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Supplementary Notes on the Crossing between Two Closely Related Phytophagous Ladybirds, *Henosepilachna vigintioctomaculata* and *H. pustulosa* (Coleoptera, Coccinellidae)*

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Synopsis F_1 fertility and F_2 and backcross viability of hybrids between Hokkaido form of *Henosepilachna vigintioctomaculata* and Sapporo form of *H. pustulosa* are briefly reported. F_1 hybrids of both sexes in two reciprocal combinations are fertile. The hatching ratios of eggs laid by intra-F_1 and backcrossing pairs are higher than those by interbreeding pairs reported previously. Both F_2 and backcross offspring can grow normally under laboratory conditions and their sex ratios do not significantly differ from 1 : 1.

Based on the preliminary crossing experiments between two closely related phytophagous ladybirds, *Henosepilachna vigintioctomaculata* (MOTSCHULSKY) and *H. pustulosa* (KÔNO) (henceforth abbreviated as Hv and Hp respectively), KATAKURA and NAKANO (1979) reported on the degree of their sexual isolation, hatching ratios of offspring by both conspecific and interbreeding pairs, and viability of their F_1 hybrids. But F_1 hybrid fertility and related items could not be examined because of the difficulty of successive rearing mainly due to the seasonal restriction of their foods, fresh leaves of their host plants. Previously YASUTOMI (1966) reported F_1 fertility and F_2 viability of hybrids between Hp and Hv, but he did not give any detailed records. Supplementing these studies, the present paper deals with the fertility of F_1 hybrids and the viability of both backcrosses and F_2 hybrids.

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Materials and Methods

Crossing experiments were intermittently made from 1978 to 1980 by using

* Contributions to the knowledge of *Henosepilachna vigintioctomaculata* complex. IX.

Table 1. Sources of the materials used.

Material	Parents or eggs collected from	on	Locality
F_1a (Hv♀×Hp♂)			
A*	*S. megacarpum*	June 26, 1978	Nopporo near Sapporo
B*	ditto	June 14, 1979	ditto
C*	ditto	June 4, 1980	ditto
D*	ditto	June 8, 1980	ditto
F_1b (Hp♀×Hv♂)			
E**	♂: *S. brioniaefolius*	July 4, 1979	Kobetsuzawa in Sapporo
	♀: thistle	June 1, 1978	Nopporo near Sapporo
F*	*S. megacarpum*	May 31, 1980	Nopporo near Sapporo
Hp			
G	thistle	May 27, 1978	Nopporo near Sapporo
H	ditto	June 1, 1979	ditto
I	ditto	June 8, 1980	ditto
Hv			
J	potato	June 10, 1978	Hiroshima-chô near Sapporo
K	ditto	June 13, 1979	Nishinosato near Sapporo
L	ditto	June 8, 1980	Nopporo near Sapporo

* Offspring of naturally interbreeding pairs.
** Offspring of a laboratory crossing pair.

Hokkaido form of Hv, Sapporo form of Hp, and their F_1 hybrids (F_1a: Hv ♀× Hp ♂, F_1b: Hp ♀×Hv ♂). Sources of the materials are listed in Table 1. Most of the F_1 hybrids used were obtained from interbreeding pairs occasionally caught at Nopporo near Sapporo, where I have been engaged in the study on a mixed population of Hp and Hv both dependent on a herb, *Solanum megacarpum* KOIDZ. (cf. KATAKURA *et al.*, 1977). Only E was obtained by laboratory crossing. Hv and Hp used for backcrossing were the laboratory reared offspring of some females (G, H, J–L) or were reared from eggs (I) both collected at field. A total of 20 pairs in eight kinds of combinations, one F_2 and seven backcrosses, could be examined (Table 2). Females and males, except those of a pair, No. 19, were separately reared soon after the emergence with sufficient fresh food (thistle or *S. megacarpum* for Hp, potato or *S. megacarpum* for Hv and F_1 hybrids) and were used for experiments after confirming their sexual maturity (cf. KATAKURA & NAKANO, 1979). In the pair No. 19, the female was kept together with four brothers from the emergence and the experiment was started without insuring her sexual maturity. Some F_1 males were used for successive crossings (each one male for pairs 1–4, 8–9, and 10–11). The results on some pairs which did not perform actual mating within the observation period (pairs 8, 9, 13, 16, 19 and 20) are also contained in Table 2. After crossing the hatching ratio of eggs produced by each pair was examined. Most of the larvae were reared with sufficient *S. megacarpum* (1978, 1979) or potato (1980) in order to obtain F_2 or backcross offspring and to check their sex ratio.

Table 2. Combinations of backcross and intra-F_1 matings examined.

Combination	Code no. for pair	Material used (cf. Table 1) (female × male)	Year
Backcross			
Hp♀ × F_1a♂	1–4	G × A	1978
	5–6	J × B	1979
	7	I × D	1980
Hv♀ × F_1a♂	8–9	H × A	1978
	10–11	L × D	1980
	12	L × C	1980
Hp♀ × F_1b♂	13	H × E	1979
Hv♀ × F_1b♂	14	K × E	1979
F_1a♀ × Hv♂	15	D × L	1980
F_1b♀ × Hp♂	16	F × I	1980
F_1b♀ × Hv♂	17–18	F × L	1980
Intra-F_1			
F_1a♀ × F_1a♂	19	A × A	1978
	20	B × B	1979

The larval periods (from hatching to adult eclosion) of some backcrosses and F_2 hybrids together with those of conspecific and F_1 offspring were measured mainly in 1979 and partly in 1980. All the rearing experiments were made under constant temperature conditions (26°C in 1978 and 24°C in 1979/1980) and a long day condition (16L8D).

Results and Discussion

1. *Fertility of F_1 hybrids and hatching ratios of eggs laid by intra-F_1 and backcross combinations.* Hatching ratios of eggs by intra-F_1 and backcross matings are summarized in Table 3. The F_1 hybrid fertility was clearly proved. Nineteen out of 20 examined pairs produced fertilized eggs. Among the six pairs whose actual mating could not be confirmed within the observation period, four pairs (pairs 9, 13, 16, and 20) produced fertilized eggs in the first oviposition, showing the occurrence of actual mating before their first oviposition. On the other hand, the first and second egg masses of pair 19 and the first egg mass of pair 9 did not hatch, though these unhatched eggs were also included in calculating the hatching ratios. Therefore, the hatching ratios obtained from these two pairs might be underestimated, if the actual mating of these pairs occurred just before the third or second oviposition respectively.

The hatching ratios remarkably fluctuated among seven backcross combinations examined, being highest in F_1a ♀ × Hv ♂ (72.2%) and lowest in F_1b ♀ × Hp ♂ (1.4%). Further, a considerable fluctuation was observed even within the pairs of

Table 3. Hatching ratios of eggs by seven backcross combinations (18 pairs) and one intra-F_1 combination (two pairs).

Parental combination	Code no. for pair	No. of egg masses	No. of eggs		Percentage hatching
			laid	hatched	
Backcross					
Hp♀×F_1a♂	1	2	86	11	12.8
(13.1)***	2	4	128	45	35.2
	3	5	187	15	8.0
	4	2	74	4	5.4
	5	6	215	25	11.6
	6	28	502	51	10.2
	7	1	23	2	8.7
	Total	48	1,215	153	12.6
Hv♀×F_1a♂	8*	3	64	5	7.8
(31.0)***	9*	2	97	31	32.0
	10	9	210	137	65.2
	11	7	126	63	50.0
	12	3	60	0	0.0
	Total	24	557	236	42.4
Hp♀×F_1b♂	13*	22	676	64	9.5
Hv♀×F_1b♂	14	18	618	76	12.3
F_1a♀×Hv♂	15	11	327	236	72.2
F_1b♀×Hp♂	16**	15	661	9	1.4
F_1b♀×Hv♂	17	13	415	127	30.6
(46.2)***	18	12	346	214	61.8
	Total	25	761	341	44.8
Intra-F_1 cross					
F_1a♀×F_1a♂	19*	12	182	42	23.1
(29.5)***	20*	33	989	354	35.8
	Total	45	1,171	396	33.8

* Actual mating not confirmed.
** Mating continued less than 60 min.
*** Mean hatching ratio per combination.

the same combination, *e.g.*, 5.4~35.2% in Hp ♀×F_1a ♂, 0~65.2% in Hv ♀×F_1a♂. The mean hatching ratio of 18 backcross pairs combined was 24.1%. On the other hand, the hatching ratios of two intra-F_1 pairs were 23.1% and 35.8% respectively, with 33.8% of the mean. Table 4 shows the mean hatching ratios of eggs laid by various combinations of Hv, Hp, and their F_1 hybrids, in which the results concerning conspecific and interbreeding pairs are cited from KATAKURA and NAKANO (1979). Although some combinations are not yet examined, the hatching ratios in backcross and intra-F_1 combinations show various states intermediate between those in conspecific and interbreeding combinations. In this experiment the hatching

backcrosses and F_2 hybrids mentioned above, and the normal growth of F_1 hybrids reported by KATAKURA and NAKANO (1979), it is likely that once F_1 hybrids hatch, subsequent growth and reproduction of these F_1 hybrids as well as their offspring may be fairly successful. For the maintenance of specific distinction, therefore, the occurrence of F_1 hybrids should be suppressed at a sufficiently low level by some isolating factors. In fact, hybrids between Hv and Hp seem rare in natural conditions. KATAKURA and NAKANO (1979) explained the rare occurrence of hybrids by the combinations of some incomplete isolating factors. I am now inclining to assume the remarkable low hatchability of eggs laid by interbreeding females of both Hv and Hp (KATAKURA & NAKANO, 1979) as the most crucial factor. Studies on the behaviour of heterogamic sperms in the female reproductive organ and/or embryonic development of hybrid eggs now in progress will clarify the factors governing the low hatchability.

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