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Preliminary observations of sperm storage in *Adalia bipunctata* (Coleoptera: Coccinellidae): sperm size and number

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Abstract

Sperm storage in *Adalia bipunctata* (L.) (Coleoptera, Coccinellidae) was examined and compared between diapausing and non-diapausing females. We observed that non-diapausing females store on average 13,688 (±722, S.E.) sperm in their spermatheca and that every female examined had sperm in storage (n=16). In contrast, most diapausing females had empty spermatheca and only two had sperm in storage (n=15). Mean sperm number in diapausing females was 250 (±112, S.E.). The mean length of the spermatozoa stored by non-diapausing females was 2,068.5 μm (±9.8, S.E.), ranging from 1,530.8 to 3,171.7 μm. Sperm length in diapausing females was only measurable in one female and was of 2,102.9 μm (±22.4, S.E.).

Key words: Coleoptera; Coccinellidae; two-spot ladybird; diapause; spermatheca

INTRODUCTION

The two spotted ladybird, *Adalia bipunctata* (L.) (Coleoptera, Coccinellidae) is multivoltine species having two to four generations through early spring to the end of summer when adults undergo a reproductive diapause, induced by short day length (Hodek and Honek, 1996). As ladybirds are diapausing insects it is important to understand the evolution of their physiology throughout the year, and several studies have addressed the reproductive physiology and biology of diapausing ladybirds (Hodek and Landa, 1971; Hemptinne and Naisse, 1987; Ceryngier et al., 1992; Nelapa et al., 1996; Hodek and Ceryngier, 2000). In most insects, females store sperm in a specialised organ (the spermatheca) often for prolonged periods. An important trait of female reproductive biology is therefore the sperm content within the spermatheca. Differential sperm storage between diapausing and non-diapausing ladybird females has been examined in a few ladybird species. In *Harmonia axiridis*, about 12 to 41% of the overwintering females were observed to have sperm in their spermatheca (Nelapa et al., 1996). In *Ceratomegilla* (syn. *Semiadalia*) *undecimnotata*, Hodek and Landa (1971) and Hodek and Ceryngier (2000) reported that from the beginning of dormancy till early April no spermatheca contained any sperm. In contrast, females *Coccinella septempunctata* are observed to copulate before dormancy and about 28 to 47% of the females collected in their dormancy sites had sperm in their spermatheca (Ceryngier et al., 1992; Hodek and Ceryngier, 2000). These sperm can be use for egg fertilization at the end of diapause and eliminate the need for female to copulate to start reproduction. In *A. bipunctata* collected in Belgium, Hemptinne and Naisse (1987) reported that the spermatheca of every female was empty in autumn.

Another important trait of adult reproductive biology is sperm competition (Parker, 1970). Although two-spotted ladybird life histories are well documented, sperm competition and patterns of sperm precedence are not fully understood in this species (De Jong et al., 1993, 1998). Last-male sperm precedence has been clearly demonstrated in two epilachnin ladybirds (Webb and Smith, 1968; Nakano, 1985). In *H. axyridis*, the last-male advantage was weak (P_2=0.55) (Ueno, 1994). In *A. bipunctata*, a high heterogeneity in the proportion of the female progeny fathered by the last-male to mate was found (De Jong et al., 1993, 1998). It therefore seems that complete sperm mixing

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mainly occurs in this species and that male reproductive success is based on the numerical representation of its sperm in the female spermatheca. The examination of ejaculate sperm number, sperm length and sperm storage can give valuable information towards understanding the processes of sperm competition and sperm precedence (Parker et al., 1990; Parker, 1998; Morrow and Gage, 2001a). However, these reproductive traits have not been examined in ladybirds.

In this paper, we compared the spermathecal content of diapausing and non-diapausing females and reported a preliminary examination of sperm storage and sperm length in the ladybird, *A. bipunctata*.

MATERIALS AND METHODS

The insects. In October 1998, diapausing two-spot ladybird females were collected in Belgium, in their dormancy sites where they stay until the end of April in our climatic conditions (Hemptinne, 1985). Seven females were collected in Gembloux in bark crevices of trees and eight females in Walhain-Saint-Paul were collected in window-frames. Non-diapausing females originated from our insect cultures (parent beetles have been collected in the field and cultured in the lab for several generations). Beetles were reared at 15±1°C and a photoperiod of 16L/8D. Twenty males and twenty females were kept in 5 *l* plastic boxes, with a piece of corrugated paper to increase the surface area. Ladybirds were fed an excess of the pea aphid, *Acyrthosiphon pisum*. Beetles were free to mate *ad libitum* and pairs were frequently observed in *copula*. Those conditions can allow females to replenish their spermathecae as soon as sperm are used for egg fertilization. After two weeks, sixteen non-diapausing females were examined for the presence of sperm in storage. Ladybirds were sexed as described in Hodek (1973) and Randall et al. (1992).

Sperm storage, length and number. Females were dissected. The entire female reproductive tract was isolated and placed on a cavity slide with 100 μl of modified Barth saline (Gage and Cook, 1994). The reproductive tract was then ruptured in several parts and teased apart with fine entomological needles and this allowed the stored spermatozoa to disperse. As the spermathecae is a chitinous structure in ladybirds (Katakura, 1981; Hemptinne, 1989), it was crushed with forceps to force the ejection of sperm. The sperm-buffer solution was then washed off the slide using ca. 5 ml Barth saline into a 50 ml Falcon tube and then diluted up to 15 ml with distilled water. Four 20 μl subsamples were retrieved using a micropipette from each 10 ml sperm solution and each 20 μl sample was placed as a smear on a flat slide and allowed to air dry under a dust cover. Sperm length and number examinations were performed as described in Arnaud et al. (2001). Twenty sperm from ten non-diapausing females were measured.

RESULTS

Most of the females collected in the dormancy sites had empty spermatheca. Only two diapausing females possessed sperm in storage. In contrast, every non-diapausing females had sperm in storage (Table 1). Non-diapausing females stored significantly more sperm than diapausing females (Two-way ANOVA, $F_{1,16}=10.96$, $p=0.004$—non-dia-

Table 1. Number and length of the sperm stored in the spermathecae of diapausing and non-diapausing females of *Adalia bipunctata*

Female	Sperm characteristics[a]	
	Number	Length (μm)
Diapausing		
1	625±375	nd
2	3,125±718	2,102.9±22.4
Non-diapausing		
1	16,750±2,016	1,993.3±26.3
2	18,875±2,824	2,096.0±24.5
3	17,625±1,533	2,077.7±16.9
4	14,000±1,696	2,198.2±53.0
5	21,125±1,434	2,156.0±15.1
6	4,875±1,329	2,011.2±23.4
7	10,000±2,179	2,062.5±22.2
8	15,875±1,625	2,050.6±24.4
9	4,875±239	2,005.2±35.9
10	14,250±2,634	2,034.1±22.0
11	10,125±427	nd
12	12,750±3,527	nd
13	19,750±2,184	nd
14	9,250±777	nd
15	16,250±1,199	nd
16	12,625±657	nd

[a] Mean±S.E., twenty sperm were measured from every female spermatheca, nd=not determined.

pausing females: 13,688±722, $n=16$, diapausing females: 1,875±603, $n=2$, mean±S.E.). The mean length of the spermatozoa stored by non-diapausing females was 2,068.5 μm (±9.8, S.E.), ranging from 1,530.8 to 3,171.7 μm. Sperm length in diapausing females was only measurable in one female (see Table 1). Too few sperm were found in the other female to allow an accurate measurement of the sperm stored in her spermatheca. No difference was observed between sperm length in the spermatheca of both kind of females (*t*-test, $t_9=0.105$, $p=0.919$). However, sample size for diapausing females is too small to give strong conclusions.

DISCUSSION

Differential sperm storage between diapausing and non-diapausing ladybird females has been examined in a few ladybirds. In some species no females were found to store sperm during domancy (Hodek and Landa, 1971; Hemptinne and Naisse, 1987; Hodek and Ceryngier, 2000). In other, about 12 to 47% of the over-wintering females were observed to store sperm in their spermatheca (Ceryngier et al., 1992; Nelapa et al., 1996; Hodek and Ceryngier, 2000). In the present study, we found that a few diapausing females of *A. bipunctata* had sperm in storage. The diapausing females with empty spermatheca may have belonged to the last generation and therefore had not copulated in the time between emergence and the onset of dormancy. The two females that had sperm in storage may have belonged to the penultimate generation. It is also possible that, as observed in *C. septempunctata* (Ceryngier et al., 1992; Hodek and Ceryngier, 2000), *A. bipunctata* females copulate just prior the onset of dormancy, but in a lower percentage.

Despite we found that two diapausing females had sperm in storage, our results are in accordance with the study of Hemptinne and Naisse (1987), and it is likely that most *A. bipunctata* females do not copulate before dormancy. Hemptinne and Naisse (1987) observed that the number of filled up spermathecae rise in March and April, meaning that mating occurred in dormancy sites before adult dispersal. This reproductive behaviour would give a reproductive advantage to females as well as to males. Large aggregations of up to 6,000 *A. bipunctata* adults could be found in a single dormancy site (see Hodek and Honek, 1996 for a review). Competition for mating is therefore potentially high at the end of dormancy. If female choice occur in *A. bipunctata*, the reproductive success of more choosy females could be favoured by this competition. High mortality can occur during dormancy in *A. bipunctata* (up to 60%—Majerus, 1994). Females that mate before dormancy may store the genes of males that are not able to survive winter, and a proportion of her progeny will inherit this negative trait. Therefore, mating before adult dispersal is more advantageous for female reproductive success than mating before dormancy. Sperm production is not cheap in animals (see Wedell et al., 2002 for a review). It could thus be costly for males to mate before dormancy, as his mate(s) could die during winter. In addition, females are likely to (re)mate before adult dispersal. Therefore it is also advantageous for males to mate after dormancy.

Sperm length is recognized as an important trait in determining male reproductive success (Bressac et al., 1991; Bressac and Hauschteck-Jungen, 1996; Otronen et al., 1997; but see Morrow and Gage, 2001b). In *Drosophila* fruit flies and in the yellow dung fly, *Scatophaga stercoraria*, a reproductive advantage is achieved by males with longer sperm: longer spermatozoa are preferentially stored and used by females (Bressac et al., 1991; Bressac and Hauschteck-Jungen, 1996; Otronen et al., 1997). In most insects, males inseminate many more sperm than the female can effectively store. Males of the beetles *Callosobruchus maculatus* and *Tribolium castaneum* inseminate about 85% more sperm than a female stores in the spermatheca (Eady, 1994; Bloch Qazi et al., 1996). *A. bipunctata* females can lay about 600 to 1,500 eggs throughout their lifetime (Iablokoff-Khnzorian, 1982). Therefore, most of the non-diapausing-females examined here had potentially enough sperm to fertilize all of their lifetime's supply of ova. However, multiple mating is observed in ladybirds (Hodek and Honek, 1996; De Jong et al., 1998; Hodek and Ceryngier, 2000). As sperm storage is under female control in *A. bipunctata* (Hemptinne, 1989), multiple mating may thus give the opportunity to the females to select between male ejaculates and to increase their reproductive success via a reduced genetic incompatibility between males and females, increased offspring viability and/or increased ability of sons to gain paternity (Jennions and Petrie, 2000).

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REFERENCES

Arnaud, L., E. Haubruge and M. J. G. Gage (2001) Sperm size and number variation in the red flour beetle. *Zool. J. Linn. Soc.* 113: 369–375.

Bloch Qazi, M. C., J. T. Herbeck and S. M. Lewis (1996) Mechanisms of sperm transfer and storage in the red flour beetle (Coleoptera: Tenebrionidae). *Ann. Entomol. Soc. Am.* 89: 892–897.

Bressac, C. and E. Hauschteck-Jungen (1996) *Drosophila subobscura* females preferentially select long sperm for storage and use. *J. Insect Physiol.* 42: 323–328.

Bressac, C., D. Joly, J. Devaux, C. Serres, D. Feneux and D. Lachaise (1991) Comparative kinetics of short and long sperm in sperm dimorphic *Drosophila* species. *Cell Motil. Cytoskeleton* 19: 269–274.

Ceryngier, P., P. Kindlmann, J. Havelka, I. Dostalkova, V. Brunnhofer and I. Hodek (1992) Effect of food, parasitization, photoperiod and temperature on gonads and sexual activity of males of *Coccinella septempunctata* (Coleoptera, Coccinellidae) in autumn. *Acta Entomol. Bohemoslov* 89: 97–106.

De Jong, P. W., P. M. Brakefield and B. P. Geerinck (1998) The effect of female mating history on sperm precedence in the two-spot ladybird, *Adalia bipunctata* (Coleoptera, Coccinellidae). *Behav. Ecol.* 9: 559–565.

De Jong, P. W., M. D. Verhoog and P. M. Brakefield (1993) Sperm competition and melanic polymorphism in the 2-spot ladybird, *Adalia bipunctata* (Coleoptera, Coccinellidae). *Heredity* 70: 172–178.

Eady, P. E. (1994) Intraspecific variation in sperm precedence in the bruchid beetle *Callosobruchus maculatus. Ecol. Entomol.* 19: 11–16.

Gage, M. J. G. and P. A. Cook (1994) Sperm size or numbers? Effects of nutritional stress upon eupyrene and apyrene sperm production strategies in the moth *Plodia interpunctella* (Lepidoptera: Pyralidae). *Funct. Ecol.* 8: 594–599.

Hemptinne, J.-L. (1985) Les sites d'hibernation de la coccinelle *Adalia bipunctata* L. (Col. Coccinellidae) en Belgique. *Acta Oecol. Appl.* 6: 3–13.

Hemptinne, J.-L. (1989) Ecophysiologie d'*Adalia bipunctata* (L.) (Coleoptera, Coccinellidae). PhD Thesis, Université Libre de Bruxelles, Belgium. 156 pp.

Hemptinne, J.-L. and J. Naisse (1987) Ecophysiology of the reproductive activity of *Adalia bipunctata* (Col., Coccinellidae). *Med. Fac. Landbouw. Rijksuniv.* Gent 52: 225–233.

Hodek, I. (1973) *Biology of Coccinellidae.* Academia, Prague & Dr W. Junk, The Hague. 260 pp.

Hodek, I. and P. Ceryngier (2000) Sexual activity in Coccinellidae (Coleoptera): a review. *Eur. J. Entomol.* 97: 449–456.

Hodek, I. and A. Honek (1996) *Ecology of Coccinellidae.* Kluwer Academic Publishers, Dordrecht. 464 pp.

Hodek, I. and V. Landa (1971) Anatomical and histological changes during dormancy in two coccinellidae. *Entomophaga* 16: 239–251.

Iablokoff-Khnzorian, S. M. (1982) "*Les coccinelles*" *Coléoptères Coccinellidae.* Société Nouvelle des Editions Boubée, Paris. 568 pp.

Jennions, M. D. and M. Petrie (2000) Why do females mate multiply? A review of the genetic benefits. *Biol. Rev. Camb. Phil. Soc.* 75: 21–64.

Katakura, H. (1981) Sperm storage place in adult females of the phytophagous ladybirds belonging to *Henosepilachna vigintioctomaculata* complex (Coleoptera, Coccinellidae). *Kontyû* (*Tokyo*) 49: 477–481.

Majerus, M. E. N. (1994) Ladybirds. In *The New Naturalist Library* (S. A. Corbet, S. M. Walters, R. West, D. Streeter and D. A. Ratcliffe eds.). Harper Collins, London. 367 pp.

Morrow, E. H. and M. J. G. Gage (2001a) Consistent significant variation between individual males in spermatozoal morphometry. *J. Zool.* (*Lond.*) 254: 147–153.

Morrow, E. H. and M. J. G. Gage (2001b) Sperm competition experiments between lines of crickets producing different sperm lengths. *Proc. R. Soc. Lond. B* 268: 2281–2286.

Nakano, S. (1985) Sperm displacement in *Henosepilachna pustulosa* (Coleoptera, Coccinellidae). *Kontyû* (*Tokyo*) 53: 516–519.

Nelapa, C. A., K. A. Kidd and K. R. Ahlstrom (1996) Biology of *Harmonia axyridis* (Coleoptera; Coccinellidae) in winter aggregations. *Ann. Entomol. Soc. Am.* 89: 681–685.

Otronen, M., P. Reguera and P. I. Ward (1997) Sperm storage in the yellow dung fly *Scatophaga stercoraria*: identifying the sperm of competing males in separate female spermathecae. *Ethology* 103: 844–854.

Parker, G. A. (1970) Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* 45: 525–567.

Parker, G. A. (1998) Sperm competition and the evolution of ejaculates: towards a theory base. In *Sperm Competition and Sexual Slection* (T. R. Birkhead and A. P. Möller eds.). Academic Press, London, pp. 3–54.

Parker, G. A., L. W. Simmons and H. Kirk (1990) Analysing sperm competition data: simple models for predicting mechanisms. *Behav. Ecol. Sociobiol.* 27: 55–65.

Randall, K., M. E. N. Majerus and H. Forge (1992) Characteristics for sex determination in British ladybirds (Coleoptera: Coccinellidae). *The Entomologist* 111: 109–122.

Ueno, H. (1994) Intraspecific variation of P2 value in a coccinellid beetle, *Harmonia axyridis*. *J. Ethol.* 12: 169–174.

Webb, R. E. and F. F. Smith (1968) Fertility of eggs of Mexican bean beetles from females mated alternately with normal and apholate-treated males. *J. Econ. Entomol.* 61: 521–523.

Wedell, N., M. J. G. Gage and G. A. Parker (2002) Sperm competition, male prudence and sperm-limited females. *Trends Ecol. Evol.* 17: 313–320.