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The effect of age on sperm stock and egg laying in the parasitoid wasp, *Dinarmus basalis*

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Abstract

Sperm quantity and quality during storage may be constraints acting on female fecundity and hence fitness. In Hymenoptera, the importance of sperm quality has rarely been considered, despite its central role in reproductive strategies and especially in sex ratio control. In these insects, fertilized eggs develop into females and unfertilized eggs into males. Experiments were conducted on the female wasp, *Dinarmus basalis,* in the laboratory with and without egg-laying resources (hosts). The first point was to test if sperm age influenced sperm storage by measuring sperm count and viability using a sperm viability test (SYBR14 : propidium iodide). The second point was the influence of prolonged storage in the female genital tract on the quantity, sex ratio and fitness of offspring produced. Results show that sperm viability in the spermatheca does not change significantly with maternal age, and that the sperm stock is not affected when females are deprived of hosts. Egg-laying is gradually restored after 21 days of host deprivation but remains at a low level after 115 days. The fitness of mated *D. basalis* females is therefore not constrained by sperm quantity or quality and seems to depend on host availability and female age.

Keywords: sperm age, fitness, spermatheca, offspring sex ratio, parasitoid

Introduction

In most insect species, reproduction involves long-term storage of sperm in a specialized storage organ of the female, the spermatheca (review in Thornhill and Alcock, 1983). Spermatozoa are released progressively from the spermatheca to fertilize ovae. Inside the spermatheca, sperm may live weeks, months, or even years in long-lived species (Taber and Blum, 1960; Neubaum and Wolfner, 1999).

Sperm quantity and quality during storage are constraints acting on female fecundity. Quantitative studies have been carried out in insect females to understand the effect of sperm management on reproductive success (Smith, 1984; Pitnick and Markow, 1994; Bressac and Chevrier, 1998; Reinhardt *et al*., 1999; Reinhardt and Meister, 2000; Chevrier and Bressac, 2002), but few have assessed sperm quality at the various stages of the reproductive process (Hunter and Birkhead, 2002; Bernasconi *et al*., 2002; Damiens *et al*., 2002), although such information is necessary for a better understanding of reproductive strategies (Tsubaki and Yamagishi, 1991; Yamagishi *et al*., 1992).

Sperm stock can decrease over time because of fertilization or processes relating to it, called "sperm use" (Gromko *et al*., 1984; Bressac and Hauschteck-Jungen, 1996; Bressac and Chevrier, 1998). Alternatively, sperm may die and disintegrate while stored in the spermathecae (Cunningham *et al*., 1971; Tsubaki and Yamagashi, 1991). In addition to a decrease in sperm stocks, a variety of environmental factors (review in Lopez-Leon *et al*., 1994) and the interaction between the male ejaculate and the female may affect sperm viability during storage (Bernasconi *et al*., 2002). In practice, the quality of a sperm population can be characterized physiologically and its fecundity predicted by its viable sperm ratio (Collins and Donoghue, 1999; Damiens *et al*., 2002).

Arguments (summarized by Reinhardt *et al*., 1999) seem to support the hypothesis that a decrease in sperm quality occurs with sperm age, leading to a decrease in probabilities of fertilization and hence female fitness. Nevertheless, there is no evidence that embryos fertilized by older sperm tend to show more developmental damage or an alteration of the offspring fitness (Byers and Muller, 1952; Reinhardt *et al*., 1999), even if this idea is widespread and documented in non-insect species (Siva-Jothy, 2000).

The fitness of parasitoids depends on two physiological parameters: the pattern of egg production (Price, 1975; Jervis and Kidd, 1986; Rivero-Lynch and Godfray, 1997) and the dynamics of sperm storage (Chevrier and Bressac, 2002). *Dinarmus basalis* (Hymenoptera, Pteromalidae) is a solitary ectoparasitoid of West African bruchid larvae and pupae. In this wasp, females produce oocytes continuously, and their individual reproductive strategy reveals a very high sperm efficiency (Chevrier and Bressac, 2002): the number of stored spermatozoa in the female's spermatheca is close to the number of oocytes fertilized in their lifetime which result in female offspring. As a consequence, the population of viable sperm detected in the spermatheca of females is a reliable predictor of fertilizations achieved in ovipositing females (Damiens *et al*., 2002). Both males and females are capable of multiple mating

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(Chevrier and Bressac, 2002). The mean lifespan of non-reproductive *D. basalis* females in the laboratory is about 115 days (unpublished data). Moreover, because females are able to copulate only at the very beginning of adult life (Chevrier and Bressac, 2002), the age of the sperm stock corresponds to the age of females.

In the present study, the quality of sperm stock was measured in laboratory in relation to age, here defined as the duration of storage within the female's storage organ. The effect of female age on the offspring quantity, sex ratio and fitness was also investigated.

Methods

Rearing conditions

Dinarmus basalis came from laboratory populations which originated in West Africa. All experiments were carried out at 33° C - 23° C (12 hrs light-12 hrs dark) in a climate-controlled chamber with *Callosobruchus maculatus* (Coleoptera, bruchidae) hosts from West Africa as described by Chevrier and Bressac (2002). In all mating experiments, virgin males were used 24 hours after emergence and virgin females 2 hours after emergence. As once-mated and three-times mated females produce the same quantity and sex ratio of offspring during the first seven days of egg-laying (Chevrier and Bressac, 2002), females were once-mated in all mating experiments.

Sperm preparation

Females and males were dissected in a drop of Beadle saline (128.3 mM NaCl, 4.7 mM KCl, 23 mM $CaCl_2$) as described by Damiens *et al*. (2002). In males, the content of both seminal vesicles were gently diluted in Beadle saline and 1/10th (3 µl) of the sperm suspension was deposited on a cleaned microscope slide. In females, the content of the entire spermatheca without any dilution was spread on a cleaned microscope slide (3 µl drop).

Sperm viability assessment was carried out on microscope slides with the live/dead™ Sperm Viability Kit (L-7011, Molecular Probes). The final concentrations of fluorochromes used were 21.5 µg/ml propidium iodide and 8000 nM SYBR-14 (Damiens *et al*., 2002). All spermatozoa were observed in each preparation. Because (1) this method only authorizes one observation from each sample, and (2) sperm counts are underestimated (Damiens *et al*., 2002), sperm counts were determined by regression adjustment. The regression was established by Damiens *et al*., 2002 from sperm suspensions stained with SYBr14/propidium iodide and DAPI: [sperm number = 1.72 (SYBR-14 : propidium iodide counts) - 15.46].

Females allowed access to hosts

One 2-hour-old virgin female was placed in a small petri dish with one 24-hour-old virgin male to ensure copulation. Only one mate was allowed to avoid mixing of spermatozoa of different males in the female's spermatheca. Once mated, each female was isolated in an oviposition box with a sucrose-saturated cotton roll fixed in the middle of the dish. The isolated females were presented daily with ten cowpea seeds each containing one or two bruchid larvae, fixed in a circle around the sucrose source as described by Bressac and Chevrier (1998) for 7 (n=15) and 14 (n=14) days. At the end of each laying period, females were dissected. Sperm viability in spermathecae was measured by SYBR-14 : propidium iodide staining. The experiment was not extended beyond 14 days because by this time the females, which mate only once, have an extremely reduced sperm stock and by 21 days the sperm are totally depleted (Chevrier and Bressac, 2002).

Host-deprived females

One 2-hour-old virgin female was placed in a small petri dish with one 24-hour-old virgin male to ensure copulation. Mated females were transferred to petri dishes containing a sucrose-saturated cotton roll for 21 (n=26) and 115 (n=26) days without the possibility of egg-laying. Twenty one days corresponds to the exhaustion of sperm stock during the egg-laying period of once-mated females (Chevrier and Bressac, 2002), and 115 days corresponds to the once-mated female lifespan (50% of females still alive) in controlled conditions with sucrose food present but without hosts. Females were then dissected and sperm quantity and viability in spermathecae were assessed by SYBR-14 : propidium iodide staining.

Aged females offspring production and fitness

Twenty one-day-old (n=36) and 115-day-old (n=12) host-deprived females were presented daily for 7 days with ten cowpea seeds as described above. The numbers of emerging hosts and parasitoid offspring were recorded every day for each female. To determine the reproductive fitness of the offspring of 115-day-old females, the quantity and viability of sperm present in seminal vesicles of sons (n=10) 24 hrs after emergence, and in the spermatheca of daughters (n=11) 24 hrs after copulation with their brothers, were measured.

Statistical Analyses

One-way ANOVA (Fisher's F test) were used to compare means (sperm survival in females, sperm counts in host-deprived females, offspring numbers). The Chi^2 test was used to compare total offspring sex ratios in both female's age.

Results

Females allowed access to hosts

For the two egg-laying periods studied (7 and 14 days), no significant change occurred in the proportion of live sperm (Table 1). The mean sperm viability was 86.26 ± 1.12 % (ANOVA $F_{1,27}$=0.32, p=0.6).

Host-deprived females

As shown in Table 1, sperm survival did not change significantly when the period without hosts increased (ANOVA $F_{1,50}$=1.71, p=0.2). Mean sperm viability was 87.24±0.75 % and did not differ significantly from that of females with hosts (ANOVA $F_{3,75}$=0.63, p=0.6). The quantity of sperm present in spermathecae after a period of 21 or 115 days without hosts also did not differ significantly (ANOVA $F_{1,50}$=1.4, p=0.2).

Aged female offspring production and fitness

After 21 and 115 days without hosts, all females started to

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Table 1. Mean viable sperm ratio (± SE) in *Dinarmus basalis* females after egg-laying during 7 and 14 days and after 21 and 115 days without egg-laying. Results with same letters are not statistically different (one–way ANOVA, p<0.05).

	With egg-laying		Without egg-laying	
Age in days	7	14	21	115
Sperm count	111.1±11.3[a]	28.1±3.9[b]	125.5±17.2[a]	101.3±11.2[a]
Viable sperm ratio	0.87±0.11[c]	0.85±0.09[c]	0.89±0.08[c]	0.87±0.05[c]

Table 2. Total offspring (males and females) and sex ratio in *Dinarmus basalis* females during 7 days of egg-laying after 21 and 115 days without hosts (± SE). Results with same letters are not statistically different (one–way ANOVA, p<0.05 for total offspring; chi^2 test, p<0.05 for sex ratio).

Age in days at the beginning of egg-laying	21	115
Total offspring/7days	40.1±2.0[a]	12.8±2.0[b]
Sex ratio	0.61±0.02[c]	0.65±0.02[c]

lay again when hosts were offered. For 7 successive days, females of all ages produced offspring (Fig. 1). The daily offspring production (Table 2) differed significantly according to the female's age (ANOVA $F_{1,12}$=18.9, p<0.05). In the 21-day-old females, daily production increased during the egg-laying period, whereas in 115-day-old females, offspring production stayed unchanged at a low level (Fig. 1). For both treatments, whatever the female's age, sons and daughters were produced, and no age effect was observed on the daily offspring sex ratios (Table 2) (chi^2=0.65, df=6, p=1).
The quantity of sperm in the seminal vesicles of the sons and in the spermathecae of daughters of 115-day-old females was respectively 2421.0 ± 218.3 and 90.7 ± 17.6. Sperm stored by daughters did not differ from their mothers (ANOVA vs 115-day-old females: $F_{1,35}$=2.4, p=0.13). Proportions of viable sperm in seminal vesicles of sons and spermathecae of daughters were respectively 56.7 ± 0.6% and 88.9 ± 0.9%.

Discussion

During the reproductive life of *D. basalis* females, whatever the physiological situation (egg-laying activity or not), the viability of stored sperm in the spermatheca remained unchanged. As no sperm depletion was found under laboratory conditions, there is no evidence of "passive loss", whereby spermatozoa die due to either physiological ageing or because of active sperm digestion by the female.

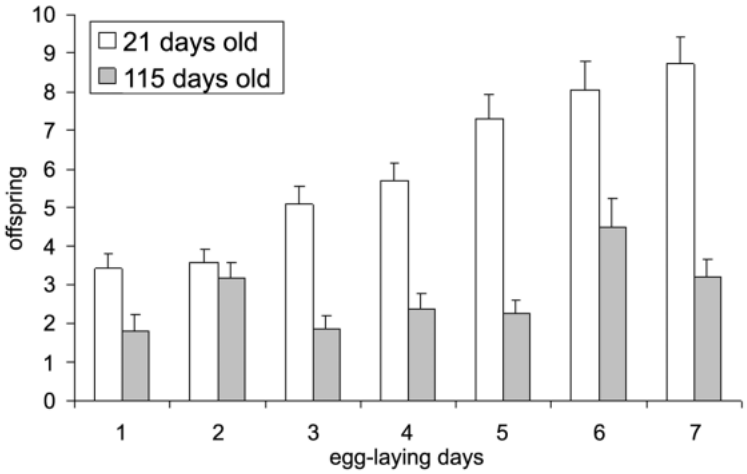

Figure 1. Offspring (males + females) laid by *Dinarmus basalis* females during 7 successive egg-laying days after a period of host deprivation of 21 and 115 days. The histogram shows mean progeny and bars show the standard error. Statistics in text.

After a period without oviposition, *D. basalis* females started to lay eggs and produced offspring when hosts were offered. While older females (115 days) produced some 68% less offspring than the younger 21 day old females, no effect was observed on the offspring sex ratio. The decrease in offspring production during the female's lifespan is physiologically age-dependent (Ode *et al*., 1997; Bressac and Chevrier, 1998), as older females lay fewer eggs than younger ones (Li and Harmsen, 1993). When investigating the effect of sperm age on fitness, two effects often overlap that can here be separated: (1) a decrease in sperm viability and/or (2) an alteration of the female's physiology. The first effect is eliminated by the present results which showed no decline in sperm viability. However, as *D. basalis* females started to lay again after a period without oviposition, oogenesis obviously slowed down but was not inhibited. Therefore the decrease of offspring production in older females could be explained by an increase of the proportion of females with resorbing oocytes due to host deprivation (Taylor and Sands, 1986). In synovigenic parasitoids, egg load increases under deprivation, but subsequently declines as eggs are resorbed (Flanders, 1942; Droste and Carde, 1992). In *D. basalis*, oocyte resorbtion has been observed by Gauthier and Monge (1999) when females were provided with low-quality hosts inducing oocyte retention. Generally, the appearance of hosts restores egg maturation (Droste and Carde, 1992), as observed here in 21-day-old females but not in 115-day-old females where oviposition remained at a low level, revealing an irreversible senescence.

In *D. basalis*, maternal age had no effect on offspring sex ratio, the total offspring sex ratio of 115-day-old females being similar to that of one-day-old females (Damiens *et al,*. 2001; Chevrier and Bressac, 2002). Unlike other species (Li and Harmsen, 1993), there was no increase with age in the proportion of unfertilized eggs laid by females. Whatever the situation, in *Dinarmus basalis* the sex ratio is constant (Damiens *et al*., 2001). Such a life-history trait would constitute an adaptation for living in leguminous seed stocks where reproductive resources are continuously present (Monge and Huignard, 1991). However, the insemination pattern of females has a key role in *D. basalis* because females mate only at the beginning of adult life and the sperm stock itself can be limited in some population situations where males constitute a limiting resource (Chevrier and Bressac, 2002).

Fitness of 115-day-old females' offspring was similar to that of mass breeding sons and daughters measured in Damiens *et al*. (2002). According to Damiens *et al*. (2002), the proportion of viable sperm in the male's seminal vesicles is lower than in the

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female's spermathecae, suggesting improved viability in the female's storage organ. But in *Apis mellifera* the viability of spermatozoa in male's ejaculate reaches 95% (Collins, 2000). Depending to the insect species considered, the viability of sperm in males can vary from 50 to about 100% (Hunter and Birkhead, 2002).

In conclusion, individual fitness of parasitoid females during their reproductive life is influenced more by egg production than sperm management. In other words, neither increasing sperm age, nor a period without reproduction, is costly for females in terms of individual fitness, even if a reproductive senescence seems to take place after 115 days. We are left with the fact that maintaining a sperm stock of good quality while other functions allowing reproduction are no longer functional may constitute a physiological cost that is not documented in other insects. This lack of information is principally due to the newness of sperm viability assessments in invertebrates (Collins and Donoghue, 1999; Damiens *et al*., 2002; Bernasconi *et al*., 2002; Hunter and Birkhead, 2002). Considering behavioural ecology, constraints acting on the reproduction of female wasps are mainly produced by their environment – principally host availability – but focusing on the physiological status of individuals could help to understand the outcome of reproduction under some sub-optimal ecological situations.

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