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Effect of Interspecific Mating on Female Fitness in Two Closely Related Ladybirds (*Henosepilachna*)

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Abstract Double-mating experiments involving conspecific and interspecific combinations were carried out with two closely related phytophagous ladybirds, *Henosepilachna vigintioctomaculata* and *H. pustulosa* in order to examine the effect of interspecific mating on the female fitness. In both species, the high level of percentage hatching of eggs after the first conspecific mating did not significantly change after the second interspecific mating, while the percentage hatching was very low after the first interspecific mating but rose to the normal level soon after the second conspecific mating. This suggests that the decrease of the female fitness by the interspecific mating is negligible in mixed populations, if females mate at least once with conspecific males before oviposition. In such circumstances, ethological isolation between the two species would not evolve.

Henosepilachna vigintioctomaculata (MOTSCHULSKY) and *H. pustulosa* (KÔNO) (abbreviated as V and P, respectively) are a pair of closely related phytophagous ladybirds widely sympatric in Hokkaido, northern Japan (KATAKURA, 1981). Although these two species generally depend on different host plants in the field (KATAKURA, 1981), interspecific mating is frequent when they coexist (NAKANO, unpubl.). Crossing experiments using laboratory-reared virgin females revealed that the percentage hatching of eggs produced by interspecific mating was much lower (average 4.0%) than that by conspecific mating (average 73.0%) (KATAKURA & NAKANO, 1979). However, the hatchability of eggs laid by females which had mated with males of the other species in the field was not always low (NAKANO, unpubl.). Moreover, most of the offspring obtained from these females showed the mother's phenotype (NAKANO, unpubl.). These observations suggest that interspecific mating between the two species does not always lower the fitness of the females, if she had previously mated with conspecific males. In order to estimate the effect of interspecific mating on the female fitness in mixed populations, the results of double crossings involving conspecific and interspecific combinations must be studied.

In the present paper, the successive change of the percentage hatching of eggs laid by the females which mated with both conspecific and heterospecific males is examined. Further, a possible relation between the ability of females to repeat mating and the lack of the strong ethological isolation between the two species is discussed.

Materials and Methods

The beetles used in the experiments were the laboratory-reared progeny of which parents were collected from thistle (P) or potato (V) in the vicinity of Sapporo, northern Japan, in 1979 and 1980. Males and females were isolated on emergence and separately kept for more than two weeks to insure their sexual maturity.

Experiment 1: Single matings

A virgin female was placed with a male in a plastic case of 6.5×5.5×2.0 cm (h) and the mating behavior was observed for 90 min. When the mating finished successfully (*cf.* KATAKURA & NAKANO, 1979), the female was reared individually with sufficient food (leaves of potato). The percentage hatching was checked for each egg mass of each female till her death. The egg masses were collected every day. The sperm longevity was represented by the number of days from mating to the last laying of viable eggs. In conspecific matings, it was the number of days from mating to the last oviposition as some eggs in the last mass laid by each female hatched. This method of estimating sperm longevity is useful since the eggs produced by virgin females have not been observed to hatch in V and P. Two conspecific (P♀×P♂ and V♀×V♂) and two interspecific (P♀×V♂ and V♀×P♂) combinations were tested.

Experiment 2: Double matings

The first mating was made as in Experiment 1. Then, each female was again mated with another male. The double-mated female was reared in isolation and the successive change in the percentage hatching of her eggs was recorded till her death. All the possible eight combinations of matings were tested. The types of the first (M_1) and the second (M_2) matings, the female species and the male species in M_1 and M_2 are as follows:

1) conspecific — conspecific (CC): P♀(P♂–P♂); V♀(V♂–V♂)
2) interspecific — interspecific (II): P♀(V♂–V♂); V♀(P♂–P♂)
3) conspecific — interspecific (CI): P♀(P♂–V♂); V♀(V♂–P♂)
4) interspecific — conspecific (IC): P♀(V♂–P♂); V♀(P♂–V♂)

Each combination was tested with 3–7 females. When the mating type was CI or IC, all larvae that hatched after M_2 were reared on *Solanum megacarpum* in 1979 and on potato in 1980. These plants are preferred and utilized in nature by both species (KATAKURA *et al.*, 1977; KATAKURA, 1981). In both experiments, the insects were reared at a constant temperature (24°C) and long day length (16L8D).

Results

The result of Experiment 1 is presented in Table 1. A single conspecific mating may be sufficient for fertilization of eggs for more than one month in both V and P. In interspecific mating, the eggs laid by seven females did not hatch at all. In the

Table 1. The longevity of sperm and percentage hatching of single-mated females.

Combination	Female no.	No. of eggs		Percentage hatching	Sperm longevity* (in days)
		laid	hatched		
P♀×P♂	1	127	112	88.2	9
	2	246	169	68.7	18
	3	209	119	56.9	10
	4	773	311	40.2	37
	5	327	122	37.3	18
	mean			(58.3)	
V♀×V♂	6	1009	645	63.9	40
	7	432	259	60.0	24
	8	228	135	59.2	16
	9	353	152	43.1	24
	10	382	97	25.4	18
	11	445	45	10.1	26
	12	330	8	2.4	16
	mean			(37.7)	
P♀×V♂	13	225	1	0.4	31
	14	390	0	0	—
	15	207	0	0	—
	16	126	0	0	—
	17	98	0	0	—
	18	87	0	0	—
	mean			(0.1)	
V♀×P♂	19	304	14	4.6	31
	20	589	2	0.3	13
	21	303	1	0.3	6
	22	507	0	0	—
	23	216	0	0	—
	mean			(1.0)	

* The successive changes in the percentage hatching of the egg masses laid by females Nos. 13, 19, 20, and 21 were (0/0/0/0/4.0/0/0/0), (0/6.4/3.0/0/0/11.4/30.3/7.1/6.3), (0/0/0/0/0/0/2.8/2.0/0/0/0/0/0), and (0/3.8/0/0/0/0/0/0), respectively. For example, the longevity of sperm in No. 13 coincides with the number of days from mating to the laying of the fifth egg mass (=31 days). In the case of conspecific mating, some eggs in the last mass laid by each female hatched.

other four females, however, sperm in interspecific mating was viable for 31, 31, 13, and 6 days. Since abrupt reduction in the percentage hatching with time was not observed in female No. 19, heterospecific sperm kept by some females might live as long as conspecific sperm.

The successive change in the percentage hatching of eggs laid by double-mated females is given in Fig. 1 and the average percentages of hatches before and after M_2 are presented in Table 2 for each combination. The average percentage hatching of conspecific mating in this experiment was lower than that previously reported (KATAKURA & NAKANO, 1979), possibly due to the different experimental design.

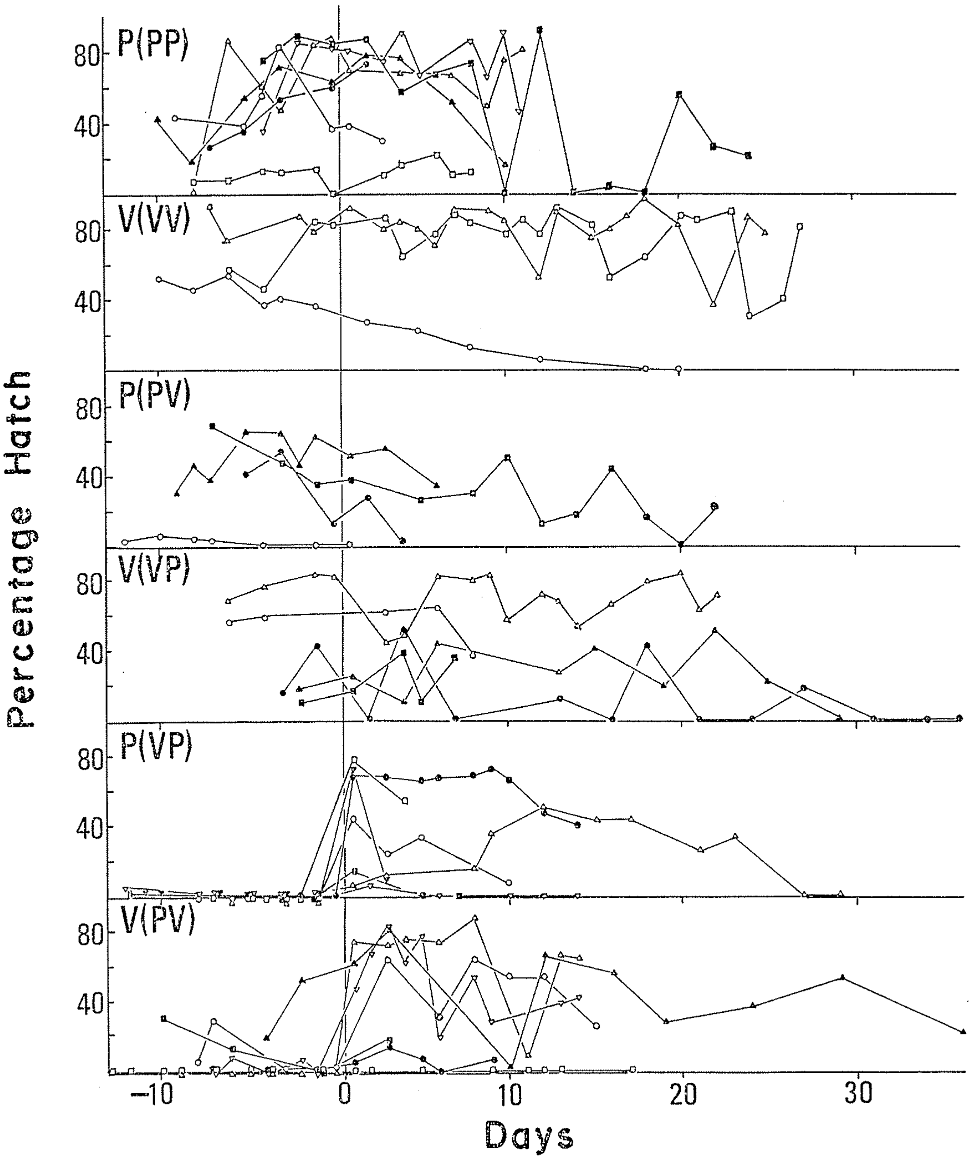

Fig. 1. Successive changes in the percentage hatching of eggs produced by double-mated females before and after the second mating. "O" denotes the day of the second mating. The percentage hatching in II combination was excluded because all egg masses in P (VV) and 32 out of 34 egg masses in V (PP) did not hatch at all. Solid and open symbols represent the results in 1979 and 1980, respectively.

Table 2 shows that the percentages of hatches in CC, II, and CI combinations did not significantly change after M_2 ($P > .05$, two-tailed *t*-test for paired comparisons), though the percentage hatchings fluctuated even in females of the same species combination (Fig. 1). In IC combination, the low percentage hatching after the first interspecific mating significantly rose ($P < .05$) soon after the second conspecific mating. Although the average percentage hatching after M_2 in IC combination was somewhat lower than that before M_2 in CC and CI combinations, the difference

Table 2. Average percentage hatching before and after M_2.

Mating type and species combination	Number of females	Before M_2		After M_2		(B)−(A)* and (range)
		Average egg number	Average percentage hatching (A)	Average egg number	Average percentage hatching (B)	
CC						
P (PP)**	7	165	52.6	212	52.4	-0.2^1 $(-42.3\sim30.2)$
V (VV)	3	207	65.0	598	55.7	-9.3^1 $(-32.9\sim7.8)$
II						
P (VV)	6	140	0.0	155	0.0	0.0^1
V (PP)	4	79	0.4	226	0.1	-0.3^1 $(-1.7\sim0.3)$
CI						
P (PV)	4	172	34.8	140	22.1	-12.7^1 $(-24.8\sim-2.4)$
V (VP)	5	70	38.7	314	37.2	-1.5^1 $(-19.0\sim15.8)$
IC						
P (VP)	7	189	0.1	165	32.4	32.3^2 $(0.5\sim66.4)$
V (PV)	7	179	8.9	242	33.7	24.8^3 $(0.0\sim65.5)$

*The *t*-test for paired comparisons (two-tailed), ^{1}not significant at .05 level, ^{2}$.01<P<.02$, ^{3}$.02<P<.05$. **♀(M_1 ♂ M_2 ♂).

was not significant ($P>.05$ by KRUSKAL-WALLIS test). This suggests that the percentage hatching after M_2 in IC females recovered to a level nearly equal to that attained by the first conspecific mating in CC or CI combination. The average percentage hatching before M_2 in V ♀(P ♂–V ♂) was higher than that in V ♀(P ♂–P ♂), since females which had produced more hybrids after M_1 were selectively used for V ♀(P ♂–V ♂) in order to examine the possibility of producing hybrid offspring in IC females.

Table 3 shows the numbers and the phenotypes of offspring produced after M_2 by the CI and IC females. Offspring of both hybrid and pure phenotypes might be expected because these females received sperm of the two species. But all offspring in 1979 (303/303) and most in 1980 (474/479) were identical to the respective mother phenotypes.

Table 3. Percentage hatching in CI and IC females before and after M_2. The phenotype and number of offspring obtained after M_2 are also given. The females whose offspring did not complete development (one female in each of P (PV), P (VP), and V (PV)) are excluded.

Mating type and species combination	Female no.	Percentage hatching		No. of adults obtained		
		before M_2	after M_2	P	V	Hybrid
CI						
P (PV)	1	35.6	15.4	7		0
	2	50.6	47.2	30		0
	3	50.7	25.9	69		0
Total (mean)		(45.6)	(29.5)	106		0
V (VP)	4	29.4	10.4		20	0
	5	18.5	27.8		36	0
	6	10.0	25.8		18	0
	7*	57.4	53.7		57	0
	8*	78.4	68.5		175	2
Total (mean)		(38.7)	(37.2)		306	2
IC						
P (VP)	9	0	62.6	77		0
	10	0	4.7	4		0
	11	0.2	40.4	23		0
	12*	0	24.4	67		0
	13*	0	66.4	9		1
	14*	0	27.6	12		0
Total (mean)		(0.0)	(37.7)	192		1
V (PV)	15	0	6.5		8	0
	16	35.3	45.5		9	0
	17	14.4	17.9		2	0
	18*	9.6	49.0		48	1
	19*	0	65.5		49	0
	20*	2.7	51.6		57	1
Total (mean)		(10.3)	(39.3)		173	2

* Studied in 1980, all other females in 1979.

Discussion

Although P and V are widely sympatric in Hokkaido, northern Japan, they are usually isolated owing to their different host plants. V mainly depends on potato (*Solanum tuberosum*, Solanaceae) or *Schizopepon brioniaefolius* (Cucurbitaceae), while P on thistle (*Cirsium kamtschaticum*, Compositae) and/or blue cohosh (*Caulophyllum robustum*, Berberidaceae) (KATAKURA, 1981). However, they often coexist on potato, *Solanum megacarpum*, and *Schizopepon brioniaefolius* when their habitats are adjacent (KATAKURA, 1981). Laboratory experiments revealed that ethological isolation between them is very weak (KATAKURA & NAKANO, 1979) and the percentage of interspecific matings on *Solanum megacarpum* during 1978–1980 reached 38%

(84/223) of the total matings observed at Nopporo near Sapporo, where nearly equal numbers of V and P coexisted (NAKANO, unpubl.). Although the percentage hatching of hybrid eggs is extremely low (KATAKURA & NAKANO, 1979), the viability and fertility of F_1 hybrid and viability of backcross and F_2 hybrids are normal (KATAKURA & NAKANO, 1979; NAKANO, 1981). Since the frequency of hybrids at Nopporo was extremely low, the major isolating mechanism between them is the low percentage hatching of F_1 hybrid eggs (NAKANO, unpubl.), mainly caused by death of hybrids at various embryonic stages (KATAKURA, unpubl.).

Then, why has ethological isolation not developed in the mixed populations at Nopporo? Although the food plant, *Solanum megacarpum* is native to Japan, the history of coexistence of the two species on it is not known. The lack of ethological isolation between V and P might be due to their recent contact. However, the present result provides another explanation based on the ability of females to repeat mating.

If the females of V and P could mate only once, the interspecific mating would cause reduction in the female fitness. In such circumstances, any trait which increases the possibility of conspecific mating would be favored and premating isolating mechanism would evolve (*cf.* DOBZHANSKY *et al.*, 1977). However, the females of both P and V mate repeatedly. Owing to the long mating period before oviposition in both V and P (KATAKURA, 1982), most females in the mixed populations would have mated with conspecific males at least once before oviposition. As mentioned above, the deleterious effect of interspecific mating on the female fitness can be eliminated by a single previous or future conspecific mating and a single conspecific mating is sufficient to fertilize eggs for more than one month in some females of both species. Thus, the interspecific mating between P and V does not exert any strong selection pressure. Consequently, ethological isolation between them is almost meaningless and difficult to develop despite frequent interspecific matings.

As far as I know, experiments on double matings involving conspecific and interspecific combinations were carried out only in two *Trogoderma* species, one of which showed the recovery of percentage hatching in IC combination (VICK, 1973). However, the above interpretation of the lack of premating isolating mechanism might be applicable to the following species pairs, *Nezara viridula* and *N. antennata* (KIRITANI & HOKYO, 1970; ITO & KIRITANI, 1971), *Oncopeltus cingulifer* and *O. unifasciatellus* (CHAPLIN, 1973), and *Trogoderma glabrum* and *T. inclusum* (STRONG & ARNDT, 1962; VICK, 1973), since these species repeat mating in both conspecific and interspecific combinations and the percentage hatching of eggs produced by interbreeding is extremely low. General accounts on the relation of multiple matings to the absence of premating isolation will be discussed in a separate paper.

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