than 1-d-old or older females (Table 2).

On July 6–7 and 8–9, 655 and 616 males were released but only 17 and 29 males copulated, respectively. The mating rate of the released males was low and the relationship between male age and mating potential was not clear. Further examination is necessary to evaluate the mating potential in males.

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REFERENCES

HOSOYA, J. (1956) *Jpn. J. Sanit. Zool.* **7**: 77–82 (in Japanese with English summary).

MINAMIKAWA, J. (1952) *Chagyo-gijutu-kenkyu* No. 6: 15–22 (in Japanese with English summary).

OYAMA, M. (1974) *Jpn. J. Appl. Entomol. Zool.* **18**: 9–13 (in Japanese with English summary).

WAKAMURA, S., T. YASUDA, A. ICHIKAWA, T. FUKUMOTO, and F. MOCHIZUKI (1994) *Appl. Entomol. Zool.* **29**: 403–411.

YOSHII, S. (1991) *Jpn. J. Appl. Entomol. Zool.* **35**: 77–79 (in Japanese with English summary).

Estimate of Multiple Insemination in a Natural Population of *Harmonia axyridis* (Coleoptera: Coccinellidae)[1]

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The degree of multiple insemination in a mating system where one female can receive sperm from several males considerably affects the sexual selection consequences. PARKER (1970) reviewed extensive literature and concluded that sperm precedence processes followed by multiple mating are under intensive sexual selection in which sperm competes for fertilization with sperm ejaculated from other males and stored in the female. In order to identify the selection consequences, along with a measurement of sperm precedence parameters, actual mating frequencies under natural conditions are necessarily estimated.

In many insect species, multiple copulation has generally been recognized under experimental conditions. However, the degree of multiple insemination under natural conditions is poorly understood. Direct observation in the field by following marked individuals could possibly underestimate copulation frequency (OSAWA, 1994).

The present study reports an estimate of multiple male sperm contribution in the ladybird beetle, *Harmonia axyridis*. This species has color polymorphism on the elytra which is controlled by four alleles (three melanic alleles and one non-melanic) at a single locus with melanic alleles being dominant over a non-melanic allele (TAN and LI, 1934). Using the variances of gene frequency within and among siblings reproduced by females collected from a natural population, the estimate could reduce the biases that occur in field observations while allowing the activities of the ladybird beetles under natural conditions to remain unaffected.

MATERIALS AND METHODS

In May, 1992, I collected overwintered adult females in a mating population from a suburb of Gifu City, Japan. Among the females collected, recessive homo females were individually reared in a plastic

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cup with freeze-dried honeybee larvae as food under conditions of 16L–8D and 25°C. Egg clutches were collected from each female and maintained until adult emergence under the same rearing conditions to determine the genotypic constitution.

An estimate of multiple paternity was made using the statistical method described by WILSON (1981). The average number of potential sires ($\hat{L}$) is given as the following equation;

$$\hat{L} = K'/2[(v-2)/v \cdot \mathrm{MSR}-1]^{-1} + 1/2,$$

where MSR is means square ratio, $\sum_{ij}(p_{ij} - p_{i\cdot})^2/\sum_{i}(p_{i\cdot} - p_m)^2$, K' is averaged sibling number after SATTERTHWAITE correction for sibling number variation, and v is degree of freedom for overall siblings. The four allele system was modified to fit a one locus two allele model by pooling the three melanic alleles into one allele. With the genetic system of a one locus two allele model, $\sum_{ij}(p_{ij} - p_{i\cdot})^2$ is the within sibling variance, and $\sum_{i}(p_{i\cdot} - p_m)^2$ is the among sibling variance, where p_{ij}, $p_{i\cdot}$ and p_m are melanic gene frequency of the jth individual in the ith family, average gene frequency in the ith family and overall average gene frequency, respectively. The general idea of the model is that a greater number of contributing males will increase the variance of male gene frequency within a sibling, while it will decrease the variance of gene frequency among siblings.

The present estimate requires genotypic constitution of siblings, all clutch members of which can be detected only when they are reproduced by recessive homo females. In the present population, the mating pattern showed no deviation from random mating expectation (UENO, unpublished). Thus, I assumed that mating was random with respect to the color morph and that the estimate using recessive homo females could represent the whole population.

RESULTS

A total of 21 sibling families were obtained from field-collected recessive homo females. And from these, 517 individuals emerged to adults. The numbers and gene frequencies are presented in Table 1 in the order of melanic/non-melanic.

The estimated mean square ratio was 0.934/0.212 = 4.409. Substituting $K' = 23.750$ and $v = 496$ into the equation yielded $\hat{L} = 3.823$. The 95% confidence limits with F-values, $F_{0.025}$ (20, 496) = 1.736 and $F_{0.975}$ (20, 496) = 0.476, were 1.864 and 7.831 matings for lower and upper limits, respectively.

Table 1. Offspring members of recessive homo females collected from a natural population of *Harmonia axyridis*

Family (i)	Melanic offspring	Non-melanic offspring	Total
1	0	13	13
2	6	3	9
3	5	4	9
4	10	1	11
5	10	5	15
6	9	6	15
7	3	6	9
8	8	1	9
9	11	7	18
10	13	18	31
11	8	2	10
12	43	20	63
13	15	9	24
14	8	5	13
15	9	5	14
16	71	32	103
17	6	8	14
18	12	4	16
19	17	10	27
20	38	11	49
21	10	35	45
Total	312	205	517

DISCUSSION

The estimation of the number of males contributing to fertilization was about 3.8 in the present study, suggesting intensive selection among sperm for fertilization. Because beetles were collected from the mating population, the opportunities for further copulations were restrained. Therefore, the present calculation may undervalue the mating frequency under natural conditions. Moreover, the present model made an assumption that sperm from all matings was used in equal proportions. If the sperm contributed unequally to fertilization, the present calculation of the number of sires would be an underestimate. The estimate would also be undervalued in the case where all copulation sires were not represented in the siblings.

P2 value, the proportion of last male sperm precedence obtained under the experimental conditions was around 0.55 (UENO, 1994). This was estimated from the genotype of the siblings produced by fe-

males which had two successive matings with different genotype males. Sperm utilization after more than three copulations is not known. However, the finite capacity of the sperm storage organ in females and a possible sperm displacement utilization pattern suggest that sperm from the last male has precedence, the proportion being 0.55, and that stored sperm from other males has a lesser contribution (UENO, 1994). Therefore, the present calculation may undervalue the mating frequency under natural conditions, and intensive sexual selection is even suggested.

OSAWA (1994) directly followed marked ladybird beetles in a different population and obtained a smaller estimation of multiple mating. Among 394 mating beetles observed, only 13.8% were observed to mate more than twice in that population. The distribution pattern of the copulation frequency was significantly different from the Poisson distribution, which, as suggested in his discussion, would be largely due to a failure to observe some copulations under natural conditions.

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REFERENCES

OSAWA, N. (1994) *J. Ethol.* **12**: 63–66.
PARKER, G.A. (1970) *Biol. Rev.* **45**: 525–567.
TAN, C.C. and J.C. LI (1934) *Am. Nat.* **68**: 252–265.
UENO, H. (1994) *J. Ethol.* **12**: 169–174.
WILSON, J. (1981) *Evolution* **35**: 664–673.

EAG and Field Responses of the Male Tea Tussock Moth, *Euproctis pseudoconspersa* (STRAND) (Lepidoptera: Lymantriidae) to (*R*)- and (*S*)-Enantiomers and Racemic Mixture of 10,14-Dimethylpentadecyl Isobutyrate[1]

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The sex pheromone components of the tea tussock moth, *Euproctis pseudoconspersa* (STRAND) were identified as 10,14-dimethylpentadecyl isobutyrate (10Me14Me-15:iBu) and 14-methylpentadecyl isobutyrate (WAKAMURA et al., 1994), and the absolute configuration of the former compound was determined as (*R*) by chiral syntheses of both (*R*)- and (*S*)-enantiomers and subsequent comparative EAG analyses of virgin female extract (ICHIKAWA et al., 1995). In the field, although many more males were captured with a trap baited with a racemic mixture of 10Me14Me-15:iBu than with three virgin females (WAKAMURA et al., 1994), the attractiveness of the enantiomers has not been evaluated. It is important to clarify which enantiomer or racemic mixture is most attractive to male moths. In the present paper, the EAG activity and attractiveness of (*R*)- and (*S*)-enantiomers and a racemic mixture of 10Me14Me-15:iBu are described.

MATERIALS AND METHODS

Insects. Larvae of *Euproctis pseudoconspersa* were collected from a wild population on camellia trees in Tsukuba, September, 1994, and reared on camellia leaves for three successive generations. Pupae were sexed and placed in separate containers until emergence. Males 1 d after emergence were used for the EAG measurement.

Chemicals. Both (*R*)- and (*S*)-enantiomers of 10, 14-dimethylpentadecyl isobutyrate (10Me14Me-15:iBu) were previously synthesized from (*S*)- and (*R*)-

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