ARTICLE

Mite dilemma: molting to acquire sexual maturity or not molting to ensure durability and dispersal ability in *Phorytocarpais fimetorum* (Parasitiformes; Gamasida; Parasitidae)

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Abstract

Dispersal from birthplace is an effective strategy to cope up with unpredictable environmental change. In many animals, dispersal is carried out prior to mating and reproduction to avoid inbreeding. However, this also means that the dispersers must find mates in the new habitat. *Phorytocarpais fimetorum* (= former name *Parasitus fimetorum*) is a free-living predatory mite inhabiting animal dung and manure. In such ephemeral habitats, the mites quickly develop from egg to deutonymph as the environmentally tolerant, dispersal stage via larva and protonymph stages. After dispersal to new habitats utilizing dung beetles as phoretic hosts, deutonymphs have to decide whether to molt to adulthood by losing their tolerance and migration ability or not to molt and continue dispersal. Here, using molt-suppressing deutonymphs that were obtained under isolated conditions, I investigated whether food conditions (amount of rearing medium bearing nematodes as prey) and encounters with conspecifics stimulate their molting. A drastic increase in food resources induced molting in both sexes, but the responses to the pairing treatment were different between sexes. Whereas female deutonymphs molted at the encounter with males regardless of whether they were deutonymphs or adults, molting of male deutonymphs was induced only at the pairing with female deutonymphs (not adult females). Because adult females are usually nonvirgin and have already started oviposition, the males seemed to discriminate against them. The decision about molting is understandable as a male mate choice, which is adaptive to the limited mating opportunities in ephemeral environments.

Keywords *Parasitus fimetorum* · Developmental arrest · Decision making · Molting condition · Bet-hedging · Mate choice

Introduction

Practically all organisms live in changing environments (Levins 1968; Stearns 1992). Dispersal and migration from birthplace to another habitat are effective ways to cope up with environmental change (Ronce 2007). In many animals, such as insects living in ephemeral habitats, dispersal occurs before reproduction because the environment in which they are born quickly deteriorates and cannot sustain the next generation (Harrison 1980). The dispersal stage also can exhibit a great tolerance against unfavorable environmental conditions such as food shortage and desiccation. If the dispersers find favorable habitats, they can enter the reproductive stage.

However, dispersal ability and environmental tolerance are often lost once reproduction begins (Harrison 1980). For example, the cricket *Modicogryllus confirmatus* shows wing dimorphism as the nonmigratory short-winged morph and migratory long-winged morph (Tanaka and Suzuki 1998). After migration, the long-winged morph absorbs the flight muscles and uses the retrieved energy for egg production and thus inevitably loses flight ability (Tanaka and Suzuki 1998). Mites also show various dispersal adaptations (Hunter and Rosario 1988; Houck and Oconnor 1991). Whereas dispersal prior to reproduction has the benefit of avoiding inbreeding, it entails the potential risk that the disperser will not find mates in the new habitat. Therefore, these organisms should change to the reproductive stage only when they ensure favorable environmental conditions, such as abundant food and the existence of mate candidates.

Phorytocarpais fimetorum (Berlese) (Parasitiformes, Gamasida, Parasitidae Fig. 1), previously commonly known as *Parasitus fimetorum*, is a free-living predatory

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Fig. 1 The developmental stages of *P. fimetorum*: **a** egg, **b** larva, **c** protonymph, **d** male deutonymph, **e** adult female, **f** adult male. Deutonymphs have a thick exoskeleton that tolerates harsh environments. Adult males are very fragile

1000μm
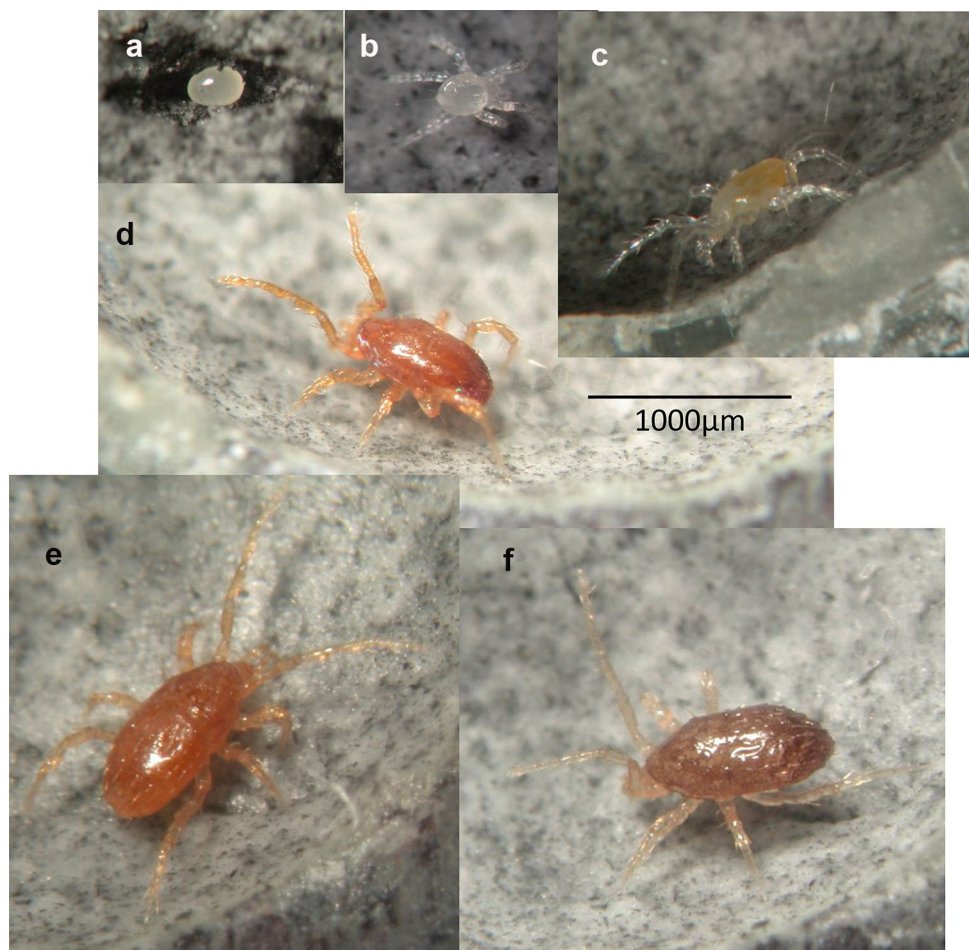
mite inhabiting animal dung and compost manure. Mites prey on fly eggs, nematodes and other small animals and quickly develop from eggs to larvae, protonymphs and deutonymphs (hereafter abbreviated to DNs) within three days in rapidly deteriorating habitats (Yasui 1993, 1997). DNs can tolerate starvation and desiccation for approximately one month, during which time they disperse to new habitats on their own legs or via phoresy on dung beetles (Aphodiinae) (Yasui 1993, 1997). In the new habitat, DNs molt to adults and lose their migration ability and tolerance (adult longevity is approximately one week Yasui 1993, 1997). In another dung-inhabiting mite, *Macrocheles muscaedomesticae* (Scopoli) (gamasida, macrochelidae), which has a haplo-diploid sex determination system, a female copulates just after adult molting with a male that has been guarding her for several hours (precopulatory mate guarding; Yasui 1988). Males die at their birth habitat after mating, and (usually mated) adult females disperse by phoresy on house flies (muscidae). In *Macrocheles*, only adult females have durability and migration ability, so developmental arrest in the immature stage does not exist. Thus, *Macrocheles* females usually finish copulation before dispersal, and even if they cannot find mates, virgin females can produce male offspring

by arrhenotokous parthenogenesis. In contrast, *Phorytocarpais* has a diploid–diploid genetic system and conducts only bisexual reproduction (no parthenogenesis) (Ito 1973, 1978; Yasui 1993, 1997), meaning that they have to find mates after dispersal. Therefore, I expected that *P. fimetorum* DNs will molt to adults only when they encounter mate candidates under promising food conditions. If DNs molt to adults without satisfying these conditions, the emerged adults will die without reproduction. Herein arises the dilemma to molt or not to molt.

In this study, I examined whether *P. fimetorum* DNs that halted development under isolated rearing conditions molt when (1) food availability was drastically improved and when (2) they encountered another conspecific individual (same or different sex, DN or adult).

Materials and methods

Mites

The population of *P. fimetorum* used in this study was collected from a compost pile in the Faculty of Agriculture,

Kagawa University, Shikoku Island, Japan (N34°16 ′35.86″, E134°07′ 29.58″, altitude 24 m) in April 2002 and established by approximately 100 wild-caught DNs. The population was maintained on an artificial medium, which consisted of moistened wheat bran, rice husks and compound food for rabbits, in a plastic container (125 mm in diameter × 50 mm in depth; lid with an opening of 40 × 50 mm and covered by fine polyester mesh; the window size could be altered with vinyl tape according to the moisture inside) and kept in an incubator at 25 ± 1 °C, 16L8D. Free-living saprophagous nematodes (Rhabditida) were allowed to reproduce in this medium as prey for the mites. Several containers, each containing hundreds of mites, were maintained as stock cultures. The experiments were carried out at the Laboratory of Entomology, Faculty of Agriculture, Kagawa University, from April 2002 to January 2003.

To collect newly laid eggs, one or two pairs of adults were introduced from the stock culture into a plastic container (36 mm in diameter × 20 mm in depth; lid with an opening of 5 mm in diameter covered by fine polyester mesh) equipped with a plaster-charcoal substratum on the bottom. A small piece of moistened rabbit food containing nematodes was fed on a 10 × 10 mm aluminum foil. Females preferred to lay eggs on loosened absorbent cotton fiber. The eggs were isolated individually in a glass vial (18 mm in diameter × 45 mm in depth, equipped with a plaster-charcoal substratum and a food piece) and incubated. I prepared ca. 500 vials in total as the sample source. Every day, I checked the vials and obtained the DNs of both sexes that molted within the past 24 h and which had not yet encountered other individuals, referred to as 0-day-old solitary DNs. They were sexed on the basis of morphological differences on the upper ventral surface of the idiosoma (the location of the genital opening in adult males; Fig. 2).

Experiment 1: molting of solitary deutonymphs when food conditions were drastically improved

I investigated whether a drastic increase in food induced the molting of DNs even under solitary conditions. Each individual from the sample source was fed a small (3 mm in diameter) piece of fresh rabbit food containing nematodes on a 5 × 5 mm aluminum foil in the glass vial. This food amount was sufficient for the development of this single individual until DN but insufficient for his/her offspring population. For individuals in the sample source, the food was renewed every day, and the molting to adulthood was checked for 10 days. This rearing condition with moderate amount of foods were used as a control. I established three treatment groups. At the various times of ontogeny (protonymphs ca. two days before molting to DN, 3-day-old and 6-day-old DNs), a mite was transferred from the sample source into a plastic container (36 mm in diameter × 20 mm in depth;
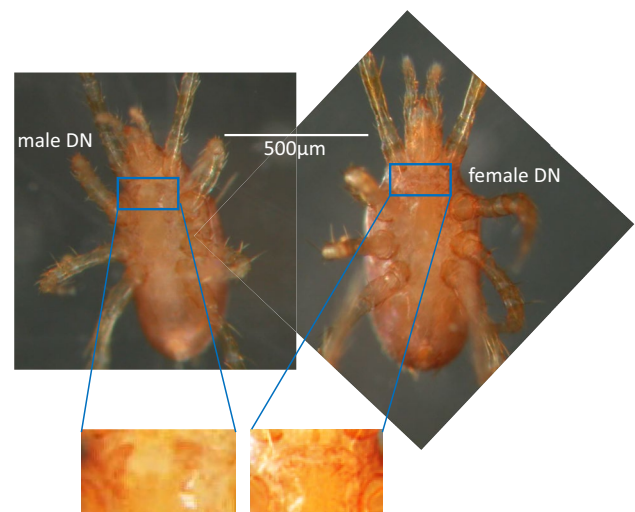

Fig. 2 Sexual differentiation in the deutonymph stage of *P. fimetorum*. A light-colored area appears between the dark-colored triangles on the ventral surface (the anterior region of sternum) in males

lid with a meshed window) full of fresh rearing medium. A piece of the nematode-bearing rabbit food was added to the container, resulting in an explosive increase in nematodes (approximately 1500 times as many as in the control). Sample sizes: protonymph, $n = 7$ females and $n = 3$ males (sexed after their molting to DNs); 3-day-old DN, $n = 4$ females and $n = 5$ males and 6-day-old DN, $n = 11$ females and $n = 13$ males. The control group consisted of 234 DNs (135 females and 99 males). I intermittently checked the molting to adulthood for two or three weeks after the start of the experiment.

Experiment 2: Molting of deutonymphs at pairing with various counterparts

In the sample source, hundreds of DNs arrested their molting for 10 days or more in the moderate food condition (i.e., as the control in Experiment 1). I placed a pair of individuals in a glass vial with a plaster-charcoal substratum in the following combinations: (1) two arresting DNs of the same sex, (2) two arresting DNs of different sexes and (3) one arresting DN + one adult of the opposite sex. As a control, DNs were kept in solitary condition (this was a replicate of the control in Experiment 1 but continued for a longer period). The mites were kept in moderate food conditions. I checked all DNs every day until their molting or death. If one of the pairing partners died within 4 days from the start of the experiment, the data were excluded from the analyses. Sample sizes: combination (1) 19 vials each containing a female pair, thus $n = 38$ females and 10 vials each containing a male pair, thus $n = 20$ males, combination (2) 18 vials each containing a heterosexual pair, thus $n = 18$ females and $n = 15$ males (3 were excluded, see Results), combination (3)

16 vials each containing a female DN and a male adult, thus $n=16$ females and 11 vials each containing a male DN and a female adult, thus $n=11$ males and control, 24 vials each containing a female, thus $n=24$ females and 9 vials each containing a male, thus $n=9$ males.

For statistical analyses of molting schedule, survival analysis (Cox proportional hazards model) in JMP ver. 10.0.2 for Windows was used. To compare the final molting rate between treatments, Fisher's exact probability test with Holm−Bonferroni sequential correction was used.

Results

Experiment 1: Effects of food conditions on molting

Figure 3 shows the cumulative molting rate of solitary DNs. Under moderate nutrient conditions (control), solitary DNs exhibited a low molting rate (Fig. 3d). The cumulative molting rate on the 10th day was 3 times higher in males (26/99) than in females (10/135). Survival analysis (Cox proportional hazards model) detected a highly significant difference between sexes (no. parameters $=1$, d. f. $=1$, likelihood ratio Chi-square $=15.3555$, $P<0.0001$). No individuals died during the 10-day observation period.

However, when food resources were drastically increased, the solitary individuals quickly responded (Fig. 3a–c). More than 50% of individuals molted to adulthood within a few days. Males showed a higher molting rate than females. Survival analysis (Cox proportional hazards model) detected a highly significant difference among the four groups (Fig. 3) (no. parameters $=3$, d. f. $=3$, likelihood ratio Chi-square $=38.4473$, $P<0.0001$) and between sexes (no. parameters $=1$, d. f. $=1$, likelihood ratio Chi-square $=21.3086$, $P<0.0001$). Even if the control samples ($n=234$, 84% of all samples) were excluded from the analysis, the differences were still significant among the three treatments (Fig. 3a–c) (no. parameters $=2$, d. f. $=2$, likelihood ratio Chi-square $=7.1877$, $P=0.0275$) and between sexes (no. parameters $=1$, d. f. $=1$, likelihood ratio Chi-square $=7.1954$, $P=0.0073$).

Experiment 2: Effect of pairing on molting

Figure 4 shows the cumulative molting rate of a DN paired with another individual (two individuals in a vial). In the pairs of two DNs, molting of both individuals was tracked. Thus, "DN♀ + DN♀, $n=38$" (Fig. 4a) means that 19 pairs (38 individuals) of female DNs (19 vials) were investigated. In the pairs with one DN and one adult (A), only DNs were tracked so that "DN♀ + A♂, n $=16$" (Fig. 4c) means that 16 DNs (16 vials) were examined.
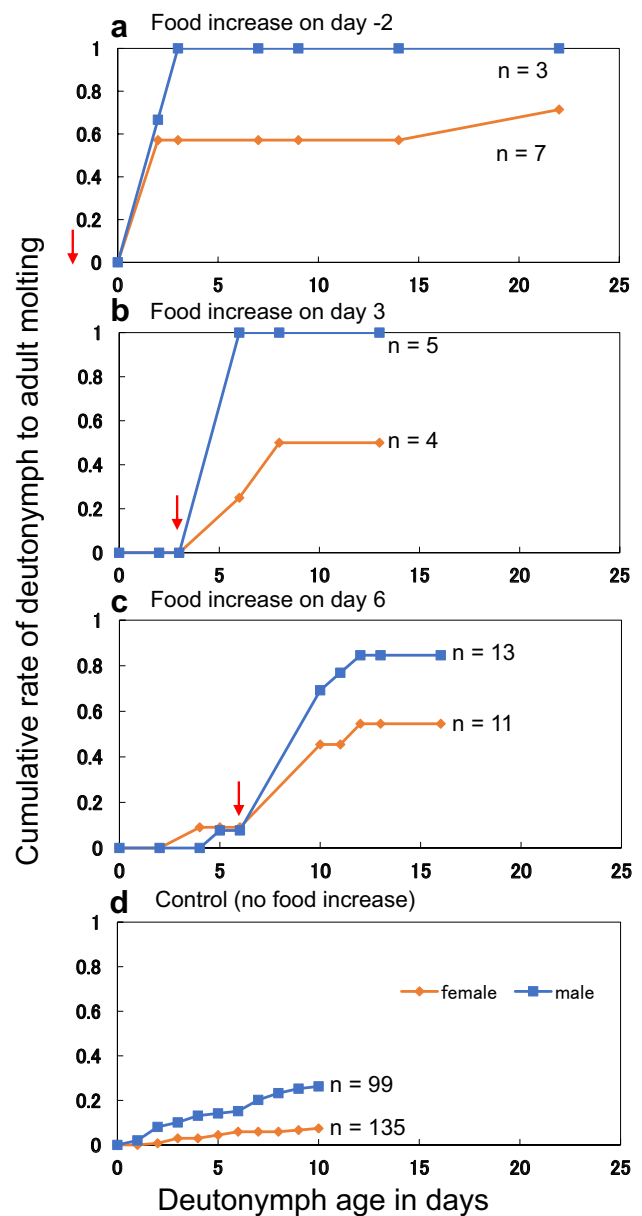

Fig. 3 The effect of nutritional improvement on the molting of solitary deutonymphs. Day 0 is the day a protonymph molts into a deutonymph. Red arrows indicate the day on which the food resource increased to a sufficient level for the deutonymphs' development and reproduction

When paired with a DN of the same sex (Fig. 4a), approximately 50% of female DNs and 40% of male DNs finally molted within a month. Survival analysis (Cox proportional hazards model) detected no significant difference in the molting rate between sexes (no. parameters $=1$, d. f. $=1$, likelihood ratio Chi-square $=0.8426$, $P=0.3587$).

Out of 18 heterosexual pairs of DNs (Fig. 4b), three females molted within a day but died before male DNs molting. Males of these 3 pairs were excluded from the analysis, so the male sample size decreased to 15. All
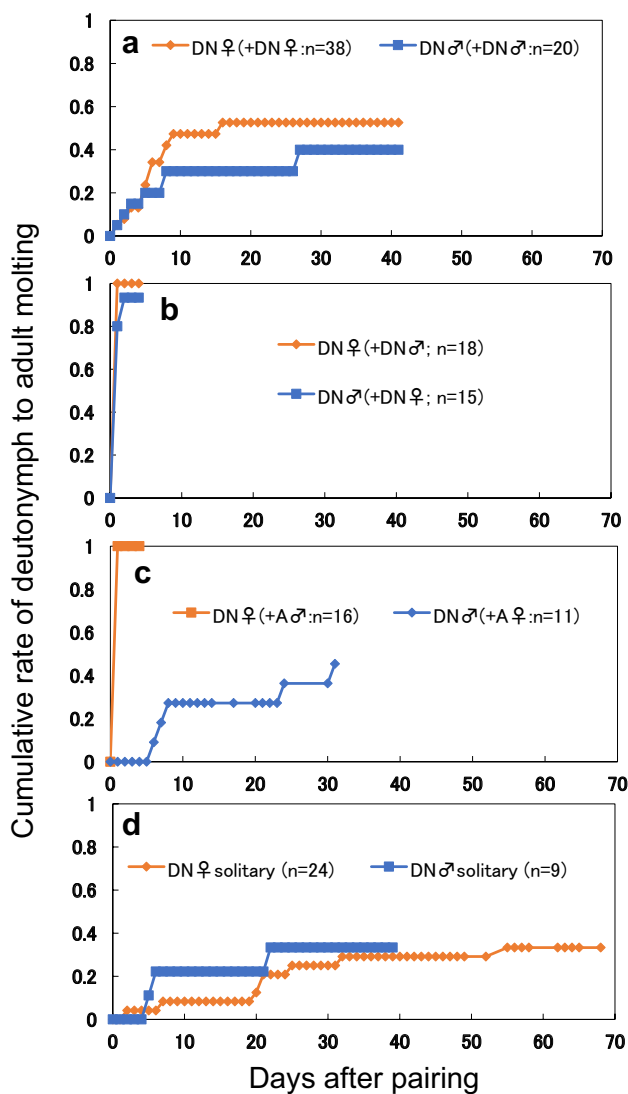
Fig. 4 The effect of pairing partner on deutonymph (DN) molting: **a** with a deutonymph of the same sex, **b** with a deutonymph of the opposite sex, **c** with an adult (A) of the opposite sex, **d** solitary condition (control). The deutonymphs were 10-days old at the start of pairing (day 0)

Fig. 5 The effect of pairing partner on female **a** and male **b** deutonymphs. DN: deutonymph, A: adult. n : no. tested individuals. Error bars show the 95% Wald confidence interval under binomial distribution. There are no significant differences between the same letters (Fisher's exact probability test with Holm–Bonferroni sequential correction; $P > 0.05$)

The solitary DNs (control; Fig. 4d) survived for longer than a month without molting. The cumulative molting rate at the end was ca. 35% for both sexes. Survival analysis (Cox proportional hazards model) detected no significant difference between sexes (no. parameters = 1, d. f. = 1, likelihood ratio Chi-square = 0.1048, $P = 0.7460$).

In the condition in which the DN was alone, male DNs tended to molt earlier than female DNs (protandry; Figs. 3, 4d), whereas in the condition in which the DN was combined with other individuals, female DNs tended to molt earlier than male DNs (protogyny; Fig. 4a–c).

In summary, the pairwise comparison of the maximum cumulative molting rate in all 6 combinations among 4 treatments using Fisher's exact probability test with Holm−Bonferroni sequential correction (Fig. 5) detected a sex difference in the response. All female DNs molted in combination with male DNs or male adults, but in pairs with another female DN, they showed a low molting rate that did not differ from the solitary condition (Fig. 5a). In contrast, the molting rate of male DNs was still low at pairing with female adults (Fig. 5b).

females and 93.3% of males molted to adulthood within a few days. The molting rate of females was slightly higher than that of males, but survival analysis (Cox proportional hazards model) detected no significant difference between sexes (no. parameters = 1, d. f. = 1, likelihood ratio Chi-square = 0.3631, $P = 0.5468$).

When paired with an adult of the opposite sex (Fig. 4c), female DNs immediately molted, but male DNs did not. Survival analysis (Cox proportional hazards model) detected a highly significant difference between sexes (no. parameters = 1, d. f. = 1, likelihood ratio Chi-square = 16.7439, $P < 0.0001$). The molting rate in these males was as low as that in the solitary condition (ca. 35%; Fig. 4d).
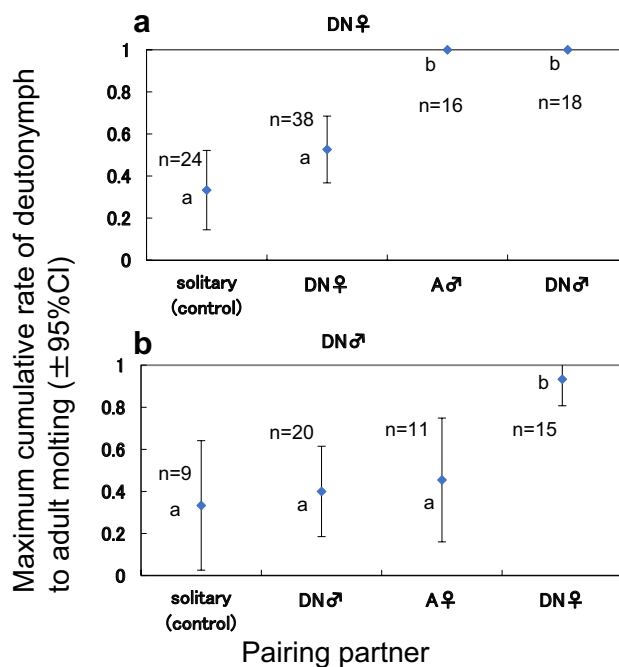
Discussion

This study clarifies that (1) a drastic increase in food resources stimulates the dormant DNs of *P. fimetorum* to molt and (2) cohabitation with another individual, especially of the opposite sex, induces the molting of dormant DNs. In addition, I established a sexing method for living DNs of this species. Hereafter, I discuss these findings in detail.

Adaptive molting decision

If a few individuals that were settled in a new habitat successfully lay eggs after molting, the hatched offspring also have to reach the deutonymphal stage to acquire tolerance and dispersal ability to the next habitats. Thus, abundant food resources sufficient for the development of the offspring population (not for the immigrated individuals) are required as a molting condition. The low molting rate in Fig. 3d and high molting rate in Fig. 3a–c are understandable for this reason. In Experiment 1, the individuals entering promising food environments quickly developed to adulthood without contact with other individuals. This may be an artifact in the experimental condition. Such a good patch containing huge food resources would also accept many other immigrants in nature so that the molted individuals can find mates sooner or later. Fast development (higher intrinsic rate of natural increase; Pianka 1970) is advantageous in an expanding population. Thus, the drastic improvement in nutritional conditions may stimulate solitary DNs to molt.

However, under moderate food conditions, which were sufficient for the development of a few mites, DNs molted only when they ensured potential mates, especially DNs of the other sex (Fig. 4b). Interestingly, whereas female DNs did not discriminate between male adults and male DNs (in the form of molting decisions), male DNs discriminated against female adults (Fig. 4c).

Male choice

Molting of DNs at pairing can be interpreted as a kind of mate choice. The present study shows that male DNs of *P. fimetorum* are more choosy than female DNs. Sexual selection (Darwin 1871; Andersson 1994) works through intrasexual competition and intersexual mate choice. Because of the intersex difference in potential reproductive rate (Clutton-Brock and Vincent 1991) due to the difference in gamete production ability between sexes, the competing sex is usually male, and the choosing sex is usually female in most animals. However, if mating opportunity is limited and if there is great variation in female quality, males cannot fully express their potential fertilization ability and thus choose mates (Johnson and Hubbell 1984; Schwagmeyer and Parker 1990; Ball and Parker 2007). For example, males of *M. muscaedomesticae* choose young virgin females because only the first male to mate with a female can fertilize her eggs (i.e., perfect first-male sperm precedence; Yasui 1988, 1996). For male DNs of *P. fimetorum*, adult females may be low-quality mate candidates because in most cases, they would have already mated with other males and laid a certain amount of eggs, meaning a low residual reproductive value (Yasui 1996, 1997). Short longevity (ca. 1 week) of adult males limits their mating opportunity. In contrast, for female DNs, males, irrespective of adult or DN, could be appropriate mates. Therefore, only male DNs exhibit mate choice by molting decisions.

How to sex deutonymphs–a breakthrough

The suspension of molting (a kind of dormancy) in DNs and its resuming conditions, especially contact with individuals of different sexes, were reported in other species of Parasitidae (Ito 1973, 1976, 1978; Nehring and Müller 2009). However, the difficulty of sexing at the living deutonymphal stage has made the pairing experiment controlling the sex combinations difficult. Ito (1973, 1976, 1978) sexed DNs of *Parasitus gregarious* based on carmine-color pigmentation of the ventral surface, which appears only in two-week-old or older male DNs. This is too late to detect the decision-making of young DNs. Nehring and Müller (2009) did not sex DNs of *Poecilochirus carabi* and instead indirectly estimated the probability that the pairing group of DNs contained both sexes under the assumption of a 1:1 sex ratio. This method is quite frustrating. Based on the newly found morphological sex difference (Fig. 2), which may be common among parasitid species, the present study enabled to clarify the molting decision of young DNs. According to Wojciech Witaliński (personal communication), such well-defined morphological differences between the sexes at the deutonymphal level are not necessarily common. The only difference is a pattern of sternal reticulation (Wrensch and Johnston 1983), which is visible only on mounted slides under a moderate power microscope. Therefore, it is not useful for stereomicroscope aid selection. The method in this study will contribute to various studies in the future.

Remaining issues

How do DNs recognize the developmental stage and sex of the encountering conspecifics? Ito (1976) showed that direct contact with heterosexual counterparts induced the molting of *Parasitus gregarious* DNs and suggested that some tactile and/or chemical stimulation might be involved. Such proximate factors are plausible in *P. fimetorum*. If they can also recognize and discriminate kin or nonkin, mate choice could

function as inbreeding avoidance. If the DNs descended from a single mother dispersed to multiple patches by riding on different dung beetles, the mother's lineage would avoid extinction unless all patches simultaneously experienced poor conditions. Thus, reproduction after dispersal could work as spatial risk-spreading or metapopulation bethedging (Hopper 1999; Yasui 2022). Nehring and Müller (2009) reported that male DNs of *Poecilochirus carabi* did not molt if adult males occurred nearby to avoid cannibalism by adults (such as male−male competition) during the vulnerable period immediately after molting. If this is also the case in *P. fimetorum*, it may explain the protandry in the solitary condition but the protogyny in the cohabited condition. Males probably molt in hiding from rivals or when rivals are not in the vicinity. Improved nutritional conditions and encounters with individuals of the opposite sex may synergistically promote DN molting, but I did not examine the interaction between these two factors for simplicity. These untested possibilities remain for future research.

Data availability The data sets supporting this article have been uploaded as part of the electronic supplementary material.

Declarations

Conflict of interest I declare that I have no competing interests with other persons.

Ethical approval The mites (*P. fimetorum*) used in this study are invertebrates and, therefore, have not been subjected to animal ethics review. The study was conducted in a manner that avoided or minimized discomfort or distress to the laboratory animals, and efforts were made to ensure that the animals did not suffer unnecessarily at any stage of the experiment. The laboratory population of *P. fimetorum* used in this study have maintained at Kagawa University. This population has been maintained on the artificial medium in which nematodes as their natural prey reproduce. We reared this population at 25 °C, which is comfortable condition for this mite. All animals in the study were handled carefully.

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