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A Further Study on the Effect of Interspecific Mating on the Fitness in a Pair of Sympatric Phytophagous Ladybirds

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Abstract As a supplement to a previous study by NAKANO (1985), the results of multiple-mating experiments involving both conspecific and interspecific combinations between *H. vigintioctomaculata* and *H. pustulosa*, a pair of closely related sympatric ladybirds, were presented. In one series, females were successively mated with conspecific males once and heterospecific males once; after that these females were reared in pair with the heterospecific males. The fertilization ratios and the hatching ratios of eggs laid before the second (interspecific) matings were high but they significantly dropped during the pair rearing with the heterospecific males. In another series, females were first mated with heterospecific males once, and then mated with conspecific males once; after that they were reared singly. In this series, the fertilization ratios and the hatching ratios in *H. pustulosa* females and the hatching ratios in *H. vigintioctomaculata* females before the second (conspecific) matings were significantly lower than those in once conspecifically mated females. Although these values considerably rose after the second (conspecific) matings they did not always reach the level attained by the females who had mated only once with conspecific males. On the basis of the present results and those given by NAKANO (1985), the relations among the number of matings, the degree of conspecific sperm precedence and the fitness of interspecifically mated individuals were discussed.

Introduction

It has been considered that the premating reproductive isolation can be reinforced at the sympatric zones of two incipient species at the expense of less fitted hybrid progeny (LITTLEJOHN, 1969; DOBZHANSKY *et al.*, 1977; HETH & NEVO, 1981). However, the reinforcement of premating isolation is possible only when interspecific matings reduce the fitness of the interspecifically mated individuals (LEVIN, 1970; NAKANO, 1985). If individuals do not suffer the reduction of fitness from interspecific matings by some postmating devices, the premating isolation between the two species would not be reinforced even though their hybrids are extremely unfitted. NAKANO (1985) believes that a pair of closely related phytophagous ladybirds, *Henosepilachna vigintioctomaculata* (MOTSCHULSKY) (henceforth abbreviated as V) and *H. pustulosa* (KÔNO) (abbreviated as P), actually represent such a situation. It was already known that the hatching ratios by the conspecific matings of these two species were high, generally attained to 70% or more, whereas those by the interspecific matings were extremely low, being only a few percents (KATAKURA & NAKANO, 1979; NAKANO, 1981; KATAKURA, 1986). According to NAKANO (1985), however, the low hatching ratio by an interspecific mating rose to the normal level soon after a subsequent conspecific mating. Further, the high hatching ratio by a preceding conspecific mating did not significantly drop after a second interspecific mating. Moreover, almost all of progeny obtained from these doubly mated females were regarded as conspecific with the mother beetles. Thus, his results strongly suggest that in this species pair there is nearly complete conspecific sperm precedence at fertilization when both conspecific and heterospecific sperm are available. Under this condition, each female does not reduce the fitness when she mates at least once with a conspecific male before the onset of oviposition. From these results, coupled with other evidence, he considered that these two species in nature do suffer little deleterious effect from the interspecific matings, and hence, the premating isolation between them is difficult to evolve in spite of the distinctly low hybrid fitness. Indeed, the sexual isolation, one type of premating isolation, between these two species measured under the laboratory condition was weak though positive (KATAKURA & NAKANO, 1979), and their interspecific matings were rather common in a natural mixed population (NAKANO, unpubl.). However, each female mated only twice in his experiments. It is still not clear whether the conspecific sperm precedence is really strong enough as supposed by him. There is a possibility that the repeated interspecific matings may lower the high hatching ratio by the foregoing conspecific mating. We actually have experienced that in another crossing series using different local forms of P and V, interspecific matings of field caught post-hibernating females and subsequent pair rearing with the heterospecific males resulted in the production of eggs with intermediate hatching ratios between those of con- and interspecific matings (KATAKURA & NAKANO, 1979). Since the field caught females undoubtedly mated with conspecific males before the collecting, this result suggests that repeated interspecific matings may lower the high hatching ratio by foregoing conspecific mating(s). In the present paper, NAKANO's finding is supplemented and reevaluated on the basis of the data obtained in 1984 by multiple mating experiments including analyses of fertilization ratios and hatching ratios.

Material and Methods

The present experiments were made as a continuation of crossing experiments focused on the relation between the fertilization ratio and the hatching ratio and their probable relation to the condition and number of sperm kept by the female (KATAKURA & SOBU, 1986). And the beetles used in the present study were a part of "single-rearing series" in that study. The provenance of beetles and their rearing condition were described in KATAKURA and SOBU (1986).

The design of the present experiments was similar to that of NAKANO (1985). Virgin females were placed with males in a plastic case and allowed to mate freely. When a female mated successfully with a male (*cf.* KATAKURA & NAKANO, 1979), the female was reared individually with sufficient food. The fertilization ratio (*cf.* KATAKURA & SOBU, 1986) and the hatching ratio were checked for each female. Then, each female was again mated with another male. After the second mating, the check of the fertilization ratio and the hatching ratio was continued. The following two series and four combinations of matings were studied:

1) Conspecific mating – interspecific mating (CI): P♀(P♂–V♂); V♀(V♂–P♂).
2) Interspecific mating – conspecific mating (IC): P♀(V♂–P♂); V♀(P♂–V♂).

When the second mating was conspecific (namely, IC), the doubly mated females were reared singly afterward, but when the second mating was interspecific (CI), the females were reared in pair with the heterospecific males to know the effect of repeated interspecific matings.

Results

The results are summarized in Table 1. Further, the successive changes of fertilization ratios and hatching ratios in each rearing combination were shown in Fig. 1.

1. *Condition before the Second Mating*

The fertilization ratios and the hatching ratios before the second matings, namely, those of once mated females, of the present rearing series were already reported together with some additional data (KATAKURA & SOBU, 1986).

C-I series. The fertilization ratios by the conspecific matings were very high, and nearly all eggs were fertilized in both species. The hatching ratios were also generally high, though variable individually.

I-C series. The fertilization ratios were considerably different between the two reciprocal combinations of interspecific matings. The fertilization ratios of eggs laid by the interspecifically mated females of V were high like the fertilization ratios by the conspecifically mated ones. On the other hand, the fertilization ratios by the interspecifically mated P females were considerably varied according to individuals, and generally decreased in the laterly laid eggs. As shown in Table 1, the fertilization ratio of No. 4 female of P♀(V♂–P♂) was very low, and only the first egg mass was partially fertilized (*cf.* Fig. 1). Further, no egg was fertilized in a female of P♀(V♂–P♂) (No. 3 in Table 1, also *cf.* Fig. 1). It is uncertain whether this female received sperm at the first conspecific mating or not. On the other hand, the hatching ratios were always very low in both combinations of interspecific matings.

2. *Change after the Second Mating*

C-I series. In this series, females were reared in pair after the second matings

Table 1. Fertilization ratios and hatching ratios before and after the second matings in four types of mating combinations. V, *H. vigintioctomaculata*; P, *H. pustulosa*; N, the number of eggs examined.

Type of mating combination and code number of female		Fertilization ratio (%)*					Hatching ratio (%)*				
		before		after			before		after		
		N	%	N	%		N	%	N	%	
V♀×(V♂−P♂)	1	194	96.4	148	91.2	+	152	86.8	145	26.9	++++
	2	93	97.8	118	89.0	++	146	93.8	106	14.2	++++
P♀×(P♂−V♂)	1	79	98.7				131	55.0	36	52.8	—
	2	116	98.3	53	90.6	++	99	70.7	50	26.0	++++
	3	101	90.1	85	88.2	—	135	91.9	114	19.3	++++
	4	99	85.9	66	84.8	—	36	83.3	147	59.9	+++
	5	53	98.1	112	96.4	—**	137	89.1	58	17.2	++++
	6	133	97.0	44	95.5	—**	174	82.8			
V♀×(P♂−V♂)	1	191	93.2	229	93.9	—	205	1.0	192	58.9	++++
	2	116	94.0	220	96.8	—**	169	0	205	67.8	++++
	3	197	100	187	98.9	—**	238	10.1	189	75.1	++++
	4	181	90.6	140	96.4	+	101	0	206	86.4	++++
	5	208	94.2	178	90.4	—	175	0	155	80.6	++++
P♀×(V♂−P♂)	1	142	35.2	82	93.9	++++	111	0	135	60.0	++++
	2	47	93.6	158	95.6	—**	126	1.6	105	51.4	++++
	3	140	0	34	100	++++	176	0	16	37.5	++++**
	4	135	3.7	72	93.1	++++	121	0	107	71.0	++++
	5	123	93.5				124	0	36	22.2	++++**

* Chi-square test: —, $0.05 \geq p$; +, $0.02 \leq p < 0.05$; ++, $0.01 \leq p < 0.02$; +++, $0.001 \leq p < 0.01$; ++++, $p < 0.001$.

** FISHER's exact probability test.

with heterospecific males. The fertilization ratios slightly but significantly dropped after the second matings (=the first interspecific matings) in three out of seven cases. Further, the hatching ratios very significantly dropped after the second matings except for one case. As shown in Fig. 1, any clear trend during the pair rearing could not be detected in the successive changes of the fertilization ratios, but the hatching ratios were relatively steeply decreased during the pair rearing.

I-C series. The changes of the fertilization ratios and the hatching ratios in I-C series were profound except for the fertilization ratios in V♀(P♂-V♂) series. The fertilization ratios of P♀(V♂-P♂) series and the hatching ratios of both P♀(V♂-P♂) and V♀(P♂-V♂) series drastically rose after the conspecific matings. To know whether these ratios rise to the level attained by the females who had mated once with conspecific males, the results after the second conspecific matings in I-C series were compared with those of once conspecifically mated ones given in KATAKURA & SOBU (1986), a part of which were subsequently used as the C-I series of the present study. As shown in Table 2, there is no significant difference in the fertilization ratios between P♀(V♂-P♂) and P♀P♂, as well as V♀(P♂-V♂) and V♀V♂. However, the statistical analysis revealed that the hatching ratios

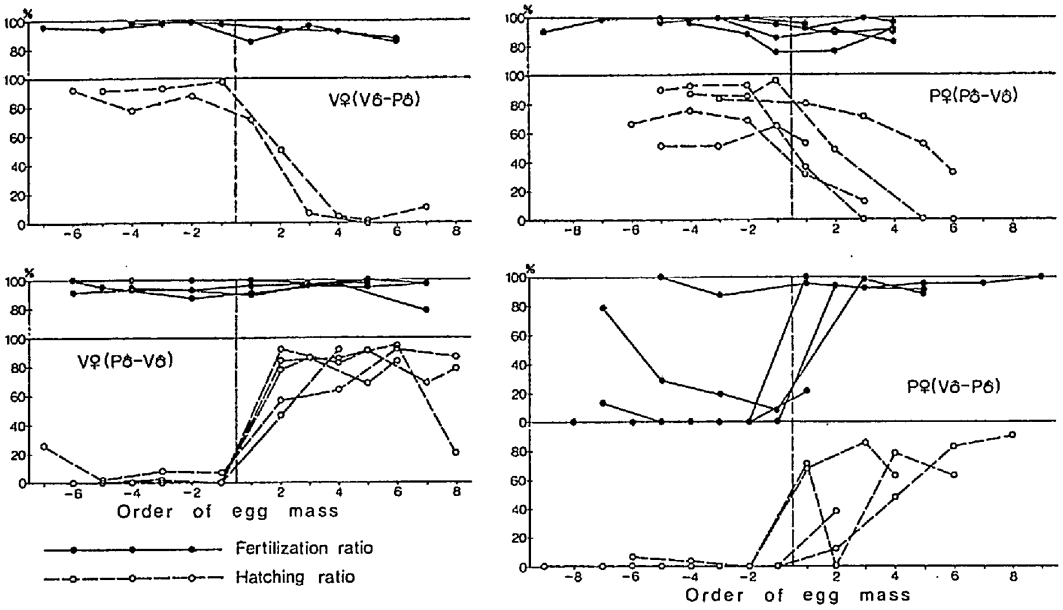

Fig. 1. Successive changes of fertilization ratios (solid circles and solid lines) and hatching ratios (open circles and broken lines) of egg masses in four types of mating combinations examined. Egg masses were numbered from the time of the second mating (vertical broken lines).

Table 2. Fertilization ratios and hatching ratios by single conspecific matings (C) and those after the second conspecific matings following the first interspecific matings (IC). The data for the single conspecific matings were cited from KATAKURA & SOBU (1986).

Species of females	C		IC		Probability*
	N	%	N	%	
H. vigintioctomaculata					
Fertilization ratio	4	97.4	5	95.3	p>0.2
Hatching ratio	4	89.0	5	73.8	p=0.02
H. pustulosa					
Fertilization ratio	8	94.3	4	95.7	p>0.2
Hatching ratio	8	75.9	5	48.4	p=0.02

* MANN-WHITNEY U-test.

did not reach the level attained by the single conspecific matings.

Discussion

On the basis of the results obtained by the double mating experiments, NAKANO (1985) concluded that in this species pair, the females would not suffer the deleterious effect from the interspecific matings if they mate at least once with the conspecific males before oviposition. However, the results of the present study are inconsistent with the results reported by him. By the pair rearing with heterospecific males after single interspecific matings, the fertilization ratios of the eggs laid by previously once conspecifically mated females decreased slightly, and the hatching ratios evidently. Further, by single conspecific matings after the preceding single interspecific matings, the fertilization ratios were raised to the normal level, but the hatching ratios did not attain the normal level though considerably increased. Contrary to NAKANO's belief that the interspecific matings do not reduce the fitness of the interspecifically mated females when they mate at least once with conspecific males, all the present results suggest that in this species pair, the interspecific matings more or less reduce the fitness of interspecifically mated individuals. In other words, the conspecific sperm precedence at fertilization in this species pair was not so strong as supposed by NAKANO. Despite the discrepancy in the empirical evidence obtained by his study and the present study above, however, the general conclusion of NAKANO (1985) seems to be valid. He considered that under the influence of complete conspecific sperm precedence, the loss of fitness of females due to the interspecific matings would be stochastically decreased with the increase in the number of matings. However, conspecific sperm precedence need not be complete. If there is substantial degree of conspecisfic sperm precedence, the loss of fitness would be stochastically decreased with increasing the number of matings. In order to showthis, I would like to examine a simple hypothetical situation below. Imagine a mixed population consisting of two closely related species which satisfy the following conditions:

1) These two species mate at random. 2) Their hybrids are completely inviable. 3) The sex ratio is equal in both species. 4) When a female mates more than once, the female preserve the same amount of sperm from each male irrespective of the species of the male. For example, when a female mated twice, a half volume of the sperm preserved by this female was from the first male and the other half from the second male.

Under these conditions, the proportion of the conspecific sperm preserved by the females who mate n times is given from the equation $(p+q)^n$, where p and q $(=1-p)$ are the proportion of conspecific individuals and that of heterospecific individuals in the mixed population. For example, if the females mate once, p of them mate with conspecific males and preserved conspecific sperm, and q with heterospecific ones and heterospecific sperm; if they mate twice, p^2 preserve only conspecific sperm, 2pq preserve the same amount of conspecific sperm and heterospecific sperm, and q^2 only heterospecific sperm; when mated thrice, p^3, conspecific sperm only; $3p^2q$, two third volumes of conspecific sperm and a third volume of heterospecific sperm; $3pq^2$, a third volume of conspecific sperm and two third volumes of heterospecific sperm; and q^3, heterospecific sperm only; *etc.* Then, let us express the strength of the conspecific sperm precedence by f $(0<f)$; when f is 1, there is no difference in the fertility between conspecific and heterospecific sperm;

Table 3. The effect of conspecific sperm precedence and the number of matings on the average fitness of females when p is 0.5. The fitness is given by the percentage ratio of viable offspring per female. Further explanations in text.

Number of matings	Strength of conspecific sperm precedence (f)							
	1	2	4	8	16	32	. . .	∞
1	0.50	0.50	0.50	0.50	0.50	0.50	. . .	0.50
2	0.50	0.58	0.65	0.69	0.72	0.74	. . .	0.75
3	0.50	0.61	0.71	0.78	0.82	0.85	. . .	0.88
4	0.50	0.63	0.74	0.82	0.87	0.90	. . .	0.94
5	0.50	0.64	0.75	0.84	0.90	0.93	. . .	0.97
6	0.50	0.64	0.76	0.85	0.91	0.94	. . .	0.98
.	.	.	.	.	.	.	. . .	.
.	.	.	.	.	.	.	. . .	.

when f is smaller than 1, the fertility of conspecific sperm is weaker than that of heterospecific sperm; and when f is larger than 1, the conspecific sperm is stronger. For example, when f is 3, the fertility of the conspecific sperm is three times as strong as that of the heterospecific sperm, namely the conspecific sperm fertilize the thrice larger amount of eggs than the heterospecific sperm of the equal volume. Since only the eggs fertilized by conspecific sperm are viable, we can tentatively define the fitness of a given female as the proportion of viable individuals in her offspring. Thus, the average fitness of females who have mated n times is given as:

$n=1$: p

$n=2$: $p^2+(2pq)(f)/(f+1)$

$n=3$: $p^3+(3p^2q)(2f)/(2f+1)+(3pq^2)(f)/(f+2)$

$n=4$: $p^4+(4p^3q)(3f)/(3f+1)+(6p^2q^2)(2f)/(2f+3)+(4pq^3)f/(f+4)$; *etc.*

When the females mate only once, the fitness of each female is 1 or 0 and their average fitness is p. Further, if there is no difference between conspecific and heterospecific sperm in the ability to fertilize, namely, f is 1, the average fitness of the females in an mixed population is again p irrespective of the number of matings made by them. However, when there is a difference between conspecific and heterospecific sperm in the fertility ($f \neq 1$), the average fitness of females in the population would be affected by the number of matings made by them. When f is larger than 1, the average fitness becomes larger with increasing the value n, and when f is smaller than 1, the opposite is true. Table 3 exemplifies the effect of n on the average fitness of females under the various f values when p is 0.5. Since the selection pressure to reinforce premating isolation is proportional to the loss of fitness* due to the interspecific mating, the result suggests that the selection pressure becomes weaker with the increase in the number of matings provided that there is substantial degree of conspecific sperm precedence. Table 3 also suggests

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* Although only the female fitness is examined here, the *average* fitness of the males must be changed strictly parallel to the average fitness of the females.

that when the female has a trait to mate many times before beginning oviposition, the loss of fitness due to the interspecific mating is weaker when f is larger. Thus, repeated matings by the female may act as a hazzard against the evolution of premating barriers under certain conditions. Theoretically, it is even possible that the number of matings prior to the oviposition or the conspecific sperm precedence (=gametic isolation) may be reinforced at the secondary contact of two cognate species whose females have an ability to store sperm for a long period of time. In the case of P and V studied in this paper and by NAKANO (1985), there may be a selection pressure for the reinforcement of the premating isolation between them. But the selection pressure must be weak owing to the strong conspecific sperm precedence and repeated matings by the females. The weak but positive sexual isolation between them must increase the frequency of conspecific matings in nature and hence, it may be sufficient to reduce the loss of fitness due to the interspecific mating.

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References

DOBZHANSKY, Th., F. J. AYALA, G. L. STEBBINS & J. W. VALENTINE, 1977. Evolution. W. H. Freeman & Co., San Francisco.

HETH, G., & E. NEVO, 1981. Origin and evolution of ethological isolation in subterranean mole rats. *Evolution*, 35: 259–274.

KATAKURA, H., 1986. Evidence for the incapacitation of heterospecific sperm in the female genital tract in a pair of sympatric phytophagous ladybirds (Insecta, Coleoptera, Coccinellidae). *Zool. Sci.*, 3: 115–121.

——— & S. NAKANO, 1979. Preliminary experiments on the crossing between two puzzling phytophagous ladybirds, *Henosepilachna vigintioctomaculata* and *H. pustulosa* (Coleoptera: Coccinellidae). *Kontyû, Tokyo*, 47: 176–184.

——— & Y. SOBU, 1986. Cause of low hatchability by the interspecific mating in a pair of sympatric ladybirds (Insecta, Coleoptera, Coccinellidae): Incapacitation of alien sperm and death of hybrid embryos. *Zool. Sci.*, 3: 315–322.

LEVIN, D. A., 1970. Reinforcement of reproductive isolation; plants versus animals. *Am. Naturalist*, 104: 571–581.

LITTLEJOHN, M. J., 1969. The systematic significance of isolating mechanisms. *In* Systematic Biology. *Proc. int. Conf. natn. Acad. Sci. Publ.*, pp. 459–482, Washington, D. C.

NAKANO, S., 1981. Supplementary notes on the crossing between two closely related phytophagous ladybirds, *Henosepilachna vigintioctomaculata* and *H. pustulosa* (Coleoptera: Coccinellidae). *Kontyû, Tokyo*, 49: 370–476.

——— 1985. Effect of interspecific mating on female fitness in two closely related ladybirds (*Henosepilachna*). *Ibid.*, 53: 112–119.