Fig. 5E).

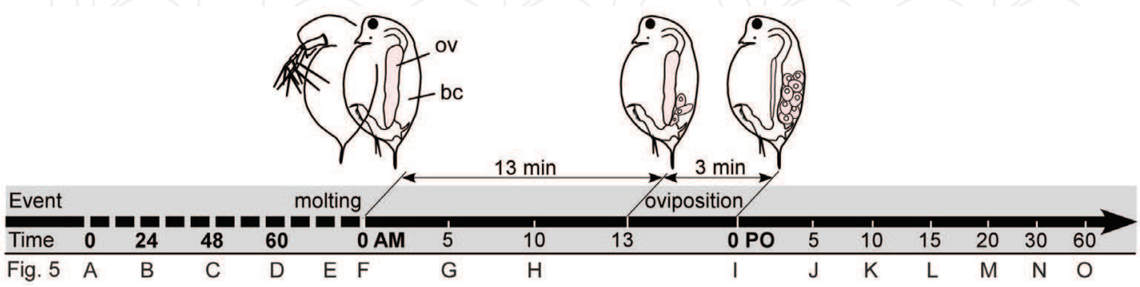


Fig. 4. The time course of parthenogenesis in *D. pulex*. The oviposition cycle is about 3 days at 18°C. The broken line of the time axis (from 0 to 60) indicates the time scale in hours, and the solid line of time axis (from 0 AM to 60 PO) indicates the time scale in minutes. Usually, after releasing all neonates that developed in the dorsal brood chamber, females molt. After this molt occurs at 0 AM (minutes after molting), a strict time course proceeds. The female begins extruding eggs into the brood chamber at 13 AM and this process is completed within about 3 min. The point of 0 PO (minutes post oviposition) indicates the time when the female extruded the last egg. Then, the parthenogenetic eggs in the brood chamber develop into juveniles. Ovarian and spawned eggs in a clutch are approximately the same size and mostly synchronized in the cell cycle. In the lower part of Fig. 4, the capital letters show the point of time when the specimens were observed in Fig. 5. ov, ovary; bc, brood chamber. Modified from Hiruta *et al*., 2010.

The chromosomes co-oriented in a position midway between the poles, and then each bivalent started to separate into two half-bivalents, one moving to each pole of the spindle by 5 AM (Fig. 5F, G). However, the movement of chromosomes from the metaphase plate to the poles was arrested at an early stage of anaphase before 10 AM (Fig. 5H). Egg laying (oviposition) began at 13 AM. The migration of all eggs from the ovary to the brood chamber was completed within about 3 min (= 0 PO). At 0 PO, the chromosomes moved back and assembled as a diploid equatorial plate around the equator of the spindle in the spawned egg (Fig. 5I). By 5 PO, the division apparatus migrated to the periphery and the cell division cycle restarted. Then the sister chromatids moved apart, one going to each pole of the spindle through metaphase and anaphase by 10 PO (Fig. 5J, K). The complete set of chromosomes was lifted above the egg surface and eventually one polar body-like small daughter cell was extruded at around 20 PO (Fig. 5L, M).

After the completion of oogenesis, the chromosomes left in the egg moved deeper inside the egg (Fig. 5N) and mitosis occurred without cytokinesis, resulting in a polynuclear syncytial embryo (Fig. 5O). Then, the nuclei migrated to the periphery, and a typical superficial cleavage proceeded.

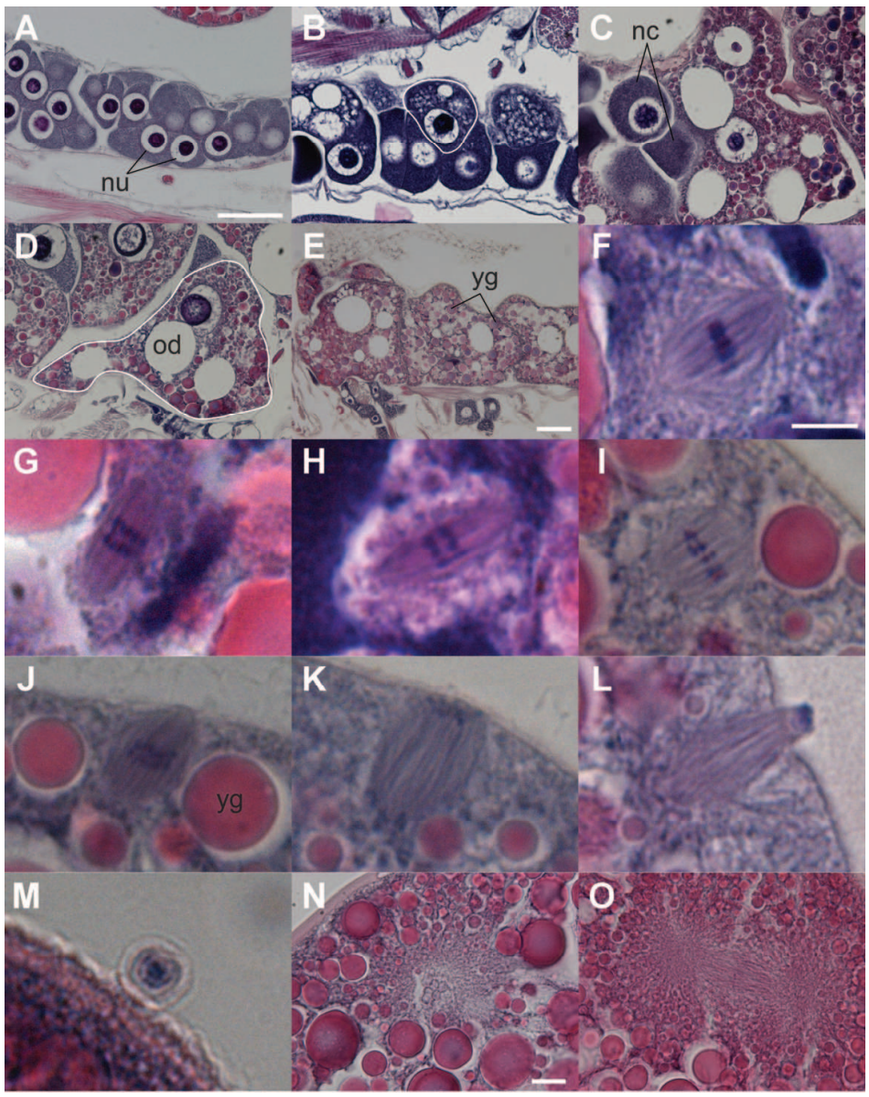


Fig. 5. Oogenesis of parthenogenetic *D. pulex*. (A-E) Oocytes and nurse cells in the ovary. (F-H) The division apparatus in ovarian eggs. (I-O) The division apparatus in eggs spawned to the brood chamber. (A) 0 h. The oocytes and nurse cells were indistinguishable. (B) 24 h. Yolk and oil droplet formation took place only in oocytes. (C) 48 h. The yolk granules and oil droplets increased. (D) 60 h. The degeneration of nurse cells proceeded. (E) Just before molting. The breakdown of the germinal vesicle (GVBD) took place. (F) 0 AM. Division apparatus appeared and chromosomes aligned at the metaphase plate. (G) 5 AM. Each bivalent is separated into two half-bivalents. (H) 10 AM. The division seemed to stop at early anaphase. (I) 0 PO. The chromosomes moved back and rearranged around the equator of the spindle. (J) 5 PO. The chromosomes started to separate. (K) 10 PO. The division proceeded to anaphase. (L) 15 PO. One complete set of chromosomes was lifted above the egg surface. (M) 20 PO. A polar body-like small daughter cell was extruded. (N) 30 PO. The swelled chromosomes left in the egg moved to a deeper part. (O) 60 PO. The first cleavage proceeded without cytokinesis. Scale bars = 100 μm in A-E; 5 μm in F-M; 10 μm in N and O. nc, nurse cell; nu, nucleus; od, oil droplet; yg, yolk granule. Solid white circle indicates an oocyte. Modified from Hiruta *et al*., 2010 and unpublished data.

5. Abortive meiosis found in parthenogenetic *D. pulex*

Parthenogenetic eggs achieve two successive divisions like normal meiosis while the first division is abortive (see section 4). In the first meiosis, bivalents align at the equatorial plate (Fig. 6A) and begin to separate into two half-bivalents (Fig. 6B). Then, each half-bivalent moves back and sister chromatids rearrange as a diploid equatorial plate around the equator of the spindle (Fig. 6C). Finally, the second meiosis-like division takes place normally, producing a single polar body-like extremely small daughter cell (Fig. 6D). Compared with meiosis, it is known that the first meiotic division is skipped there.

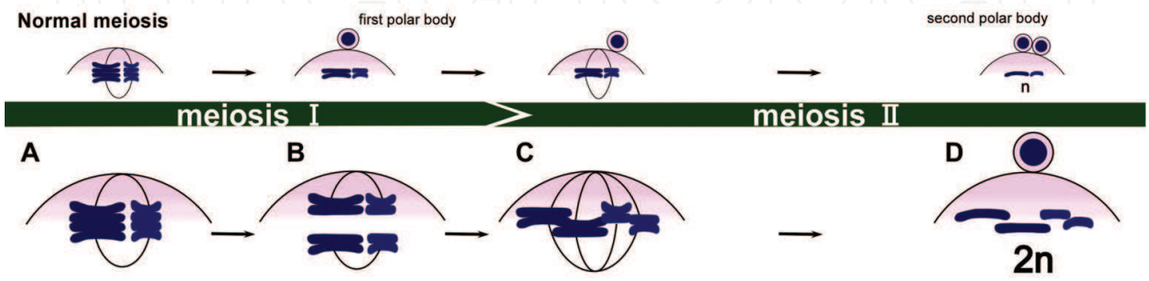


Fig. 6. Schematic illustration of parthenogenesis in *D. pulex*. The top row shows the process of meiosis. In the first meiosis, bivalents aligned at the metaphase plate and separate into two half-bivalents, producing the first polar body. Then, the second meiosis takes place, producing the second polar body. As a result, a haploid egg is produced. By contrast, a diploid egg is produced by parthenogenesis in *D. pulex*. (A) In the first meiosis, bivalents align at the equatorial plate and begin to separate into two half-bivalents. (B) However, the division is arrested at early anaphase. (C) Then each half-bivalent moves back and sister chromatids rearrange as a diploid equatorial plate around the equator of the spindle. (D) Finally, the second meiosis-like division takes place normally, producing a single polar body-like extremely small daughter cell. Illustration adopted from Hiruta *et al*., 2010.

5.1 Hypothetical model for reproductive strategy in *D. pulex*

We hypothesized that *D. pulex* switches its reproductive mode (sexual or parthenogenetic) depending on whether the egg is fertilized or not. It is highly plausible that, if the egg is not fertilized, the first meiosis is aborted and, subsequently, a second meiosis-like division takes place as observed in our study. On the other hand, normal meiosis may well occur if the ovarian egg is fertilized. If this is true, it seems appropriate to assume that fertilization occurs at the stage between first metaphase and anaphase in the ovarian egg.

Since the two reproductive modes (sexual reproduction or parthenognesis) are not strictly associated with the two types of egg (resting or non-resting egg) as mentioned in section 2.1.2, a study will need to be conducted to verify the hypothesis including whether the non-resting egg is produced by sexual reproduction. If this hypothesis is true, daphnid species adopt the least waste system in the production of eggs, because eggs are able to develop regardless of fertilization. In order to verify this hypothesis, we are currently trying to establish the methods for *in vitro* maturation of ovarian eggs and artificial insemination.

6. Spindle assembly and spatial distribution of γ-tubulin during abortive meiosis in parthenogenetic *D. pulex*

During the analysis of nuclear maturation division, which resulted in either parthenogenetic or sexual development of the egg, we found that the spindle in abortive meiosis was barrel-shaped, anastral, and organized without centrosomes (Fig. 7, Hiruta C., unpublished). Corresponding to our results, a barrel-shaped meiotic spindle without centrosomes has been reported in several animal species including parthenogenetic pea aphid (Riparbelli *et al*., 2005). The centrosomes are present in cleavage division (mitosis) of both reproductive modes. In the case of sexual reproduction in various animals, the sperm supplies the centriole after fertilization. On the other hand, in the case of parthenogenesis in insects, centrosomes are spontaneously assembled in mitotic spindle microtubules. We suspect that *de novo* assembled centrosomes could be an evolutionary conserved process leading to parthenogenetic development.

Even more surprisingly, gamma (γ)-tubulin is localized along spindle microtubules for the duration of abortive meiosis, while it is present only on the centrosomes in parthenogens' cleavage division (Fig. 7, Hiruta C., unpublished). The results from *D. pulex* are identical with those from pig oocytes, but not universal among animals (Lee *et al*., 2000). Incidentally, the localization of γ-tubulin to centrosomes corresponds to a typical spindle formation which is highly conserved in animals. Comparative research needs to be conducted to reveal whether sperm entry affects the localization of γ-tubulin in *D. pulex* or not.

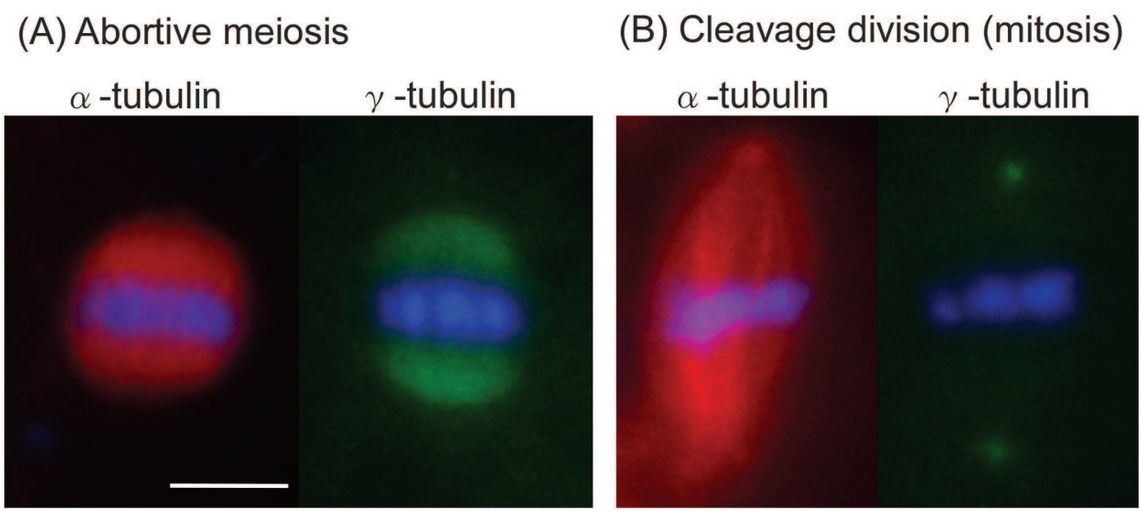


Fig. 7. Immunofluorescence localization of α- and γ-tubulin during abortive meiosis and cleavage division (mitosis). Scale bar = 5 μm. Chromosomes were counter-stained with DAPI (blue). (A) Spindle formation was barrel-shaped, anastral, and organized without centrosomes. Gamma-tubulin was distributed along spindle microtubules during abortive meiosis. (B) Spindle formation was spindle-shaped, astral, and had organized centrosomes. Gamma-tubulin was present only the spindle poles. Hiruta C., unpublished.

7. Molecular basis of the transition from sexual reproduction to parthenogenesis

There are variations of the meiotic program that would produce diploid oocytes by skipping a division, by fusion of a haploid oocyte with a polar body, or by premeiotic endoreplication of chromosomes (Suomalainen *et al*., 1987; Schön *et al*., 2009). At any rate, it is thought that parthenogenesis evolved from sexual reproduction by changing the meiotic program. It seems likely that a relatively simple deviation from the established program of oogenesis, *i.e.*, meiosis, is sufficient to permit parthenogenesis. Schurko *et al*. (2009) reported that expression patterns of meiosis-related and meiosis-specific genes (*e.g.*, SMCs, REC8) during sexual reproduction of *D. pulex* are similar to that during parthenogenesis. Some of these genes are present in multiple copies and might be expressed differently in sexual reproduction and parthenogenesis. To cite one example, there are seven RECQ2 copies, which limit crossing over, which could have evolved novel roles in parthenogenesis. In fact, as if to correspond to the expectation, we have so far failed to observe chiasmata where crossing over occurred. In addition, it is suggested that PLK1, which is involved in orienting kinetochores during mitosis and meiosis, controls the localization within the spindle of γ-tubulin for mitotic spindle formation through the augmin complex in human and *drosophila* cells (Goshima *et al*., 2008; Uehara *et al*., 2009). As mentioned in section 6, the γ-tubulin is localized along the spindle in abortive meiosis. There is a possibility that a PLK copy is expressed in parthenogenesis specifically to operate the localization of γ-tubulin. We expect to reveal how the meiotic program is altered resulting in parthenogenesis. On the other hand, the absence of meiosis-specific DMC1 suggests that innovations for recombination in meiosis and parthenogenesis in *D. pulex* may have evolved. In the last paragraph, we stated factors associated with regulation of chromosomal organization. The following discussion is about the cell cycle control factors. There are several copies of cell cycle proteins, such as cyclins, cdks and polo kinases in *D. pulex*. In particular, the Mos-MAPK (mitogen-activated protein kinase) pathway, which is responsible for metaphase arrest in meiosis I or II before fertilization in many animal species (Sagata, 1996), might be a candidate for cell cycle arrest in parthenogenetic *D. pulex*. We have preliminary data that MAP kinase is expressed in *D. pulex* oocyte in metaphase of meiosis I.

Consequently, a precise description of the reproductive modes in *D. pulex* allows us to understand the mechanism which is widely preserved in eukaryotes and in the *Daphnia*-specific mechanism, namely, commonality and diversity of the pattern of division.

8. How does the transition from sexual reproduction to parthenogenesis occur during evolution?

Parthenogenesis, which is thought to arise from the alteration of meiosis, has been found in various animal species (Suomalainen *et al*., 1987; Schön *et al*., 2009). In many taxonomic groups, parthenogenesis was independently acquired during evolution. By comparing those cases, we could gain insight into the transition from sexual reproduction to parthenogenesis, many of which resulted from the change made during meiosis. According to Suomalainen *et al.* (1987), the following cases have been categorized as the type of 'skipped the first meiosis' (Fig. 8).

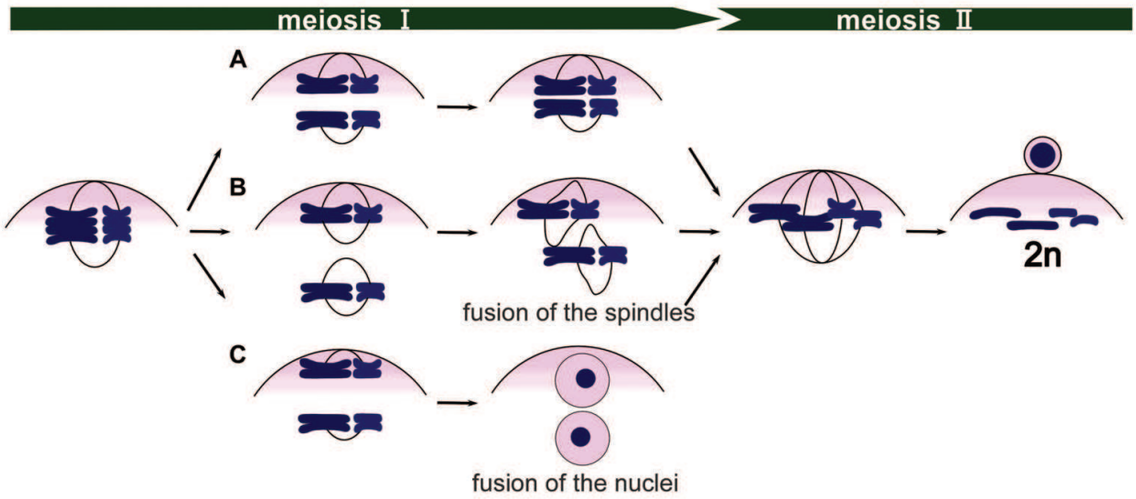


Fig. 8. Different types of abortive meiosis. These are categorized as 'skipped the first meiosis'. (A) *Daphnia pulex*. Two half-bivalents move back to the equatorial plate. (B) *Apterona helix*. Two spindles, an inner and outer, come together side by side and fuse. (C) *Fasciola hepatica*. Two haploid nuclei fuse.

In Lepidoptera *Apterona helix* (Fig. 8B), the first meiosis is aborted at the end of anaphase and two metaphase plates, an inner and outer, are formed. Both of these contain the haploid number of chromosomes and have separate spindles. Before the second meiosis, the inner metaphase spindle with its chromosome plate moves to the side of the outer spindle. Finally, the two spindles and the metaphase plates lie side by side and fuse (Narbel, 1946). In Crustacea *Artemia salina,* Lepidoptera *Solenobia lichenella, Luffia ferchaultella* and *L. lapidella*, meiosis is interrupted at some stage between the end of the first anaphase and the second metaphase (Narbel-Hofstetter, 1950, 1963, 1965; Stefani, 1960). Then the two haploid plates reunite, forming a new metaphase spindle, and the second diploid meiosis is accomplished. In Trematoda *Fasciola hepatica* (Fig. 8C), the first meiosis occurs without cytokinesis, giving rise to two haploid nuclei. These nuclei fuse and form a diploid cleavage nucleus (Sanderson, 1952).

There are differences at the stage of division arrest and restoration or maintenance of diploidy even in the same division that skipped the first meiosis. At any rate, if first meiosis is completely skipped, parthenogenetic division becomes congruent with mitosis and finally turns out to be obligate parthenogenesis. A comparative and detailed study of the parthenogenetic mechanism will bring us to a better understanding of the possible alteration of meiosis during evolution.

9. Conclusion

In conclusion, *D. pulex* is suitable for experimentation to understand the evolution of reproductive modes from a viewpoint of evolutionary developmental biology. The case study in *D. pulex* will contribute to a discussion of challenges such as how does parthenogenesis work and how evolution of sexual reproduction and parthenogenesis occurs.

10. Acknowledgments

We are grateful to the members of the Tochinai laboratory for their helpful advice and discussion. We also thank a great number of *D. pulex* that made a sacrifice for our research. CH was supported by a JSPS Research Fellowships for Young Scientists (No. 09J01653). This work was supported by a Grant-in-Aid for Exploratory Research, financed by the Ministry of Education, Culture, Sports, Science and Technology, Japan to ST (No. 21657054).

11. References

Colbourne, JK., Pfrender, ME., Gilbert, D., Thomas, WK., Tucker, A., Oakley, TH., Tokishita, S., Aerts, A., Arnold, GJ., Basu, MK., Bauer, DJ., Cáceres, CE., Carmel, L., Casola, C., Choi, JH., Detter, JC., Dong, Q., Dusheyko, S., Eads, BD., Fröhlich, T., Geiler-Samerotte, KA., Gerlach, D., Hatcher, P., Jogdeo, S., Krijgsveld, J., Kriventseva, EV., Kültz, D., Laforsch, C., Lindquist, E., Lopez, J., Manak, JR., Muller, J., Pangilinan, J., Patwardhan, RP., Pitluck, S., Pritham, EJ., Rechtsteiner, A., Rho, M., Rogozin, IB., Sakarya, O., Salamov, A., Schaack, S., Shapiro, H., Shiga, Y., Skalitzky, C., Smith, Z., Souvorov, A., Sung, W., Tang, Z., Tsuchiya, D., Tu, H., Vos, H., Wang, M., Wolf, YI., Yamagata, H., Yamada, T., Ye, Y., Shaw, JR., Andrews, J., Crease, TJ., Tang, H., Lucas, SM., Robertson, HM., Bork, P., Koonin, EV., Zdobnov, EM., Grigoriev, IV., Lynch, M. & Boore, JL. (2011). The ecoresponsive genome of *Daphnia pulex*. *Science*, Vol.331, No.6017, pp.555-561

Cristescu, MEA., Colbourne, JK., Radivojac, J. & Lynch, M. (2006). A microsatellite-based genetic linkage map of the waterflea, *Daphnia pulex*: On the prospect of crustacean genomics. *Genomics*, Vol.88, No.4, pp.415-430

Decaestecker, E., Meester, LD. & Mergeay, J. (2009). Cyclical parthenogenesis in *Daphnia*: Sexual versus asexual reproduction, In: *Lost sex: The evolutionary biology of parthenogenesis*, Schön, I., Martens, K. & Dijk, PV., pp.295-316, Springer, ISBN 978-90-481-2769-6

Goshima, G., Mayer, M., Zhang, N., Stuurman, N. & Vale, RD. (2008). Augmin: a protein complex required for centrosome-independent microtubule generation within the spindle. *J Cell Biol.*, Vol.181, No.3, pp.421-429

Hebert, PDN. (1978). The population biology of *Daphnia* (Cruatacea, Daphnidae). *Biol. Rev.*, Vol.53, pp.387-426

Hiruta, C., Nishida, C. & Tochinai, S. (2010). Abortive meiosis in the oogenesis of parthenogenetic *Daphnia pulex*. *Chromosome Res.,* Vol.18, No.7, pp.833-840

Hobæk, A. & Larsson, P. (1990). Sex determination in *Daphnia magna*. *Ecology*, Vol.71, No.6, pp.2255-2268

Jenner, RA. & Wills, MA. (2007). The choice of model organisms in evo-devo. *Nat Rev Genet*, Vol.8, No.4, pp.311-319

Kato, Y., Shiga, Y., Kobayashi, K., Tokishita, S., Yamagata, H., Iguchi, T. & Watanabe, H. (2011). Development of an RNA interference method in the cladoceran crustacean *Daphnia magna*. *Dev Genes Evol*, Vol.220, pp.337-345

Kühn, A. (1908). Die Entwicklung der Keimzellen in den parthenogenetischen Generationen der Cladoceren *Daphnia pulex* de Geer und *Polyphemus pediculus* de Geer. *Arch Zellforsch*, Vol.1, pp.538-586

Lee, J., Miyano, T. & Moor, RM. (2000). Spindle formation and dynamics of γ-tubulin and nuclear mitotic apparatus protein distribution during meiosis in pig and mouse oocytes. *Biol Reprod*, Vol.62, No.5, pp.1184-1192

Narbel, M. (1946). La cytologie de la parthénogenèse chez *Apterona helix* Sieb. (Lepid, Psychides). *Rev. Suisse Zool*, Vol.53, pp.625–681

Narbel-Hofstetter, M. (1950). La cytologie de la parthénogenèse chez *Solinobia* sp. (*lichenella* L.?) (Lépidoptères, Psychides). *Chromosoma*, Vol.4, pp.56–90

Narbel-Hofstetter, M. (1963). Cytologie de la pseudogamie chez *Luffia lapidella* Goeze (Lepidoptera, Psychidae). *Chromosoma*, Vol.13, pp.623-645

Narbel-Hofstetter, M. (1965). La variabilité cytologique dans la descendance des femelles de *Luffia ferchaultella* Steph. (Lepidoptera, Psychidae). *Chromosoma*, Vol.16, pp.345–350.

Ojima, Y. (1954). Some cytological observations on parthenogenesis in *Daphnia pulex* (de Geer). *Jour Fac Sci Hokkaido Univ*, *Ser.VI, Zool.12*, pp.230-241

Ojima, Y. (1958). A cytological study on the development and maturation of the parthenogenetic and sexual eggs of *Daphnia pulex* (Crustacea, Cladocera). *Kwansei Gakuin Univ*, Annual Studies 6, pp.123-176

Olmstead, AW. & Leblanc, GA. (2002). Juvenoid hormone methyl farnesoate is a sex determinant in the crustacean *Daphnia magna*. *J Exp Zool*, Vol.293, No.7, pp.736-739

Riparbelli, MG., Tagu, D., Bonhomme, J. & Callaini, G. (2005). Aster self-organization at meiosis: a conserved mechanism in insect parthenogenesis?. *Dev Biol*, Vol.278, No.1, pp.220-230

Sagata, N. (1996). Meiotic metaphase arrest in animal oocytes: its mechanisms and biological significance. *Trends Cell Biol*, Vol.6, No.1, pp.22-28

Sagawa, K., Yamagata, H. & Shiga, Y. (2005). Exploring embryonic germ line development in the water flea, *Daphnia magna*, by zinc-finger-containing VASA as a marker. *Gene Expr Patterns*, Vol.5, pp.669-678

Sanderson, AR. (1952). Maturation and probable gynogenesis in the liver fluke, *Fasciola hepatica* L.. *Nature*, Vol.172, pp.110-112

Schön, I., Martens, K. & Dijk, PV. (2009). *Lost sex: The evolutionary biology of parthenogenesis*. Springer, ISBN 978-90-481-2769-6

Schurko, AM., Logsdon, JM Jr. & Eads, BD. (2009). Meiosis genes in *Daphnia pulex* and the role of parthenogenesis in genome evolution. *BMC Evol Biol*, Vol.9, No.78

Stefani, R. (1960). L'*Artemia salina* parthenogenetica a Cagliari. *Riv Biol*, Vol.52, pp.463-490

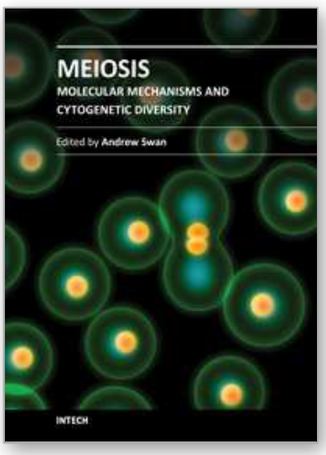
Suomalainen, E., Saura, A. & Lokki, J. (1987). *Cytology and evolution in parthenogenesis*. CRC Press, ISBN 0-8493-5981-3, Boca Raton, FL

Tollrian, R. & Dodson, SI. (1999). Inducible defenses in Cladocera: Constraints, costs, and multipredator environments, In: *The ecology and evolution of inducible defenses*, Tollrian, R. & Harvell, CD., pp.177-202, Princeton, ISBN 0-691-00494-3, UK

Tsuchiya, D., Eads, BD. & Zolan, ME. (2009). Methods for meiotic chromosome preparation, immunofluorescence, and fluorescence *in situ* hybridization in *Daphnia pulex*, In: *Meiosis, Volume 2: Cytological Methods, vol. 558,* Keeney, S., pp.235-249, Springer, ISBN 978-1-60761-103-5, USA

Uehara, R., Nozawa, RS., Tomioka, A., Petry, S., Vale, RD., Obuse, C. & Goshima, G. (2009). The augmin complex plays a critical role in spindle microtubule generation for mitotic progression and cytokinesis in human cells. *Proc Natl Acad Sci*, Vol.106, No.17, pp.6998-7003

Zaffagnini, F. & Sabelli, B. (1972). Karyologic observations on the maturation of the summer and winter eggs of *Daphnia pulex* and *Daphnia middendorffiana*. *Chromosoma*, Vol.36, No.2, pp.193-203



Meiosis - Molecular Mechanisms and Cytogenetic Diversity
Edited by Dr. Andrew Swan

ISBN 978-953-51-0118-5
Hard cover, 472 pages
Publisher InTech
Published online 29, February, 2012
Published in print edition February, 2012

Meiosis, the process of forming gametes in preparation for sexual reproduction, has long been a focus of intense study. Meiosis has been studied at the cytological, genetic, molecular and cellular levels. Studies in model systems have revealed common underlying mechanisms while in parallel, studies in diverse organisms have revealed the incredible variation in meiotic mechanisms. This book brings together many of the diverse strands of investigation into this fascinating and challenging field of biology.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Chizue Hiruta and Shin Tochinai (2012). How Does the Alteration of Meiosis Evolve to Parthenogenesis?- Case Study in a Water Flea, Daphnia pulex -, Meiosis - Molecular Mechanisms and Cytogenetic Diversity, Dr. Andrew Swan (Ed.), ISBN: 978-953-51-0118-5, InTech, Available from: http://www.intechopen.com/books/meiosis-molecular-mechanisms-and-cytogenetic-diversity/how-does-the-alteration-of-meiosis-evolve-to-parthenogenesis-case-study-in-a-water-flea-daphnia-pule

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen