Sexual activity in Coccinellidae (Coleoptera): a review

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Abstract. Although there are few studies of the sexual life of coccinellids these phenomena have attracted the interest of isolated groups of coccinellidologists. Probably the most important finding is that at least some coccinellid species (*Adalia bipunctata* and *Harmonia axyridis*) do not mate at random with the females prefering certain males. This phenomenon was first observed in *Adalia bipunctata* by Lusis and then studied in detail by Majerus, O'Donald, de Jong and others. In Japan, *Harmonia axyridis* was similarly studied by Osawa and Ueno. While the former author found that in this species (as in *A. bipunctata*) the colour of the elytra is most important in mate choice by females, the latter stresses that size and activity are important. Sperm competition is another interesting phenomenon, most often the sperm of the last male fertilizes the eggs (Ueno, Katakura). Obata and Hidaka have contributed in an important way to elucidating the function of the spermatophore in mating. The studies by Hodek and Ceryngier recorded the maturation and regression of testicular follicles and the relation of mating activity to diapause in four coccinellid species. In contrast to females, where induction of diapause prevents maturation of ovaries, in diapausing males the tissue of testicular follicles remains active until the temperatures decrease in late autumn. Dissection of spermathecae revealed principal difference in autumn mating activity between *Coccinella septempunctata*, in which 40–60% of the beetles mated before hibernation and *Ceratomegilla* (syn. *Semiadalia*) *undecimnotata*, which does not mate in autumn.

INTRODUCTION

The studies on coccinellids have naturally concentrated on their relation to prey: prey specificity, quantitative aspects, prey foraging behaviour and impact. Thus the development of gonads and mating have always been much less studied, although they also have some practical relevance, particularly when coccinellids are reared as predators to be used in biological control. The little interest in the sexual activity of ladybirds, shown by a limited number of authors quoted here, inspired us to write this review. While the review does not attempt to be exhaustive, the aim of authors has been to stimulate further research in this field, or at least provide information. The very limited range of coccinellid species studied so far needs to be widened before general predictions about evolutionary trends in mating behaviour can be made.

1. MATING IN RELATION TO DORMANCY

Contrary to what is often assumed the activity of the tissue of coccinellid testicular follicles is not arrested when diapause is induced. Testes mature in the pupa and the males are ready to mate shortly after adult emergence. In some species diapause induction differentially affects males and females in respect of the maturation of their gonads. While the males of *Coccinella septempunctata* acquire all other characteristics of diapause syndrom, such as accumulation of reserves and ethological events, i.e. migration and change of taxes, they are nevertheless able to fertilize females just prior to entering dormancy. This is not true for all species, as will be shown in *C. undecimnotata*. Also in the subspecies *Coccinella septem-*

punctata brucki diapause in central Japan differs in its physiology from that in *C.s. septempunctata* in Europe.

Incidence of pre-winter mating may be checked by dissection of spermatheca, where females store sperm. In the common European subspecies, *C. s. septempunctata*, the incidence of females that overwinter with the spermatheca containing sperm is high. In that part of the population that ceases feeding very early, so that the first adults arrive at dormancy sites in August, 38% of the females (n = 50) had sperm in their spermathecae. These adults were already in their dormancy shelters (grass tussocks on the hill Raná near Louny, in N. Bohemia), when collected on 22 Sept. 1998. The males had still highly active testicular follicles (at stage 3.5 on aver.), in spite of being dormant in the tussocks (Fig. 1, *C. sept.*, Rana).

C. septempunctata populations are heterogenous in central Europe (Hodek & Honěk, 1996; Hodek & Okuda, 1997); some active, feeding beetles can still be found on vegetation with aphids in autumn. Such beetles were collected in C. Budejovice (S. Bohemia) from *Impatiens parviflora* infected with aphids at the same time as those from dormancy sites at Rana.. While the activity of testicular follicles was the same (stage 3.4 on aver.), the percentage of full spermathecae was much higher (58%; n = 48) in the feeding beetles (Fig. 1, *C. sept.*, CB). Evidently more males searched for mates when foraging for prey, than did those at the dormancy sites when still active on hot sunny days in Aug.–Sept., prior to hiding in tussocks.

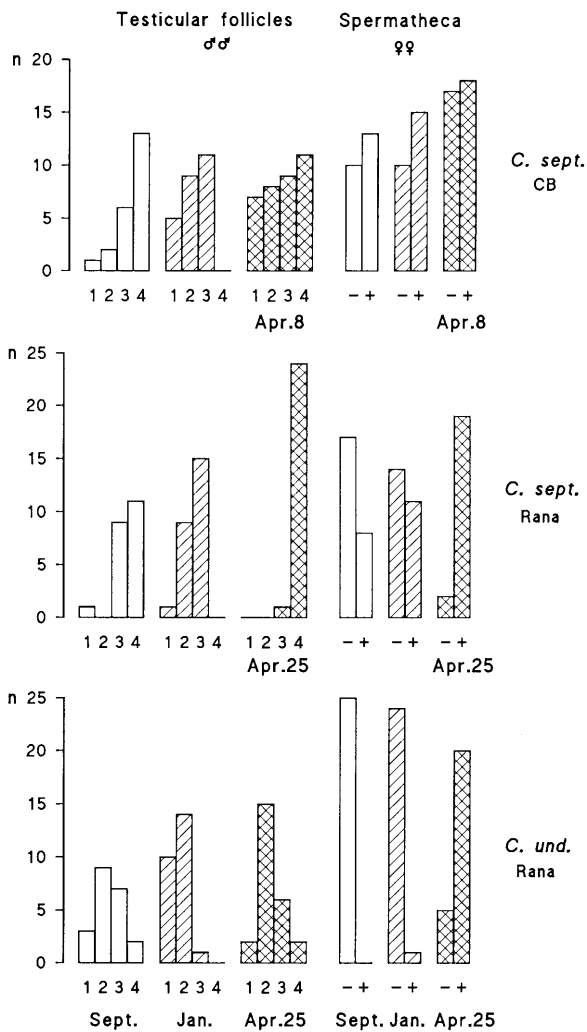In another coccinellid, which also overwinters on the hill Rana, *Ceratomegilla (= Semiadalia) undecimnotata*,

Fig. 1. Condition of testicular follicles and spermatheca in *Coccinella septempunctata* and *Ceratomegilla (= Semiadalia) undecimnotata*. Horizontal axis: 1 – lowest activity, 4 – highest activity; - – empty, + –filled with sperm; vertical axis: distribution

ticular follicles was much lower than in *C. septempunctata* (Fig. 1, January).

In spring, the increase in ambient temperature (to aver. temperature above about 12°C - Hodek 1960, 1973) initiates the opposite process. In April (earlier or later dependent on ambient temperature) the activity of testicular follicles is resumed; this occurs in *C. septempunctata* earlier than in *C. undecimnotata* (Fig. 1, Rana). The spermatogenesis begins: the spermatogonies begin to divide, groups of growing spermatocytes, enveloped by cyst-cells are formed and quickly fill the whole follicle. In some years the tissue of testicular follicles is fully active and spermiogenesis complete in all males after mid-April. Such a situation has previously been reported (Hodek & Landa, 1971) and was the case in spring 1999 (Ceryngier, Hodek, Havelka, unpubl.). However, the males do not have to wait for the new sperm to mature before mating, as they have kept reserves of sperm in their vesiculae seminales. If they disperse early, about one half of the females could use sperm from their spermathecae for fertilizing eggs.

no females were mated before dormancy or in early dormancy (n = 25). One female (n = 25) was mated between September and January (Fig. 1, *C. und.*). Two further species had intermediate incidence of pre-diapause meeting. About a third of *C. magnifica* females had full spermathecae in September (n = 13) and in *C. quinquepunctata* it was only 10% (n = 50) (Fig. 2).

In the next 4 months of the cold period, up to 22 Jan., the incidence of full spermathecae in *C. septempunctata* did not change, but in most males the activity of testicular follicles regressed to the medium stage of 2.2, on average, due to the low temperature prevailing over that period: there were loose spermatocytes and spermatids in the centre of the testicular follicle, with spermatogonies on its distal top and spermatodesms (spermbundles) on the proximal end. Spermatogenesis ceases at the stage of the division of spermatogonies or of young spermatocytes. In *C. undecimnotata* the average state of activity of tes-
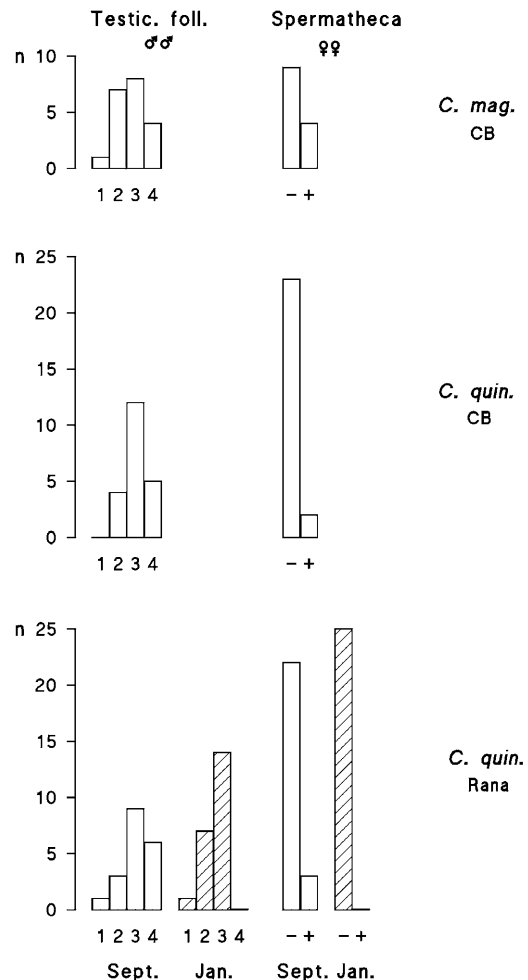

Fig. 2. Condition of testicular follicles and spermatheca in *Coccinella magnifica* and *C. quinquepunctata* (for explanations see Fig. 1)

Whether the viability of sperm that has been stored for more than 8 months - either in spermathecae or in vesiculae seminales - is lower than that of the "de novo produced" sperm, still has to be investigated.

As the females of *C. undecimnotata* overwinter with empty spermathecae, they all mate in spring (Hodek & Landa, 1971; Ceryngier, Hodek, Havelka, unpubl.). Mating at the dormancy sites might be advantageous in central Europe as this species is on the northernmost boundary of its distribution area and rather rare, so that the probability of the sexes meeting away from the hibernation sites is much lower than in *C. septempunctata*.

In spring almost all *C. septempunctata* females from Rana contained sperm in their spermathecae in late April, whereas only half of the females from C. Budejovice did in early April (Fig. 1). At the hill Rana the condition of spermathecae was similar in both *C. septempunctata* and *C. undecimnotata*, although the activity of testicular follicles was much lower in the latter species (Fig. 1). Most females of *C. undecimnotata* were apparently inseminated by males using the reserves of sperm in their seminal vesicles (Fig. 1, *C. und.*) (Ceryngier, Hodek, Havelka, unpubl.).

A biochemical study on the Japanese subspecies *C. septempunctata brucki* (Okuda, unpubl.) has given slightly different results. In central Japan, this subspecies has summer diapause and winter quiescence, i.e. the reverse of the situation in Europe (Hodek & Okuda,1997). In the laboratory at 25°C and long-day (i.e. conditions inducing estivation diapause) Okuda found that DNA synthesis in testicular follicles decreased within 30 days of adult life to less than a half of the pre-diapause (adult age 1 day) and post diapause (age 120 days) levels. The synthesis of DNA in beetles in deep diapause was at the same level as in beetles one day before adult emergence from pupae. Thus DNA synthesis never ceased completely and spermatocytes, although in reduced number, were present in diapausing males. In fact, this is not so much surprising, because the activity of the tissue of testicular follicles is also affected by the ambient temperature. The metabolic rate of Okuda's beetles in the laboratory remained much higher as they did not experience the marked decrease in temperature to which the beetles in our samples were exposed outdoors. In the mild winter of central Japan, similar results might be found in the field samples.

2.1 PREFERENTIAL MATING IN *ADALIA BIPUNCTATA*

Non-random mating is not only found in ladybirds. However, this aspect of their sexual life was studied rather actively as species with melanic and non-melanic forms are a convenient experimental model.

Already in the early sixties, Rothshild & Lane (1962) suspected that "mating of black and red forms of *Adalia bipunctata* in spring might not be completely at random". At about the same time Lusis (1961) observed matings of *A. bipunctata* at several localities in Riga and Moscow and recorded much fewer matings between red females and red males than would be expected in case if mating were random (Table 1). Reciprocally, significantly more "black" matings were recorded. He calculated an index of sexual activity for typical and black forms (Fig. 3). Lusis (1961) hypothesized that the higher sexual activity of the black forms is due to their higher metabolic rate, produced by higher absorbance of solar energy. Creed (1975) did not observe this phenomenon in a population of *A. bipunctata* near Birmingham and suggested that the increased mating activity in melanics may occur only in some parts of the ladybird's distribution area, i.e. for example in Eastern Europe.

However, within the next 10 years the existence of non-random mating was corroborated for English populations, and it was recorded that the females choose melanic males. Muggleton (1979) reported that the mating preference is affected by the frequency of a certain form: regardless of colour, the rare morph is preferred over the common morph. Majerus et al. (1982 b) reported a preference for melanic males in a population of *A. bipunctata* in N. Staffordshire in 1981: while amongst the non-mating males 34% were melanics, among the mating males the incidence of melanics was 49%, i.e. 15% higher. Incidence of melanic females, however, was the same, about 35%, for both mating and non-mating females. When compared to that expected from random mating, the calculated excess of melanic males in mating pairs was 20%. When the authors checked these field results in the laboratory they obtained similar results: about 23% of females preferentially mated with melanic males (Table 2). By means of selection experiments the authors demonstrated that the preferential mating has a large genetic basis (Majerus et al., 1982 a): the incidence of females mating with melanic males increased to 57% in the 4th generation (Table 3).

To discriminate between two possible mechanisms of non-random mating, i.e. between (1) competition among males and (2) choice by female, the beetles from the selected line were mated with unselected insects; in one replicate the males were from the 4th generation of the selection experiment, and the females from the unselected
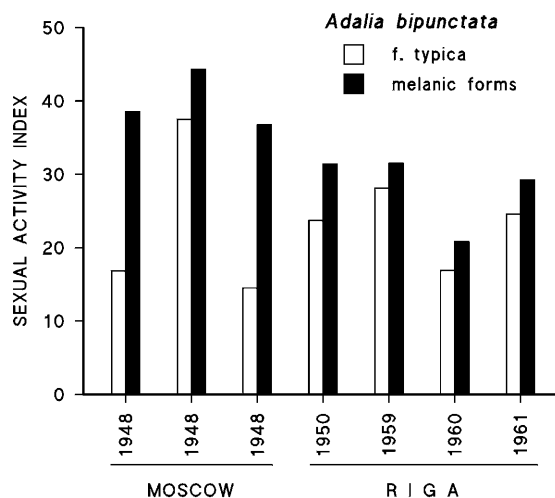

Fig. 3. Index of sexual activity in *Adalia bipunctata* (Lusis, 1961)(calculated from the results given in Table 1)

Place	Year	Host-plant	Time	Genera-tion [1]	Recorded beetles			Matings (n)		
					n	red(%)	black(%)	red × red [2]	black × black [2]	Σ [3]
Moscow	1948	*Padus*	5/5-19/5	0	604	90.5	9.5	38 47.4	3 0.4	57
Moscow	1948	*Caragana*	31/5-7/6	0	1479	89.5	10.5	130 147.4	4 1.8	182
Moscow	1948	*Lappa, Atriplex, Chaenopo-dium*	2/7-30/7	1	1621	96.5	3.5	105 115.5	2 0.1	124
Riga	1950	*Tilia*	19/6-9/7	1	2216	55.8	44.2	75 93.3	82 57.2	301
Riga	1959	*Humulus*	2/7-9/7	1	1550	63.2	36.8	82 90.0	34 30.4	225
Riga	1960	*Humulus*	24/6-2/8	1	8652	63.2	36.8	268 314.8	136 108.6	793
Riga	1961	*Humulus*	12/6-24/7	0, 1	10000	58	42	390 448.0	294 235.0	1331

1) overwintered beetles = 0

2) 1st line - observed mating, 2nd line - expected for the case that mating was random

3) mixed matings represent the difference

stock; males from the unselected stock and females from the selected line were in the other. In the third, control replicate, both sexes were from the unselected stock. Only in the case of selected females had the progeny the incidence of preferential mating typical for the selected line, i.e. above 50% (Table 4). Thus it was proved that the female preferred the melanic males (Majerus et al., 1982 a). The outcome is not caused by male competition. It appears that these studies confirmed Darwin's (1871) ideas and Fisher's (1930) theory of sexual selection by discovering "choosy females" in *A. bipunctata*.

Table 2. Preferential mating in *A. bipunctata*. In all cases the representation of melanic males in the matings is higher than would be expected were mating random (Majerus 1994, from Majerus et al. 1982b)

A Cage experiment

Number of ladybirds [1]	Matings observed				
	♂**mel.** ×♀**mel.**	♂**mel.** ×♀typ.	♂typ. ×♀mel.	♂typ. ×♀typ.	Total
30 mel., 70 typ.	13	21	12	27	73
30 mel., 70 typ.	16	27	14	34	91
50 mel., 50 typ.	**24**	**20**	9	9	62
50 mel., 50 typ.	**18**	**13**	7	7	45
70 mel., 30 typ.	71	24	15	8	118
70 mel., 30 typ.	60	27	12	7	106

B Small box experiment

Number of ladybirds	Matings observed				
	♂**mel.** ×♀**mel.**	♂**mel.** ×♀typ.	♂typ. ×♀mel.	♂typ. ×♀typ.	Total
5, 5, 5, 5	**47**	**39**	26	28	140

1) mel. = f. *quadrimaculata*; typ.= f. *typica*

Preferential mating with melanic males should help to maintain variation since it is frequency dependent. In the 1980s, the proportion of melanic forms was declining in Britain. As the females then had fewer melanic males to mate with, each melanic male would have to mate with many females. This tendency would be reversed when the melanic males become less rare. Negative frequency dependence of the advantage will thus help maintain the polymorphism (O'Donald & Majerus, 1984, 1985). Several years later the preferential mating was not found (Kearns et al., 1990, 1992) in the populations studied by Majerus, O'Donald and co-workers.

However, we still do not know how females recognize melanic males. It would be interesting to continue the analysis by searching for the mechanism of choice: is the female preference for melanics based on visual cues, or smell ? The study by O'Donald & Majerus (1989) suggests that the preferential mating in *A. bipunctata* is not based on visual discrimination by females of melanic from non-melanic males. It is to be hoped that the recent studies by Hemptinne et al. (1998) indicating the role of the species specific alkanes for the orientation of males towards females in *A. bipunctata* will help the further progress. Perhaps the hydrocarbons on the elytrae of melanics differ from those on non-melanics. Also the studies on functional morphology in coccinellids (Barbier et al., 1992; Jourdan et al., 1995) may help in the solution of the above question.

2.2 PREFERENTIAL MATING IN *HARMONIA AXYRIDIS*

Obata (1988a) described in *Harmonia axyridis* that the male's mating attempts sometimes fail: the female refused to mate. This is one factor that could determine non-random mating. Obata analysed some reasons for the refusal, such as bad food conditions or presence of a sper-

TABLE 3. Preferential mating in *A. bipunctata*. The excess of melanic males amongst mating pairs has a genetic basis (Majerus, 1994, from Majerus et al., 1982a)

Generation [2)3)]	Number of ladybirds used		Matings				Estimate of female preferences for mel. males [1)]
	typ.	mel.	♂♂mel. ×♀♀mel.	♂♂mel. ×♀♀typ.	♂♂typ. ×♀♀mel.	♂♂typ. ×♀♀typ.	
Selection line							
1	224	96	21	31	24	46	**0.18**
2	112	48	13	18	9	14	**0.39**
3	84	36	12	14	6	10	**0.46**
4	140	60	18	21	5	12	**0.57**
Control line							
1	98	42	13	15	8	21	**0.27**
2	70	30	7	11	7	12	**0.27**
3	112	48	10	18	10	18	**0.29**
4	112	48	11	22	13	28	**0.21**

1) assuming the excess of melanic males amongst mating pairs is due to female choice

2) 70 ♂♂ + 70 ♀♀ typ. + 30 ♂♂ + 30 ♀♀ mel. (gen. 1); ratio 7 typ.: 3 mel. restored at the start of gen. 2–4

3) next gen. of the selected line always founded by ♀♀ that mated with mel. ♂♂; all females, regardless of the form of their male mate, provided offspring in the control line

matophore in the bursa copulatrix. Another factor might be a difference in the activity of the males of different forms.

Osawa & Nishida (1992) studied mating preference in a Kyoto population of the polymorphic Asian species, *H. axyridis*. In this population, there occur three melanic forms (*axyridis*, *spectabilis* and *conspicua*) and one non-melanic form (*succinea*). Osawa combined field observations with laboratory mate-choice experiments. The prevalence of melanics recorded in spring decreased in summer due to non-random mating. This behaviour was corroborated by laboratory experiments: in spring both melanic and non-melanic females preferred mating with non-melanics. However, in summer non-melanics were less successful at mating than melanics. It was found that the sexual selection is mainly due to females (and only to a lesser degree to males) and that the colour of elytrae is the most important factor in female selection of males for mating.

These findings might indicate that also in *H. axyridis* the polymorphism is maintained by preferential mating. The alternation of forms in the course of seasons (non-melanics in spring vs. melanics in summer) echoes the data for *A. bipunctata* in the classic paper by Timofeeff-Ressovsky (1940).

Ueno et al. (1998) studied the overwintered beetles in two years in Gifu, only about 100 km distant, but isolated by mountains from Kyoto (the origin of Osawa's beetles). In non-melanic coccinellids they found a greater number of large males mating (size of mating males in 1992: 6.60, 1993: 6.54 mm, vs. non-mating 6.23 and 6.15 mm, resp.) and assumed that the difference in body size reflected a different level of activity. They did not find any significant difference from random mating expectation for colour morphs.

3.1 SPERM COMPETITION IN EPILACHNINAE

Multiple mating has been recorded in many coccinellid species, and its beneficial effects have already been reported (Hodek, 1973; Hodek & Honěk, 1996). Because the female can store viable sperm in the spermatheca, there is competition for fertilization between sperm from different males (Parker, 1970). Similar to other insects the earlier sperm is usually displaced by the last sperm. This was recorded in the Mexican bean beetle, *Epilachna varivestis* (Webb & Smith, 1968), and later in another epilachnin, *Henosepilachna pustulosa* (Nakano, 1985). Two morphologically distinct forms, the nominate form from central Hokkaido and the other from Sapporo, were used. A virgin female of the nominate form was mated first with a male of the nominate form, and after oviposition to a male of the Sapporo form. In reversed replicates a nominate female was first mated with a Sapporo male and then with a nominate male. A similar procedure followed with Sapporo females. The average percentage of offspring fertilized by the second male was 71.7%.

According to Walker's (1980) review, dealing with 30 insect species, only those with elongate or tubular spermathecae should show last sperm precedence, while species with spheroid spermathecae should show first sperm precedence. It is assumed that in an elongate spermatheca it is easier to displace prior sperm away from the spermathecal duct.

In both epilachnins, however, sperm precedence was recorded, although they have spheroid spermathecae. The so called spermatheca of Epilachninae is vestigial and has apparently lost its primary function. Sperm is stored in a pair of swellings in the common oviduct which may function in a similar way to an elongate spermatheca (Katakura, 1981).

TABLE 4. Preferential mating in *A. bipunctata*. Formal proof that the excess of melanic males amongst mating pairs is due to female choice. In each case the level of preference is characteristic of the females used in the experiment. (Majerus 1994, from Majerus et al., 1982a)

Stocks and sexes of lady-birds used [1,2]	Numbers of matings				Estimate of female preferences for mel. males
	♂♂ mel. × ♀♀ mel.	♂♂ mel. × ♀♀ typ.	♂♂ typ. × ♀♀ mel.	♂♂ typ. × ♀♀ typ.	
Sel ♂ × K ♀	11	28	17	34	0.191
K ♂ × **Sel** ♀	16	28	6	15	**0.539**
K ♂ × K ♀	18	35	21	42	0.224

1) 70 typ. and 30 mel. from specific stocks (equal numbers of males and females of each form), were placed in cages.

2) "Sel": ladybirds from the progeny of the fourth generation of the selected line with preference > 50%
"K": ladybirds from unselected Keele stocks - preference around 20%

3. 2 SPERM TRANSFER AND COMPETITION IN *HARMONIA AXYRIDIS* AND *ADALIA BIPUNCTATA*

The often observed regular body shaking that occurs during copulation in coccinellids was described in detail in *H. axyridis* by Obata (1988b) and its function analysed. Body shaking was essential for insemination: when the copulation was interrupted before body shaking started, the female laid a few eggs that did not hatch. Dissections confirmed that a spermatophore is formed in the bursa copulatrix during copulation (Obata, 1988b). Within one hour after the genital separation the spermatophore was emptied, ejected and usually eaten by the female (Obata & Hidaka, 1987). In *Chilocorus discoideus* the material from the male accessory glands that is injected into the bursa copulatrix, before the sperm, forms the spermatophore (Fisher, 1959). After ejection the spermatophore is not eaten in this species.

In *H. axyridis*, both Osawa (1994) and Ueno (1994, 1996) reported multiple mating and the latter author found last male precedence (P2 value = 0.55). He found positive correlation between the precedence and the duration of the second sperm transfer.

In an earlier study of *A. bipunctata*, de Jong et al. (1993) performed one type of experiment (scenario I): a virgin *typica* female was mated once with a *typica* male (1st phase) and after a week interval with a melanic male (2nd phase). An almost complete second male sperm precedence was recorded, although in several cases the possibility of first male sperm precedence was not completely excluded and some evidence for sperm mixing was obtained. Later de Jong et al. (1998) made a more detailed study in which two situations were compared. It was assumed that a greater ejaculate load from the 1st phase matings with *typica* males might affect the situation for the 2nd phase mating with melanic males. Another scenario (II) was therefore arranged with multiple matings in the 1st phase: one *typica* female was left for six days with three *typica* males. In the 2nd phase, however, there was no change compared to the 1993 scenario I: the female was only mated once with a melanic male. The

results of this scenario II were compared with those of the scenario I, the same as in the earlier study (de Jong et al., 1993).

The multiple matings (3–15 observed, aver. 8.4) in the first phase (scenario II) did not change significantly the percentage of melanic morphs in the progeny: 26.2% in comparison with 24.0% in the scenario I. The chance that the last male fertilizes an egg was not increased; this again provides evidence that in *A. bipunctata* mechanisms have evolved to reduce sperm competition, as was concluded in the earlier study (de Jong et al., 1993). In the 1998 study, there was a highly significant heterogeneity in the proportion of melanic offspring across families in both scenarios, indicating thus a high incidence of sperm mixing, higher than was shown by the fewer and smaller families in the 1993 study. However, the duration of successful mating (2nd phase) was much longer in the scenario II (73–280 min, med. 153), than in the scenario I (35–235 min, med. 108.5). Also the frequency of female rejection behaviour in the 2nd phase mating was higher in scenario II (in 16 of 20 females) than in scenario I (7 of 23 females). These results bring evidence in favour of the assumption that multiple mating changes the condition for the next phase of matings.

3.3 SPERM TRANSFER IN *CRYPTOLAEMUS MONTROUZIERI*

Recently this process and the related structures were described in great detail for a predator of mealybugs, *Cryptolaemus montrouzieri*, one of the most important agents of biological control (Kaufmann, 1996). It is a unique study of these aspects and thus the observations cannot be compared with those for other species and may not apply to the whole family. The author observed the movements of sperm in the spermatophore and spermatheca in 900 individuals. The ejaculatory system in *C. montrouzieri* consists of two chitinous structures and a large powerful muscle block; it is very efficient and highly specialized in comparison with other beetles, e.g. carabids. Although spermatozoa are produced in testicular follicles within about a week of emerging from pupae, the males do not mate before the age of at least 3 weeks; only then is the muscular block fully operational. Already this character appears to differentiate the scymnin *C. montrouzieri* from the ladybirds belonging to other subfamilies, e.g. from the most often studied Coccinellinae.

During mating the muscle block pushes sperm into the spermathophore, which is driven into the vagina of the female, where it is enveloped by the fertilization sac. The proximal tip of the sac is connected to the seminal duct of the spermatheca. The sperm is then pumped in; the force generated by the muscle block is indicated by the very visible swirling whirlpools. The transfer of sperm takes about 30 min. The spermathecal air pump, already 60–80% inflated at emergence of the female, is further inflated when the sperm is driven into the spermatheca. Usually the contents from three spermatophores (from three males) are accumulated together in the spermatheca (occasionally even four).

When the spermatheca is filled, the air pump is 90–100% inflated and ready for action. It blows out air, which circulates the sperm, at first to the posterior end. Again a whirlpool is visible around the opening of the pump. Sperm moves fast round the spermatheca until the pump is deflated to about 40%. Thus the sperm of 3–4 males is thoroughly mixed before fertilization (Kaufmann, 1996). These observations indicate that sperm precedence does not occur in *C. montrouzieri*.

Citations of results from Ransford's (1994) unpublished thesis indicate that his study brings important information on the transfer of spermatophors in *A. bipunctata*. It is a pity that this has not yet been published. A comparison with Kaufmann's (1996) observations on *C. montrouzieri* would be interesting.

Further similar studies on other coccinellid species might reveal important findings. A comparison, at least at the level of subfamilies, is needed.

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