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The effect of female mating history on sperm precedence in the two-spot ladybird, *Adalia bipunctata* (Coleoptera, Coccinellidae)

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Effects of two different mating regimes on sperm precedence in the two-spot ladybird, *Adalia bipunctata*, were studied using the polymorphic gene for melanism as a marker for paternity. Virgin nonmelanic females (homozygous recessive) were mated to nonmelanic male(s) and then, after laying fertilized eggs, were mated to a melanic male of known genotype. The results after the two successive single matings showed a highly variable degree of paternity of the second male. Initial multiple mating with nonmelanic males did not alter the pattern of paternity after the subsequent single mating with a melanic male, but it had two other effects: (1) the female showed an increase in rejection behavior, and (2) a longer copulation was required for high success of the melanic male. Additional observations in which families were reared from beetles collected in copula in the field demonstrated that sperm competition also occurs under natural conditions. The outcome of the competition was variable with frequent sperm mixing. *Key words:* copulation duration, mating history, rejection, sperm competition, sperm precedence. *[Behav Ecol 9:559–565 (1998)]*

Repeat matings by females occur in many insects, giving scope for sperm competition (Parker, 1970; Ridley, 1989). This competition within a female among the sperm from two or more males for the fertilization of the ova (Parker, 1970) has given rise to a wide array of adaptations and counter-adaptations within males that enhance the chance of a male's own sperm fertilizing the eggs or that reduce the chance of another male's sperm (see Thornhill and Alcock, 1983). It has become clear, however, that females do not necessarily play a passive role and are sometimes able to control paternity in several, perhaps subtle, ways (Arnqvist and Rowe, 1995; Birkhead and Møller, 1993; Sakaluk and Eggert, 1996). The outcome of multiple matings can be influenced by several factors including mating history. These need to be understood before any predictions can be made about the genetic or evolutionary consequences of sperm competition.

Female ladybird beetles are highly polygamous and store sperm in a spermatheca (Hodek, 1973). Two-spot ladybirds, *Adalia bipunctata* (L.), mate many times, both in the field (Brakefield, 1984a) and in the laboratory (de Jong PW, unpublished data). This species shows color polymorphism; the most abundant morphs are black with four or six red spots (melanic *quadrimaculata* and *sexpustulata*, respectively), which are primarily determined by a single dominant allele, or red with two black spots (*typica*) (Majerus, 1994). To evaluate the consequences of any deviation from random mating among these morphs for the maintenance of this polymorphism (e.g., Brakefield, 1984b; Muggleton, 1979; O'Donald and Majerus, 1984), knowledge is needed about the occurrence of sperm precedence under conditions relevant in the field. In this study we used laboratory experiments on two-spot ladybirds to examine the influence of mating history on the outcome of sperm competition and raised the offspring of pairings collected in the field to examine sperm competition within a natural population.

A previous experiment (de Jong et al., 1993) found that in many double matings of a virgin *typica* female to a *typica*, followed by mating with a melanic male, eggs were fertilized by exclusively the first or the last male. Females where only the first male was successful exhibited some rejection behavior toward the second, which could have been attributable to high ejaculate loads. Under field conditions, the chance that a female mates just twice is extremely low. If a single insemination does not always fill a female's spermatheca, variation in sperm load is expected, and possibly also variation in sperm precedence.

We designed the following laboratory experiments to determine whether the number of previous matings of a female influences a male's probability of fathering her offspring. The gene for melanism was used to determine paternity. We compared two states of mating history: series 1: two successive single matings of a *typica* female with, respectively, a *typica* and a melanic male; series 2: repeated matings of a *typica* female with *typica* males, followed by a single mating with a melanic male. Any influence of ejaculate load is expected to result in a lower success of melanic males in series 2.

In addition, we examined the incidence of sperm competition in a natural population by collecting beetles in copula and raising the offspring in the laboratory. These observations on field-collected pairings were performed to determine whether our observations on sperm competition in laboratory conditions also apply in the field and whether similar patterns of sperm competition to those in *typica* females occur following matings of melanic females.

METHODS

The laboratory experiments

Female *typica* ladybirds were from the F_3 of a laboratory stock originating from Utrecht, The Netherlands (melanic frequency = 31.1%, total n = 1182). Experimental males were collected in May 1994 at Zevenbergen, The Netherlands (22.6%

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melanics, $n = 190$; sexing as in de Jong et al., 1991). Rearing and experiments were carried out in a climate room at 20°C, 70% relative humidity, and an 18 h:6 h light:dark cycle. Males were kept at 2°C for 3 weeks, and subsequently for 1 week at 11°C until the day before the experiment. Experimental females were fully mature at the start of the experiment. Ladybirds at 20°C were fed daily on pea aphids.

On day 1 of the experiment (beginning of phase A), we randomly chose about 30 virgin *typica* females and allocated them to each series of crosses. Series 1 was similar to an earlier experiment (de Jong et al., 1993). We put each female in a clean, 9-cm petri dish (with a filter paper), together with one *typica* male each. We recorded time until start and end of copulation for each pair, as well as the occurrence of any female rejection behavior such as retracting their abdomens, running away, rolling over, and dropping from the lid of the petri dish (de Jong et al., 1993). Males were removed immediately after one mating. Females were then held for 7 days during which eggs were collected; females were transferred to a clean petri dish when eggs were found. We scored numbers of eggs and egg fertility per egg batch in each series. In series 2, we put the individual females in clean petri dishes at the same time on day 1, together with three *typica* males each. They were kept together for 6 days during which the number of matings was recorded by regular daytime observation (the data are minimum values), and eggs were collected and scored (see above).

On day 7 (beginning of phase B), we placed females of each series separately into clean petri dishes with one randomly allocated melanic male (form *quadrimaculata* or *sexpustulata*) each. Thus, all phase A copulations were with *typica*, and phase B with melanic males, and all females were *typica*. Time elapsed until start and end of copulation and rejection behavior were recorded. Melanic males were allowed to mate only once. Eggs were then collected daily, and larvae were divided into small groups with ad libitum feeding to reduce cannibalism. We scored the morph of each beetle, yielding a measure of sperm precedence assuming equal preadult viability. The experimental melanic males were genotyped by test-crossing each one to three virgin *typica* females. At least 7 offspring (usually >40) were raised per male to determine the paternal genotype with 99% certainty.

Field-collected pairings

We collected 23 female *A. bipunctata* at Zevenbergen (site 32 in Brakefield, 1984a) on 20 April 1995, soon after the resumption of activity and dispersal to shrubs following winter hibernation (Brakefield, 1985). These beetles were used to rear virgin *typica* females for test crosses. Two of the 23 females laid no fertile eggs and were presumably unmated.

Subsequently, at the same locality, we collected two further samples of mating pairs, one on 26 April and another on 25 May. The population was at high density on plantings of the shrub *Rosa rugosa*, the most important plant used for posthibernation feeding, mating, and egg laying in The Netherlands (Brakefield, 1984a). Some pairings were also collected on *Prunus padus* at the same locality. We also scored samples of solitary beetles for melanic morph on each date. Estimates of melanic frequencies were 20.9% on 26 April ($n = 287$) and 30.8% on 24 May ($n = 325$). The latter sample is more consistent with others from Zevenbergen, which indicate around 30% melanics. We collected 85 and 31 mating pairs on the two dates, respectively. There was no evidence of nonrandom mating in either sample (i.e., the proportion of the various mating combinations between morphs was not significantly different from that expected from the morph frequencies in the population (chi-square tests, ns).

Each pair of beetles in copula was carefully transferred from the plant to a clean 9-cm petri dish (with a filter paper) and placed in the shade. We allowed the pairings to separate when the male was immediately removed (the few pairs that broke up within 1 min of capture were discarded). Melanic males were later test-crossed with virgin *typica* females to establish their melanic genotype.

We used females from pairings in which one partner was melanic to rear individual families as in the laboratory experiments. Each female produced more than 50 offspring which were scored for color morph. The three families reared from homozygote melanic females are excluded from analysis (each gave 100% melanic offspring). No male mating partners were homozygote melanics. We released females from the 46 *typica* × *typica* pairings collected on 26 April in two outdoor population cages (3 × 4 × 2 m) planted with *R. rugosa* and *Tilia vulgaris*. Pupae and freshly emerged adults were collected 4 weeks later from the cages and scored. The *typica* × *typica* pairings ($n = 14$) from 24 May and all melanic × melanic pairings ($n = 8$) were discarded. All statistical tests performed in this study are two-tailed.

RESULTS

The laboratory experiments

After allowing the *typica* females to mate with the *typica* male(s) in phase A, 27 of 31 females in series 1 produced fertilized eggs; four laid only infertile eggs (an additional female failed to copulate and one died). In series 2, a similarly high proportion of females produced fertilized eggs (28 of 30). After remating with the melanic male (phase B), 25 series-1 females continued to produce fertilized eggs; 1 died before mating, and 1 stopped laying. In series 2, 20 females continued to lay fertilized eggs after mating B. The other eight females failed to mate even when the male was replaced with one that had already copulated successfully with another female. Therefore, these females were removed from the experiment (but see Discussion). In series 1, 2 of the 25 remaining females produced fewer than 10 offspring and were excluded from the analysis.

Determination of sperm precedence

Table 1 gives details of the successful crosses. To compare the overall data sets, we first examined the potential influence of family size. Including only crosses involving heterozygous melanic males and those with copulations longer than 30 min (shorter matings have never been fertile; de Jong PW, personal observation), family-sizes did not differ between series (1: $n = 21$, mean ± SE = 67.9 ± 20.4; 2: $n = 17$, mean = 78.3 ± 20.8; $t = 1.55$, df = 34, ns). No significant correlation occurred between family size and proportion of melanic offspring (1: $r = -.04$; 2: $r = -.21$). Pooling offspring showed no significant overall difference in melanic proportion between series ($n = 2756$; $\chi^2 = 1.79$, df = 1, ns; with 26.2% and 24.0% melanics in series 1 and 2, respectively). Moreover, these overall proportions of melanics did not deviate significantly from a 1:3 ratio expected if the chance of the last male to fertilize an egg is equal to that of a previous male (series 1: $\chi^2 = 1.08$, df = 1, ns; series 2: $\chi^2 = 0.78$, df = 1, ns; combined data: $\chi^2 = 0.017$, df = 1, ns). A Mann-Whitney U test showed no difference in the median proportion of melanic offspring between the series ($n_1 = 21$, $n_2 = 17$, $z = -.04$, ns). There was, however, highly significant heterogeneity in the proportion of melanic offspring among crosses within series 1 ($\chi^2 = 272.8$, df = 20, $p < .001$) and series 2 ($\chi^2 = 212.9$, df = 16, $p < .001$). Thus, although each series when

Table 1
Details of series 1 and series 2 crosses

Female no.	Male genotype	No. of offspring		Proportion melanic	No. of A matings	Mating duration (min)		Rejection behavior	
		mel	*typ*			A	B	A	B
Series 1									
1	Hom	33	0	1.0	1	131	106	–	–
2	Hom	72	26	0.73	1	138	220	–	+
3	Het	24	37	0.39	1	116	214	–	–
4	Het	20	19	0.51	1	73	94	–	–
5	Het	29	38	0.43	1	107	111	–	–
6	Het	39	34	0.53	1	88	81	+	–
7	Het	32	39	0.45	1	75	82	–	–
8	Het	21	21	0.5	1	109	87	+	–
9	Het	46	40	0.53	1	70	106	–	+
10	Het	25	27	0.48	1	107	135	–	+
11	Het	26	70	0.27	1	107	60	+	–
12	Het	22	42	0.34	1	140	130	–	–
13	Het	11	62	0.15	1	273	235	–	–
14	Het	28	61	0.31	1	92	58	–	+
15	Het	22	54	0.29	1	93	195	–	–
16	Het	13	68	0.16	1	96	115	+	–
17[a]	Het	1	42	0.02	1	108	35	–	–
18	Het	14	76	0.16	1	232	226	+	+
19	Het	0	68	0	1	116	144	–	–
20[a]	Het	0	19	0	1	120	30	–	+
21	Het	0	77	0	1	110	42	–	+
22[a]	Het	0	99	0	1	101	46	–	+
23[b]	Het	0	59	0	1	73	75	–	–
Series 2									
a[a]	Hom	13	35	0.27	4	nd	211	nd	+
b	Het	44	41	0.52	9	nd	235	nd	+
c	Het	23	35	0.40	8	nd	264	nd	+
d	Het	30	45	0.40	8	nd	160	nd	–
e	Het	43	32	0.57	6	nd	241	nd	–
f	Het	36	55	0.40	15	nd	223	nd	+
g	Het	17	64	0.21	9	nd	160	nd	+
h	Het	15	45	0.25	9	nd	97	nd	+
i	Het	7	24	0.23	10	nd	90	nd	–
j	Het	16	38	0.30	8	nd	126	nd	+
k	Het	33	68	0.33	10	nd	132	nd	+
l[a]	Het	17	68	0.20	3	nd	73	nd	+
m[a]	Het	1	112	0.01	6	nd	153	nd	–
n[a]	Het	3	104	0.03	12	nd	100	nd	+
o[a]	Het	10	73	0.12	10	nd	100	nd	+
p	Het	11	72	0.13	9	nd	133	nd	+
q[a]	Het	13	77	0.14	9	nd	282	nd	+
r[a]	Het	0	59	0	8	nd	83	nd	+
s[a]	Het	0	108	0	8	nd	24	nd	+
t	Het	0	65	0	6	nd	6	nd	+

Frequencies of *typica* (*typ*) and melanic (mel) offspring produced following mating B with a melanic male (homozygote: Hom; heterozygote: Het), duration of copulations and the occurrence of rejection behavior are given. For series 2 the minimum number of A matings is also indicated; nd, no data.

[a] Control mating with melanic male yielded one or more infertile egg batches.

[b] Control mating with melanic male yielded only infertile egg batches.

pooled showed apparently random use of sperm, there was extreme heterogeneity between crosses with respect to the outcome of sperm competition. Additionally, the crosses involving homozygous melanic males, where the offspring could unequivocally be allocated to a particular father, also show a variable proportion of melanic offspring (Table 1).

Duration of copulation

Durations of successful A matings within series 1 did not differ from those of B matings (analyzing crosses in which both A and B matings had definitely been successful; mating A: median = 107 min, range = 70–273 min; mating B: median = 108.5 min, range = 35–235 min; Wilcoxon matched-pairs signed-ranks test, $n = 18$, $T = 80.5$, ns). There was a significant correlation between the duration of the single initial A mating and the B mating in series 1 (Figure 1; Spearman rank correlation, $r_s = .57$, $n = 18$, $p < .05$), but exclusion of the two cases with exceptionally long A matings removes the correlation ($n = 16$, $r_s = .39$, ns).

Durations of successful B matings tended to be longer in series 2 (Mann-Whitney U test, series 1: median = 108.5 min, range 35–235 min, $n = 18$; series 2: median = 153 min, range 73–282 min, $n = 17$; $U = 100.5$, $p < .10$, two-tailed test).

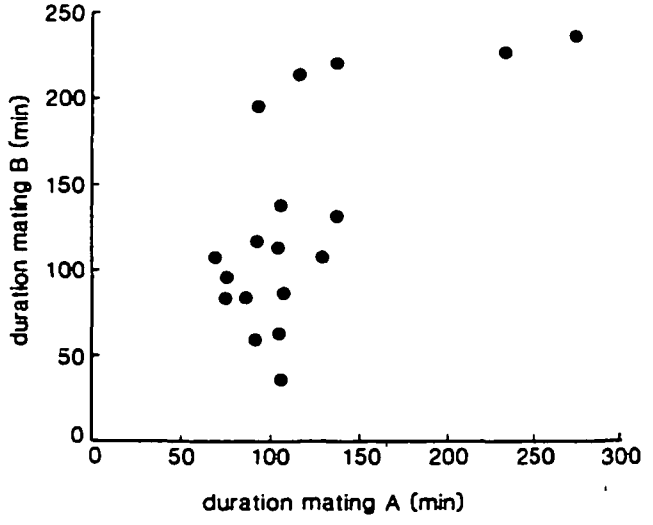

Figure 1
Duration of mating B against mating A in series 1.

Figure 2 indicates that the duration of B matings in which at least 80% of offspring were sired by the B-male (i.e., where the proportion of melanic offspring is at least 40%; note that with complete melanic male precedence, the expected proportion of melanic offspring is 50% when the melanic male is heterozygous) in series 1 is about half that in series 2. The average durations of these highly successful B matings are significantly longer in series 2 (Mann-Whitney U test, median = 100 min, range 81–135 min, n = 8 and 238 min, range 160–264 min, n = 4 for series 1 and 2, respectively; $U = 0$, $p < .01$, two-tailed test). Figure 3 shows that the frequency-distribution of copulation durations is apparently bimodal (although not significantly different from a normal distribution; Kolmogorov-Smirnov test, ns); the majority of A matings lie within the first peak. Most of the highly successful B matings in series 1 lie within the first peak, while those of series 2 lie within the second one. Within series 1, no significant correlations were found between the proportion of melanic offspring after mating B and the duration of matings A and B (Spearman rank correlation, $r_s = -.31$, $n = 23$ and $r_s = .22$, $n = 23$, respectively). There was, however, a significant correlation between the proportion of melanic offspring and the duration of mating B relative to A (B/A; $r_s = .43$, $n = 23$, $p < .05$).

Rejection behavior

In series 1 there was no difference between matings A and B in the frequency of female rejection behavior ($\chi^2 = 2.27$, df = 1, ns). Comparison of mating B between series 1 and 2 revealed a significantly higher frequency of rejection behavior in series 2 ($\chi^2 = 10.49$, df = 1, $p < .01$; excluding incidents of failed copulation without rejection behavior; see Table 1).

The field-collected pairings

The 46 *typica* females collected in copula with *typica* males on 26 April produced 185 offspring in the two population cages. Sixteen of these were melanics, demonstrating that some sperm from earlier pairings with melanic males were successful in fertilizing ova.

The offspring of matings of the homozygote *typica* × heterozygote melanic combination are given in Table 2. Many families are very large. A single family involving a melanic male mating partner produced only *typica* offspring, indicat-

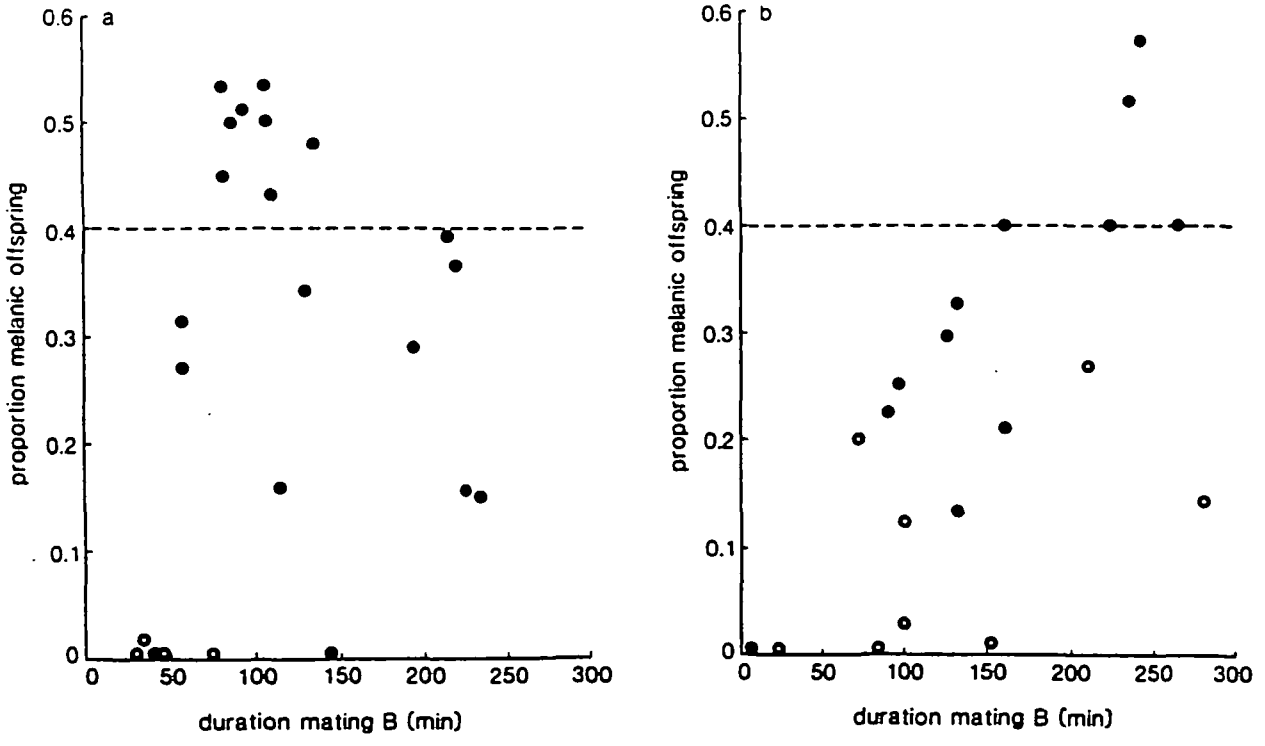

Figure 2
The proportion of melanic offspring after mating B against the duration of mating B in (a) series 1 and (b) series 2. A white star in a dot indicates that the control mating with the melanic male yielded one or more infertile egg batches. The area above the dashed line represents a high proportion of offspring sired by the B male.

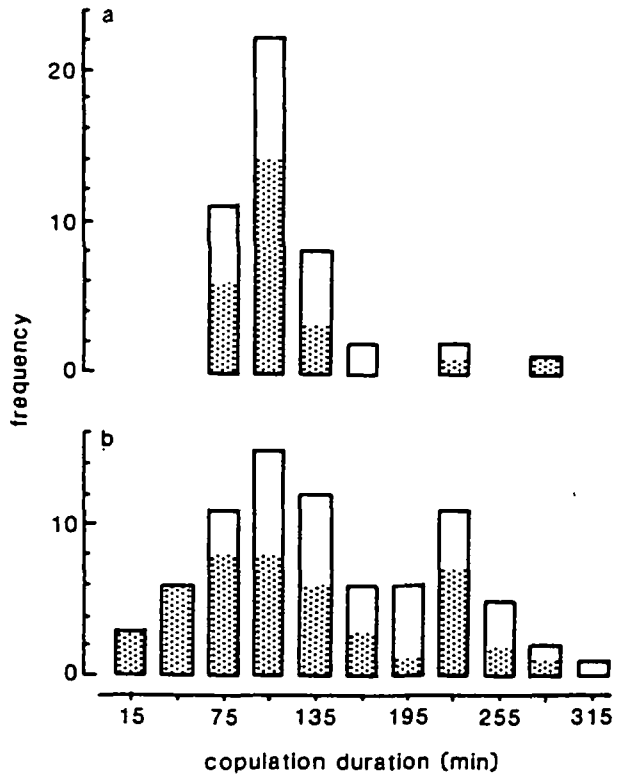

Figure 3
Frequency distribution of mating times for (a) mating A; (b) mating B; the figure presents the combined results of the present study (stippled area) and earlier experiments (de Jong et al., 1998 and unpublished results).

ing the complete failure of the final mating. This may be an example of first (earlier) male sperm precedence but could also be due to disturbance at time of collection. The analysis of this pairing combination is insensitive for detecting instances of first-male sperm precedence.

If eggs laid by the females shown in Table 2 were fertilized only by sperm from the observed final pairing, an equal frequency of each color morph would be expected in each family. Some families show frequency distributions close to equality; 7 of the 43 families show a proportion melanics of between 0.49 and 0.52. However, besides final male sperm precedence, this type of result could also arise after a single mating (which is unlikely), or a series of earlier (successful) matings which were all of the same pairing combination.

Many other families in Table 2 are clearly biased toward one color morph, indicating that sperm mixing is frequent. Variability among families occurs whether the male mating partner is melanic or *typica* (values from heterogeneity chi-square tests for three of the four groupings in Table 2 are highly significant with $p < .1$ for the first group involving *typica* males). There are fewer strong biases from matings involving *typica* males. Series of matings of the same combination are not expected to influence the segregation, and thus sperm mixing will only be detectable after a change in the mating combination within a sequence. Such a change is less likely for the final pairing combination of *typica* male × melanic female because only about 30% of males are melanic.

Additional evidence for the importance of sperm mixing comes from analysis of the pooled data for each mating combination (overall melanic frequencies in offspring are similar across collecting dates; the single family with only *typica* offspring is excluded). Both groups of offspring show a highly

Table 2
Details of families reared from *typica* × melanic pairings collected in the field

Pairing (M × F)	Date	Offspring mel	Offspring *typ*	Offspring *n*	Proportion melanic
mel × *typ*	26 April	27	44	71	0.38
		31	68	99	0.31
		34	73	107	0.32
		57	46	103	0.55
		11	74	85	0.13
		17	91	108	0.16
		26	70	96	0.27
		8	79	87	0.09
		43	51	94	0.46
		45	9	54	0.83
		45	54	99	0.45
		56	9	65	0.86
		60	41	101	0.59
		35	61	96	0.36
		27	63	90	0.30
typ × mel	26 April	38	44	82	0.46
		52	67	119	0.44
		58	33	91	0.64
		47	36	83	0.57
		55	51	106	0.52
		39	37	76	0.51
		67	55	122	0.55
		63	49	112	0.56
		42	49	91	0.46
		33	31	64	0.52
		52	39	91	0.57
		56	51	107	0.52
		43	42	85	0.51
		76	52	128	0.59
		51	72	123	0.41
mel × *typ*	24 May	42	94	136	0.31
		94	140	234	0.40
		55	119	174	0.32
		0	140	140	0
		100	100	200	0.50
		78	101	179	0.44
		34	71	105	0.32
		64	119	183	0.35
typ × mel	24 May	85	50	135	0.63
		112	31	143	0.78
		96	101	197	0.49
		106	93	199	0.53
		126	91	217	0.58

Frequencies of melanic (mel) and *typica* (*typ*) offspring are given for families grouped by collecting date and morph of female parent. Only data from pairings involving melanic heterozygotes are included. The proportion of melanic offspring is given.

significant departure from a 1:1 ratio of melanics:*typica* (melanic mating male: $\chi^2 = 134.7$; *typica* male: $\chi^2 = 25.1$, $p < .001$ in each case, df = 1). Furthermore, the offspring segregation differs greatly according to the mating combination (for melanic males = 38.5% melanics; *typica* males = 54.7% melanics). Rough estimates of the proportion of sperm from previous matings that are successful in fertilizing ova after the final pairing can be obtained by taking these overall proportions and assuming (1) all females are mated at least twice; (2) mating is random for color morph; (3) sperm from previous matings carry the melanic allele with a probability of one-sixth (approximate expectation with 30% melanic frequency and Hardy-Weinberg proportions); and (4) one-half of female gametes in families involving melanic females carry the melanic allele. The estimates are 34% and 55% for the

pairings involving melanic males and *typica* males, respectively. These estimates confirm the importance of sperm competition and the frequent occurrence of sperm mixing in field conditions. Indeed, together with the rearing of melanics from *typica* × *typica* pairings and the early spring onset of mating (including in hibernacula; Brakefield 1984b), these observations suggest that incidences of eggs laid between matings being fathered by single males are probably rare.

DISCUSSION

Proportion of melanic offspring

There was highly significant heterogeneity in the proportion of melanic offspring across families in each series of the laboratory experiment. Similar variability was also evident in families reared from the same pairing combination collected in the field. The results indicate a higher incidence of some degree of sperm mixing than was evident in the 11, comparatively small, families examined by de Jong et al. (1993), which all involved double matings. The data from field pairings show a lower frequency of families where few, if any, progeny resulted from the final pairing than in series 2 of the laboratory experiment (e.g., *m*, *n*, *r*, *s*, and *t* in Table 1). This probably reflects a more fully effective expression of rejection behavior by females under field conditions (see below).

Despite the large individual variation in sperm precedence, the pooled data for the laboratory experiment show no heterogeneity in proportion of melanics or difference in the median proportion of melanics between the series. De Jong et al. (1993) found that the average time in which singly inseminated females laid fertile eggs was 23.5 days, which is much longer than the time interval between our A and B matings (1–7 days). We also found no loss of fertility before the B mating, so that the chance that complete sperm exhaustion played a role when B males fertilized the eggs is extremely low. Also, no correlation was found between the number of eggs laid between mating A and B in series 1 and the proportion melanic offspring after mating B (data not shown). However, the gap between A and B matings may have caused a general bias in sperm precedence toward B-males: for both series the overall probability of an egg being fertilized by the last male was equal to that of being fertilized by a previous male (cf. approximately 0.3–0.5 chance for the field pairings). The apparent lack of an effect of a large number of previous matings on the chance that the last male fertilizes an egg is strong evidence that the two-spot ladybird has indeed evolved mechanisms to reduce sperm competition, as previously concluded by de Jong et al. (1993); a mere accumulation and proportional use of sperm from different matings is extremely unlikely to explain the results of this experiment. However, two other results, in addition to the data from field pairings, show that the mating history of a female does have an effect on the chance of a successful insemination.

Rejection behavior in females

We observed more female rejection behavior for the B mating in series 2, in which almost all females showed strong rejection. The high number of previous matings in series 2 (Table 1) or the short interval between the final mating A and the B mating might have induced this rejection, perhaps mediated by a high ejaculate load. As mentioned above, in a natural situation, rejection behavior is likely to decrease the chance of a successful copulation. In our laboratory experiments, however, the ladybirds were confined in a petri dish, which might have masked such a potential component of decreasing male mating success after multiple insemination of the female. Although we observed eight cases in series 2 where the B male failed to copulate and no active rejection behavior was observed, these might be examples of a decrease in male success due to multiple insemination of the female. Once in copula, a male is capable of fertilizing a high proportion of the subsequent eggs, even if the female has been inseminated many times (see Table 1). Thus, multiple initial matings do not preclude a successful subsequent insemination, supporting the view of behaviorally mediated effects of female mating history.

Duration of copulation

A second influence of female mating history on male mating success is revealed by comparing the dependence of B-male success on copulation time across series 1 and 2. The apparent bimodality of copulation times (Figure 3) supports the description of mating in *A. bipunctata* as involving one or more cycles (see, e.g., Cordero et al., 1995), each characterized by a specific behavioral sequence and the transfer of one spermatophore (Ransford, 1994). The position of the peaks in Figure 3 correspond closely with the durations of one- and two-cycle matings found by Ransford (1994). Although almost all A matings and highly successful B-matings from series 1 lie within the one-cycle peak, the highly successful B matings in series 2 are significantly longer and may involve two cycles. In Figure 2 it appears that the best a male can do in one cycle with a multiply inseminated female is to add some sperm and achieve some degree of sperm mixing. Apparently at least two cycles are required to manipulate previous males' sperm and fertilize all the subsequent eggs (note that full paternity of a heterozygous melanic male is expected to produce 50% melanics). The melanic frequency from pooled matings in series 2 of <150 min (presumably one cycle) is significantly lower than in series 1 ($\chi^2 = 23.2$, df = 1, $p < .001$), supporting our interpretation. For singly inseminated females in series 1, the amount of sperm present in the spermatheca might be sufficiently low to be manipulated successfully within one cycle. The time required to manipulate previous sperm and inseminate the female might also be influenced by the intensity of female rejection behavior. Both this effect and non-normal distributions of copulation durations have been observed in a number of other studies (see Lorch et al., 1993; Thornhill and Alcock, 1983).

The scenario described above is also supported by the correlation in series 1 between the proportion of melanic offspring after mating B and the duration of mating B relative to mating A. Our data show that the duration of mating A tends to be negatively, and the duration of mating B positively, correlated with the success of the B male. Thus, a long mating A relative to B seems to impede the B male. Furthermore, in series 1 the only two A-matings with a duration falling within the two-cycle peak (Figures 1 and 3) were associated with a duration of copulation B consistent with two cycles. Thus, a relatively long single mating may, like multiple insemination, require and induce a longer subsequent mating in order for the latter male to be successful. If the B male is able to assess female mating history, he may be able to adjust his copulation time. This may also be reflected in longer matings for B males in series 2. A similar influence of relative mating time on mating success was found for the water strider, *Gerris remigis* (Rubenstein, 1989).

Variation in sperm precedence

The chance of a copulating two-spot ladybird male of fathering offspring is clearly variable both under laboratory and field conditions. It is influenced by his partner's mating his-

tory and possibly by the amount of sperm or other material from previous male ejaculates in her spermatheca. Although many previous studies have reported variation in sperm precedence (see Lewis and Austad, 1990), it is only recently that intraspecific variation in sperm precedence patterns has been examined explicitly (e.g., Eady, 1994; Lewis and Austad, 1990; Sakaluk and Eggert, 1996; Simmons and Parker, 1992). To evaluate the evolutionary significance of sperm competition in a particular species, variation needs to be taken into account, and the mere calculation of mean P_2 (mean proportion of offspring sired by the second or last male; Boorman and Parker, 1976; Gwynne, 1984) may be of limited value or even misleading (see Lewis and Austad, 1990). The evaluation of the consequences of sperm precedence patterns is further complicated if individual patterns are not constant through time. Other factors that potentially influence variation in sperm precedence in ladybirds include variation in sperm quality (e.g., across morphs) and body size. Male mating history could also play a role, although the fertility of our wild males as used in the laboratory experiments was similar to that observed in earlier experiments with unmated, laboratory-bred males (de Jong et al., 1993).

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