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**Disappearance of Diapause in Hybrid Pupae
between Two Swallowtail Species, *Papilio*
xuthus and *P. machaon*¹**

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Abstract Crossing experiments were undertaken between male *Papilio xuthus* and female *P. machaon*. Hybrids obtained generally resembled *P. machaon* in larval stages, while the adults showed intermediate characters between parents. It is remarkable that the hybrids did not enter the pupal diapause nevertheless they were reared under short-day condition (8L-16D) throughout the larval stages.

I

Papilio xuthus and *P. machaon* are common and widespread butterflies over Japan, but they significantly differ each other in the capacity of resistance to low temperatures in the pupal diapause stages. The diapause pupae of *P. xuthus* are only tolerant to prolonged cold above supercooling temperature (about -20°C) (7). While, those of *P. machaon* are able to survive ice formation in their bodies, which occurs below supercooling temperature (about -20°C) (2). For the purpose of studying genetic factors affecting the physiological features characteristic to the pupal diapause, preliminary crossing experiments were attempted between the two species. Contrary to the expectation all hybrid pupae did not enter the diapause even if they were cultured in the formative larval stages under short-day photoperiodic condition which completely leads *P. xuthus* (4) and *P. machaon* (6) to pupal diapause. In the present paper, the life history of hybrids is reported together with some notes on the taxonomic characters in the adult obtained.

II

Crossing experiments were undertaken between male *P. xuthus* and female *P. machaon*. Adult males of *P. xuthus* were collected on the campus of Hokkaido University. Adult female *P. machaon* were taken from labo-cultured colonies which have started from eggs or first instar larvae collected outdoor and reared under long-day condition (16L-8D per day at 22°C) throughout larval stages. Adults were artificially mated by hand-pairing (1). Oviposition was stimulated by modified Richard's method (1). Females, after

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mating, were put into a glass cylinder (15 cm in diameter, 15 cm high) covered with the wire netted top. The cylinder was placed on potted goutweeds (*Aegopodium podagraria*, *Umbelliferae*), and butterflies were continuously exposed to artificial light.

III

One day or two after mating, females began to lay eggs on the both sides of goutweed leaves, and 186 eggs were obtained from three females. Freshly laid eggs were almost spherical, about 1 mm in diameter and lustreously pale yellow. Twenty-nine out of 186 eggs remained their pale yellow colour unchanged, i. e., they were unactivated eggs (unfertilized) (Fig. 1). In the remaining eggs (activated=fertilized), the brownish germ band was visible through the chorion two or three days after laying. During the growth of embryo, the activated eggs changed from brown to black.

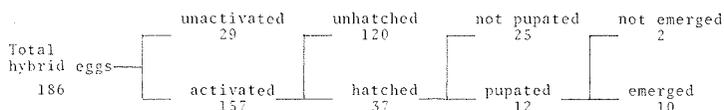


Fig. 1. Survival of hybrids

Six days after laying, young hybrids began to hatch out. However only 37 larvae could normally hatch out (Fig. 1). The other 120 activated eggs seemed to have completed their embryonic development. Actually the movement of embryos was seen through egg shells but they could not break the chorion. Some of such eggs were surgically broken open by forceps under a binocular and 22 embryos were extracted out from eggs, but all died within one day or two after the artificial hatching. Hatching rate of the hybrid larvae was thus 37/186 or 19.89%.

Normally hatched larvae were reared with leaves of goutweed (=food plant of mother species) and Amur cork tree (*Phellodendron amurese*, *Rutaceae*=food of father species) under short-day condition (8L-16D per day at 22°C) throughout larval stages. The hybrid larvae preferred goutweed leaves to Amur cork tree leaves.

IV

In the first and second instar larvae, the colour patterns of all *P. machaon*, *P. xuthus* and the hybrids were similar. Since the third instar, hybrid larvae generally resembled *P. machaon* larvae throughout the larval stages, but some recognizable differences were detected. The third instar hybrid larvae had remarkable white bands and spots on the dorsum. Among them, white spots near the spiracles of 10th and 11th segments were more conspicuous in hybrids than in *P. machaon* larvae, though less than in *P. xuthus* larvae. The 5th instar hybrid larvae had black and green stripes



Fig. 2. Third instar hybrid larvae

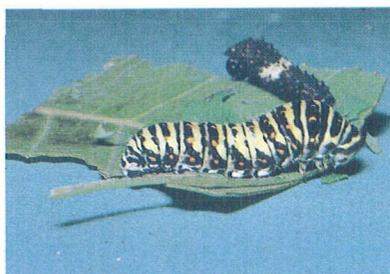


Fig. 3. Fifth instar hybrid larva with third instar larva

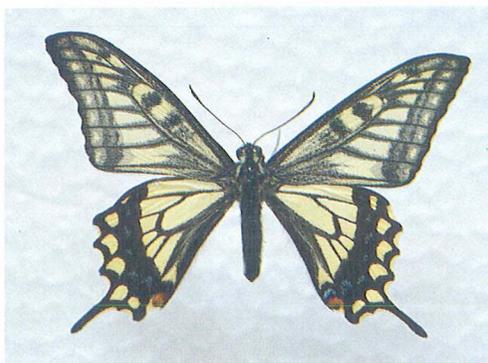


Fig. 4. Adult hybrid

around the abdomen as in *P. machaon* larvae, but the green stripes were paler than those of *P. machaon* (Fig. 3).

More than the half of hybrid larvae died during the larval stages. Only 12 out of 37 hybrid larvae successfully pupated (Fig. 1). Although not quantitatively compared, the larval mortality and growth rate of hybrids were far inferior to those of two parental species.

V

Hybrid pupae were stored under short-day condition (8L-16D per day at 22°C). They were expected to enter the diapause, because they had been reared in formative larval stages under short-day condition which completely leads *P. xuthus* and *P. machaon* into pupal diapause. However, 10 adult hybrids, all being males (5), emerged from 12 stored pupae about 10 days after pupation (Fig. 1). Remaining 2 pupae failed to emerge and died.

Hybrid butterflies were smaller than *P. machaon* and *P. xuthus* (Fig. 4). Fore-wing length from base to apex varied from 2.3 to 3.0 cm. The hybrids appeared intermediate in wing colour pattern. The ground colour of fore-wings was pale yellow resembling that of *P. xuthus*. Patterns in discoidal cell (Fig. 5, a) and first anal vein (Fig. 5, c) of fore-wing also resembled *P. xuthus*. But the black point, which is present in the space between *radius-5* and *media-1* of *P. xuthus*, were absent (Fig. 5, b). In

hind wings, the ground colour was deep yellow as in *P. machaon*, but black spot, which is distinct within a red circle near the anal angle of *P. xuthus* was nominally observed (Fig. 5, d).

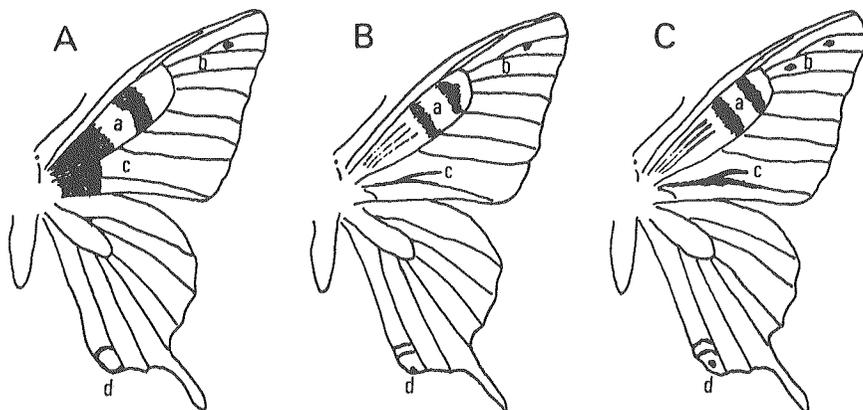


Fig. 5. Venation and some characteristic colour patterns of wings. A, *P. machaon*. B, Hybrid. C, *P. xuthus*. a, discoidal cell. b, space between *radius-5* and *media-1*. c, first anal vein. d, anal angle

Intermediate characteristics of hybrids were also observed in *harpes* of the external male genitalia. *Harpes* of the two parental species of Papilionidae and the hybrids basically resembled each other in the morphological appearance (Fig. 6). The serrate structure at the frontal part of *Harpes* differed in length, width and tooth number among the three kinds. The ratio of width to length in the hybrid showed intermediate value (0.37) between those of *P. machaon* (0.10) and *P. xuthus* (0.67).

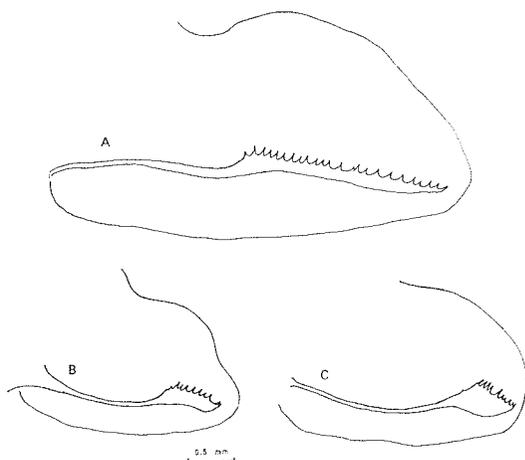


Fig. 6. *Harpes* of *P. machaon* (A), Hybrid (B) and *P. xuthus* (C)

VI

The photoperiodic reactions for the onset of diapause in several kinds of hybrids between local populations of the same species has been reviewed by Danilevskii (3). According to him, the first generation hybrids always have a photoperiodic reaction intermediate between those of the parent forms. He also quoted the results of crossing experiments by Kuznetsov and Geisprits (3) between different species of Pine Moth, *Dendrolimus pini* and *D. sibiricus*, which are very similar in the photoperiodic reactions each other. Their results show that the photoperiodic reactions of the interspecific hybrids have the same type of reaction as the parent forms.

In contrast to the results described above, the present experiment shows that the hybrids between *P. xuthus* and *P. machaon* cannot enter the diapause under the same photoperiodic condition which leads parental species into pupal diapause. However, it is not yet clear whether the hybrids completely lost the photoperiodic reactions for the onset of the pupal diapause or not, until more experiments are performed under various conditions.

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