A STUDY OF INTERSPECIFIC HYBRIDS IN BLACK SWALLOWTAILS IN JAPAN

by Shigeru Albert Ae

There are four species of black swallowtails in Japan, which resemble each other somewhat closely. They are *Papilio protenor demetrius* Cramer, *P. macilentus* Janson, *P. helenus nicconicolens* Butler, and *P. memnon thunbergii* von Siebold, and all are Rutaceæ feeders. Any combination of interspecific crossing of these swallowtails may produce hybrid butterflies and they may give some data on genetics of characters which separate species, and on evolutionary processes in these species, as in the study of the *Papilio machaon* group (Clarke & Sheppard, 1953, 1955a, 1955b, 1956a, 1956b; Remington, 1958, 1960; Ae, 1960). There are two other species of black swallowtails in Japan, *P. bianor dehaanii* C. & R. Felder and *P. maackii tutanus* Fenton, which have brilliant green and blue scales all over the upper side of the fore and hind wings. They resemble each other, and both are Rutaceæ feeders. Hybridization studies of these species may also give data of the kind which was noted above.

The writer started to work on hybridization of the above black swallowtails at Nanzan University, Nagoya, Japan, in 1959 and obtained hybrid adults (Figs. 2a & 5a) between *P. helenus* (Fig. 1a) and *P. proten*or (Fig. 3a) and between *P. bianor* (Fig. 4a) and *P. maackii* (Fig. 6). The work will be continued to obtain more data. However, the obtained data clarified the main characters of the F_1 hybrids of the above two kinds and gave some indications of interspecific relations. Therefore, the writer presents these data here.

P. protenor is very common and *P. helenus* is somewhat uncommon in Japan. A mixed flight of several individuals of each species on a flowering plant is not an unusual sight in certain localities. *P. helenus* is distributed in Japan, Taiwan, Philippines, South and West China, Thailand, Burma, India, Malaya, Sumatra, Java, and Borneo, etc., and divided into many subspecies. *P. protenor* is distributed in Japan, Korea, Taiwan, Middle and West China, Burma, and North India, etc., and divided into several subspecies. *P. bianor* is common all over Japan and *P. maackii* is also found all over Japan except most of the lowland of the southern half, but is not so common as *P. bianor*. *P. bianor* is distributed in Saghalien, Korea, Manchuria, China, Taiwan, and North Burma besides Japan. *P. maackii* is distributed in Korea, Manchuria, Amur, and Taiwan besides Japan.

MATERIALS

In May, two females of P. protenor, R-7-8 and R-7-10, were successfully hand-paired with a wild P. helenus male which was collected at Jokoji, near Nagoya. The above two females emerged from overwintering pupæ of Brood R-7, which was obtained from eggs laid by a female collected in Nagoya. The larvæ of this brood were reared on Inuzansho (Fagara schinifolia Engl.). In July, six females of P. protenor, R-15-1, R-15-2, R-16-5, R-16-8, R-16-14 and R-16-16, were successfully hand-paired with six males of *P. helenus*, of which five were collected at Hiravama and Nagao region near Shizuoka, Shizuoka Prefecture, and one at Jokoji. These six females emerged from pupe of Broods R-15 and R-16, which were obtained from eggs laid by females collected respectively in Nagoya, and at Jokoji. In September, a female of P. helenus was successfully hand-paired with a wild male of *P. protenor*, which was collected in Nagoya. This female emerged from pupze of Brood N-9, which was obtained from eggs laid by a female collected at Hirayama and Nagao region. The individual designation of a female was also used as the designation of its brood. The designation of a brood was used as the prefix of individuals in the brood.

On May 30, the writer collected a male of *P. maackii*, spring form, at Tsubame Hot Spring, Mt. Myoko, Niigata Pref. The butterfly was brought back to Nanzan University in good condition and hand-paired with 3 females of Brood D-9 of *P. bianor*: to D-9-13 on May 31, to D-9-14 on June 1, and to D-9-15 on June 2. The mother of Brood D-9 was collected at Jokoji in August, 1958. The larvæ, which hatched from eggs laid by this female, were reared on Inuzansho and they overwintered in the pupal stage. The pupæ were kept in the ice box until May 7 and brought out to the laboratory.

DURATION OF COPULATION

The technique of hand-pairing (Clarke & Sheppard, 1956b) was used for all matings. Minutes *in copulo* in Table 1 shows the approximate duration of copulation in *protenor* \times *helenus* and *maackii* \times *bianor* matings, from which eggs were obtained later. It is not certain what is a minimum effective duration of copulation. In the writer's many interand intra-specific matings in hand-pairings as well as in usual cage matings, a sufficient duration is usually 60 minutes, when copulation is normal. However, some copulations of less than 60 minutes were also fertile. REMINGTON'S data in *Papilio* (1960) show a sufficient duration can be 30 minutes. There may be a great difference according to the kinds of crossing. In the writer's experiments reported here, when a couple separated within 15 minutes, another attempt at hand-pairing was made and was usually successful immediately or some time later. The five *P. protenor* females which copulated with *P. helenus* males for more than 30 minutes did not lay any eggs and these data are omitted from the Table 1. The *P. bianor* female, D-9-14, above mentioned, also laid no egg.

FERTILITY OF MATINGS

Table 1 shows the fertility of matings. An egg has yellowish white color, when it is laid. If it starts to develop, a brown mottling or ring appears in *P. protenor* and *P. helenus* and in the hybird between them. This color change was used for an indicator of fertilization. However, no color change appears in *P. bianor* and *P. maackii* nor the hybrid between them in an early stage of development. The writer has as yet found no way to detect fertilization from superficial observation in these species. When a larva is well formed within the egg shell, the egg of these species and their hybrid turns black.

All or a part of the copulations in Table 1 which resulted in no fertile eggs may have been abnormal, although they are indistinguishable from other copulations which resulted in some fertile eggs. P. protenor females R-7-8 and R-7-10 were hand-paired with the same wild P. helenus male, N-1, firstly to R-7-8 and secondly to R-7-10. This male was previously hand-paired with three P. xuthus Linné females, and two of these females laid a few fertile eggs in spite of the wide crossing. The mate of P. helenus female, N-9-4, was a wild P. protenor male and was previously also hand-paired with a P. bianor female. This male may have copulated previously in the field. Therefore, the low fertility of the above three matings could have been the result of the shortage of spermatozoa. However, the data are by no means sufficient to conclude this. Although no data are available on intraspecific hand-pairings of the parental species at present, fertility of wild females shows some indication as a control (Table 1). Wild females of P. protenor had high egg fertility and hatchability in this experiment. However, a female of P. helenus, N-20, had very low fertility. This may also probably be the shortage of spermatozoa, since this female was collected toward the end of the flying season. Egg fertility, 98.2%, in Brood R-16-8 may indicate that egg fertility in hybrid P. protenor \times helenus is as high as in intraspecific

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	Table 1. FEI	RTILITY OF	THE MATING	SS.	
Brood	Eggs laid	Eggs fertile	Eggs blackened	Eggs hatched	Minutes in copulo
	A. P.	protenor $ imes$ 1	P. helenus		
R-7-8	47	23	22	22	140
R-7-10	34	6	1	0	65
R-15-1	33	0	_		50
R-15-2	106	0		_	120 +
R-16-5	1	1	1	1	68
R-16-8	113	111	98	93	60
R-16-14	17	0	-	-	60
R-16-16	42	0	-	_	
N-9-4	29	13	12	9	60
totals	422	${154}$	134	125	
	B. Con	trols (wild <i>F</i>	P. protenor)		
R-15	112	112	112	112	
R-16	138	136	136	135	
R-28	38	38	36	36	
	C. Co	ntrols (wild I	P. helenus)		
N-9	55	50	50	50	
N-10	6	5	4	3	
N-20	76	9	9	4	
	D. ♀ <i>P</i> .	bianor \times 3	P. maackii		
D-9-13	38	_	7	4	60 -120
D-9-15	2	_	0	0	60-120
	E. Co	ntrols (wild	P. bianor)		
D-14	69	_	57	57	

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matings in the best situation. The low egg fertility in other cases may be a mechanical failure and/or an effect of different compatibility against a partner of hybrid crossing by individual hereditary differences of parental species. Mechanical failure could involve hereditary differences in copulating organs besides a mere shortage of spermatozoa. Therefore, at present one could say that a crossing between *P. helenus* and *P. protenor* in Japan has a high egg fertility in the best condition environmentally and probably hereditarily. However, using the technique of hand-pairing means a complete bypass of courting behavior, which is very important in natural matings. Therefore, wild hybrids may not be produced at all in spite of wide sympatry between the two species.

Data are too small to discuss fertility of matings between *P. bianor* and *P. maackii* in general. Hatchability of one crossing obtained is as low as 10.5%.

DEVELOPMENTAL AND SURVIVAL RATES

Table 2 shows the developmental rates of the hybrids and their controls. The rates of the fastest developing individuals on different foodplants were recorded. Because of irregular death rates due to virus diseases during development, reliable data on average and slowest developmental rates could not be obtained.

The rearing was carried out at the window side of the laboratory room. The morning sun shone in the room until a little before noon. Since the rearing was carried out at the room temperature, temperature differences during development must be considered. Comparison of four broods of *P. protenor* reared on Inuzansho gives an indication concerning the temperature differences; lengths of their larval stages were 36 days in May-June, 30 days in July - August, and 22 and 28 days in August - early September. The differences of developmental rates due to foodplant differences are prominent in *P. helenus* and the hybrid *P. helenus* \times *P. protenor*. Only Inuzansho was used for the rearing of *P. protenor*, because of the shortage of the other food plants. The developmental rates of the hybrids between *P. helenus* and *P. protenor* seem to have no prominent difference in comparison with the parental species, although constant temperature rearing conditions are desirable for more accurate data.

P. bianor and the hybrid between *P. bianor* and *P. maackii* seem to have approximately the same developmental rate. A little delay in *P. bianor* may be seasonal, because the rearing of *P. bianor* was started 9 days earlier than the hybrid.

Brood	Kind of matings*	Date first egg laid	E egg	Ouration larva	n of pupa	Foodplant used
R-7-8	$\mathbf{R} imes \mathbf{N}$	May 21	4	38	13	Natsumikan
"	$\mathrm{R} imes \mathrm{N}$	May 22	4	28	_	Inuzansho
"	$\mathbf{R} imes \mathbf{N}$	May 23	4	33	-	Karatachi
"	$\mathbf{R} imes \mathbf{N}$	May 23	4	29	12	Unshumikan
R-16-8	$\mathbf{R} imes \mathbf{N}$	July 26	4	14	10	Natsumikan
"	$\mathbf{R}\times\mathbf{N}$	July 26	4	15	9	Karatachi
"	$\mathbf{R} imes \mathbf{N}$	July 27	4	14	10	Kihada
N-9-8	N imes R	Sept. 9	4	22	16	Unshumikan
"	N imes R	Sept. 9	4	24	20	Kihada
R-16	R imes R	May 18	5	36	14	Inuzansho
R-28	$\mathbf{R} imes \mathbf{R}$	July 12	5	30	12	Inuzansho
R-32	R imes R	Aug. 8	4	28	12	Inuzansho
R-33	$\mathbf{R}\times\mathbf{R}$	Aug. 12	4	22	11	Inuzansho
N-9	N imes N	July 18	3	28	13	Natsumikan
"	$\mathrm{N} imes \mathrm{N}$	July 19	3	35	-	Inuzansho
"	$\mathrm{N} imes \mathrm{N}$	July 18	3	26	13	Karatachi
N-20	$\mathrm{N} imes \mathrm{N}$	Aug. 17	3	28	diapause	Kihada
D-9-13	$\mathrm{D} imes \mathrm{K}$	June 3	6	30	10	Inuzansho
D-14	$\mathrm{D} \times \mathrm{D}$	May 25	7	31	13	Inuzansho

Гable 2.	DEVELOPMENTAL	RATES	IN	PAPILIO	HYBRIDS	AND	CONTROLS.

*Symbols in Tables 2-4: R=P. protenor; N=P. helenus; D=P. bianor; K=P. maackii.

Table 3 shows the survival rates in larval and pupal stages in the parental species and the hybrids when reared on the different foodplants. *P. helenus* and *P. protenor* are both Rutaceæ feeders. However, some specificity in foodplants is known in nature. According to SHROZU (1959), in Japan *P. helenus* larvæ feed on Kihada (*Phellodendron amurense* Rupr.), Karasuzansho (*Fagara ailanthoides* Engl.), Karatachi (*Poncirus trifoliata* Rafin.), Sansho (*Xanthoxylum piperitum* DC.), Yuzu (*Citrus junos* Tanaka), Unshumikan (*Citrus unshiu* Marcov.), etc. *P. protenor* larvæ feed on Yuzu, Unshumikan, Karatachi, Sansho, Inuzansho,

Brood	Kind of matings	Foodplant used	lst instar larvæ	Resulting pupæ	Survival rate
R-7-8	$\mathbf{R} imes \mathbf{N}$	Inuzansho	12	1	8.3%
R-7-8	$\mathbf{R} imes \mathbf{N}$	Natsumikan	3	1	33.3%
R-7-8	$\mathbf{R} imes \mathbf{N}$	Unshumikan	2	1	50.0%
R-7-8	$\mathbf{R} imes \mathbf{N}$	Karatachi	2	1	50.0%
R-16-8*	$\mathbf{R} imes \mathbf{N}$	Inuzansho	49	0	0.0%
R-16-8*	$\mathrm{R} imes \mathrm{N}$	Karatachi	10	2	20.0%
R-16-8*	$\mathbf{R} imes \mathbf{N}$	Natsumikan	12	5	41.7%
R-16-8*	$\mathbf{R}\times\mathbf{N}$	Kihada	2	1	50.0%
N-9-4	N imes R	Unshumikan	4	2	50.0%
N-9-4	N imes R	Kihada	2	1	50.0%
R-28	$\mathbf{R} imes \mathbf{R}$	Inuzansho	35	5	14.3%
R-16	$\mathbf{R} imes \mathbf{R}$	Inuzansho	53	23 (ads.)	43.4%
R-15	$\mathbf{R} imes \mathbf{R}$	Inuzansho	29	10 (ads.)	34.4%
N-9	$\mathrm{N} imes \mathrm{N}$	Inuzansho	30	1	3.3%
N-9	$\mathrm{N} imes \mathrm{N}$	Natsumikan	15	5	33.3%
N-9	$\mathrm{N} imes \mathrm{N}$	Karatachi	2	1	50.0%
D-9-13	$\mathrm{D} imes \mathrm{K}$	Inuzansho	4	2	50.0%
D-14	$\mathrm{D}\times\mathrm{D}$	Inuzansho	49	32	65.3%

Table 3. LARVAL SURVIVAL RATES IN PAPILIO HYBRIDS AND CONTROLS.

* Larvæ of this brood reared on mixed food plants were omitted from this table.

Miyamashikimi (Skimmia japonica Thunb.), Karasuzansho, Goju (Evodia rutæcarpa Hook. fil. & Thoms.), Kinkan (Fortunella japonica Swingl.), etc. Data indicate that while P. protenor larvæ have rather high survival rates on Inuzansho, P. helenus larvæ have rather low survival rates on it. P. helenus larvæ have higher survival rates on Natsumikan (Citrus natsudaidai Hayata) and Karatachi than on Inuzansho. The hybrids showed the same tendency as P. helenus. The hybrids also showed good development on Kihada.

Keeping these results in mind, and reconsidering the data on Table 2, the slower developmental rates of *P. helenus* and the hybrids between

P. protenor and P. helenus on Inuzansho in comparison with the other food plants may also indicate the unsuitability of foodplant.

P. bianor larvæ feed on Kokusagi (*Orixa japonica* Thunb.), Karasuzansho, Kihada, Sansho, Inuzansho, Karatachi, etc. and *P. maackii* larvæ feed on Kihada, Hirohanokihada, Karasuzansho, Hamasendan, etc. *P. maackii* does not seem to be able to feed on more kinds of foodplants than *P. bianor*. It is known that in Japan *P. maackii* is distributed in warm lowlands only where its food plants are found and is absent from other warm lowlands in which only the foodplants used by *P. bianor* are found (Shirozu, 1959).

The larval hybrids between *P. bianor* and *P. maackii* were reared on Inuzansho and fed on it readily. However, since Inuzansho is not a usual foodplant of *P. maackii*, this plant may not be the most suitable foodplant for the hybrid.

EXPRESSION OF INTERSPECIFIC DIFFERENCES IN HYBRIDS

1. P. protenor \times P. helenus.

Larval differences between Papilio protenor and P. helenus are very slight. A detailed study of large series will be necessary to show the differences. The writer's superficial observations on each of more than 20 larvæ of the parental species and the hybrids during the rearings showed a somewhat prominent difference only on the 5th instar larvæ (Figs. 1b, 1c, 2b, 2c, 3b, 3c); the stripe on the 6th abdominal segment is almost or entirely discontinuous at three points on P. helenus and is continuous on P. protenor. This stripe on the hybrid is usually discontinuous, and therefore it resembles P. helenus.

Pupæ of *P. helenus* and *P. protenor* are distinguishable only on the mid-ventral bend, on which the angle is sharper on *P. helenus* than on *P. protenor* (Figs. 1d & 3d). Pupæ of the hybrids (Fig. 2d) seem to be indistinguishable from pupæ of *P. helenus*.

Most of the hybrids emerged during the summer. They are much smaller than summer forms of wild *P. helenus* and *P. protenor*. However, butterflies reared in a laboratory are usually smaller than wild butterflies. Therefore, a comparison should be made with laboratory reared specimens. Pupal lengths of the reared specimens were measured for this purpose. Table 4 shows the results. The hybrids are not necessarily smaller than the parental species according to these data, although more data are necessary for a final conclusion.

In all, 15 hybrid butterflies were obtained, 2 from Brood R-7-8, 10 from Brood R-16-8, and 3 from Brood N-9-4 (Fig. 2a). All of them were males and some of them failed to extend their wings fully.

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Brood	Kind of matings	Foodplant used	Pupæ obtained	Average pupal length (mm.)
R-7-8	$\mathbf{R} imes \mathbf{N}$	Natsumikan	1	31.0
R-7-8	R imes N	Unshumikan	1	34.0
R-7-8	R imes N	Inuzansho	1	29.1
R-16-8	$\mathrm{R} imes \mathrm{N}$	Natsumikan	5	31.38 ± 0.97
R-16-8	$\textbf{R} \times \textbf{N}$	Karatachi	2	26.75 ± 4.31
R-16-8	$\mathrm{R} imes \mathrm{N}$	Kihada	1	33.0
N-9-4	N imes R	Unshumikan	2	27.55 ± 0.78
N-9-4	N imes R	Kihada	1	30.1
R-16	$\mathbf{R} imes \mathbf{R}$	Inuzansho	16*	35.64 ± 1.81
R-28	$\mathbf{R} imes \mathbf{R}$	Inuzansho	5	29.64 ± 3.95
N-9	N imes N	Natsumikan	5	33.02 ± 3.76
N-9	N imes N	Karatachi	1	32.0
N-9	$\mathrm{N} imes \mathrm{N}$	Inuzansho	1	30.9
D-9-13	$\mathrm{D} imes \mathrm{K}$	Inuzansho	2	32.55 ± 0.91
D-14	$D\timesD$	Inuzansho	12**	33.48 ± 0.76

Table 4. PUPAL LENGTHS OF HYBRIDS AND PARENTAL SPECIES.

* Only part of the pupæ obtained were measured.

** Over-wintering pupæ were not measured.

The most prominent difference between adult *P. helenus* and adult *P. protenor* is the presence of white patches on the hind wings of *P. helenus*. These patches cover a part of cells $Sc-R_1$, R_2 , and M_1 , and extend quite often to a part of cells M_2 , M_3 , and M_4 (Fig. 1a). The "Comstock-Needham" system is applied here to name the cells. These patches do not exist in *P. protenor* (Fig. 3a), but in *P. protenor* the male has a white band in cell $Sc-R_1$ of the hind wing. The white patches of *P. helenus* are also seen on the under side of the wing, but the dorsal white band of *P. protenor* is not seen on the under side. These white patches and band appear in the hybrid, but their development is not as clear as in the parental species, especially on the boundaries of the patches and the band. In the hybrid the patch and the band on $Sc-R_1$ overlap but only partially; therefore the presence of both characters is

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obvious. The white patches on cells M_2 and M_3 are very scant or disappear in the hybrids (Fig. 2a). In one hybrid specimen, all white patches are reduced to a trace on the under side of the wings, although they are clear on upper side.

The general shape of both the fore and hind wings is easily distinguishable between parental species. This shape in the hybrids resembles P. *helenus*.

All hybrids have more or less red scales on the basal parts of the white patches of the hind wings. These red scales are not seen either on P. *helenus* nor on P. *protenor*.

On the under side of the fore wing of both species, white scales are partially superimposed on the blackish scales which cover the whole wing. On the cells of R_5 , M_1 , M_2 , M_3 , and Cu_1 of *P. protenor*, white scales are superimposed on all areas except the wing margin and the central part of each cell (Fig. 3a). On the same cells of *P. helenus*, a scant white band of the same width is formed through the central part of each cell (Fig. 1a); therefore, no white scales are seen on the basal part of each cell, a clear difference from *P. protenor*. In the hybrids (Fig. 2a), the expression of these white scales is quite close to that of *P. protenor*; but the basal part of cell Cu_3 has no white scales, as in *P. helenus*.

On the abdomen of *P. helenus*, one lateral and three ventral white lines are usually distinct. The same lines are not clear or are absent on the abdomen of *P. protenor*. In the hybrids, the expression of these lines is somewhat intermediate between the parental species.

2. P. bianor \times P. maackii.

Larval differences between *P. bianor* (Fig. 4b) and *P. maackii* are very slight. Although there are many minor differences, the writer failed to compare them precisely, since he could not rear larvæ of *P. maackii* with *P. bianor* and the hybrid. The most prominent difference may be a pair of small processes at the 9th abdominal segment. These processes are more prominent in *P. maackii* than *P. bianor*, especially at the late larval stages. The hybrid seems to be intermediate or *maackii*-like (Fig. 5b).

EXPLANATION OF PLATE 1

PAPILIO HELENUS: la) adult & (underside at right); lb) mature larva, dorsal; lc) same, lateral; ld) pupa, lateral.

 $[\]begin{array}{l} \textit{PAPILIO F}_1 \; \textit{HYBRID ($$$$ $$ PROTENOR $$$$$ $$ $$ HELENUS): $$$ 2a) adult $$$ (underside at right); $$$ 2b) mature larva, dorsal; $$$ 2c) same, lateral; $$$ 2d) pupa, lateral. \\ \end{array}$

PAPILIO PROTENOR: 3a) adult & (underside at right); 3b) mature larva, dorsal; 3c) same, lateral; 3d) pupa, lateral.



 $\label{eq:PAPILIO BIANOR: 4a) adult $$ $$, summer form (underside at right); 4b)$ mature larva, lateral; 4c) pupa, dorsal.$

*PAPILIO F*₁ HYBRID (\bigcirc *BIANOR* × \circlearrowright *MAACKII*): 5a) adult \circlearrowright , summer form (underside at right); 5b) mature larva, lateral; 5c) pupa, dorsal. *PAPILIO MAACKII*: 6) adult \circlearrowright , spring form (underside at right).

The shapes of pupæ of *P. bianor* (Fig. 4c) and *P. maackii* are indistinguishable. However, coloration of pupæ is variable within and between species. Pupæ of *P. bianor* are usually green or brown, and the overwintering and non-overwintering forms are distinguishable. Besides these colorations, yellowish-brown and greenish-brown colors appeared in the writer's rearings. Yellowish-brown pupæ have a prominent median dorsal brown line, and this coloration resembles both of the hybrid pupæ (Fig. 5c) obtained. However, published figures of pupae of *P. maackii* seem to resemble more closely the hybrid pupæ.

The sizes of the two hybrids are smaller than wild summer specimens of P. maackii and most wild summer specimens of P. bianor. However, they are as large as P. bianor of Brood D-14 which were reared in the laboratory at approximately the same time as the hybrids.

Adults of P. bianor and P. maackii resemble each other very closely (Figs. 4a & 6). There is no single character which separates both species clearly. However, one can identify typical specimens easily. The characteristics which separate the species are as follows. 1) P. maackii generally has well developed bands of light color along the inside of the outermargin of the upper side of fore and hind wing; however, these bands become very scant or completely disappear from hind wing in some individuals. P. bianor lacks these bands completely. 2) P. maackii has a vellow band along the inside of the outer margin on the under side of the hind wing, and P. bianor never has this band. However, P. maackii lacking this band are sometimes found in the warmer regions of Japan. 3) A white band on the under side of the fore wing is wide at the apical area and becomes narrow toward the outer angle in *P. bianor*. This band has a uniform width in P. maackii. Since these characteristics of P. maackii are clearer in the spring form than in the summer form, a spring form is used for Fig. 6: the hybrids emerged in summer.

The expression of these characters in the two hybrids (Fig. 5a) obtained is as follows. 1) The bands of light color on the upper side are prominent in one individual and scant in the other. In both the boundaries of the bands are not as clear as in *P. maackii.* 2) The yellow band on the under side of the hind wing is absent in both. 3) The white band on the under side of the fore wing is somewhat wider toward the apical area and is intermediate between the condition in the typical parental species.

THE PROBLEM OF OVER-WINTERING

If *Papilio* larvæ are reared toward the end of the summer in a laboratory, usually part of the resulting pupæ emerge after a normal pupal period and others pass the winter and emerge the following spring. In the writer's 1959 rearing, pupæ of Brood R-33 of *P. protenor*, which resulted from eggs laid at the middle of August, produced 6 adults from Sept. 13 to October 1; the remaining 14 pupæ overwintered. Pupæ of Brood N-9 of *P. helenus*, which resulted from eggs laid at the end of July, produced 5 adults from August 29 to Sept. 10; the 2 remaining pupæ overwintered. However, all three pupæ of Brood N-9-4 (hybrid *P. protenor* \times *P. helenus*), which resulted from eggs laid at the beginning of September, emerged from October 21 to November 5. Although the data are still too small, the hybrid pupæ seem to lack the ability to "diapause", which is very important for survival of a species in winter.

BACK-CROSS ATTEMPTS

Three hybrid males of Brood R-18-6 (*P. protenor* \times *P. helenus*), which were reared on Natsumikan, were successfully hand-paired with females of the parental species. The first one copulated with a *P. protenor* female for about 30 minutes. The second also copulated with a *P. protenor* female, but this couple failed to separate from each other and were forced to separate after about 15 hours. These two females failed to lay any eggs. The third hybrid male copulated with a *P. helenus* female for about 1 hour. The formation of a spermatophore was observed from the outside just after separation. However, this female was very weak and died the next day without laying eggs.

One of the hybrid males between P. bianor and P. maackii was handpaired with 2 females of P. bianor, Brood D-14. Both copulations lasted about one hour and the formation of spermatophores was confirmed just after the separation at the second mating. From the first and the second matings, 17 and 39 eggs respectively, were obtained. However, no egg turned black, and of course none hatched.

DISCUSSION

The average egg fertility (35.5%) and the average egg hatchability (20.6%) of the hybrids between *P. protenor* and *P. helenus* may indicate that the relationship of the two species is not close. However, the high egg fertility (98.2%) and the somewhat high egg hatchability (82.3%) from one cross may indicate that at least some individuals of the two species still retain a somewhat close relationship. Since all 15 hybrid butterflies were males, Haldane's Rule applies in this hybrid combination. The female is presumed to be heterogametic in butterflies. The red scales on the basal parts of the white patches of the hind wing in the hybrid

between *P. protenor* and *P. helenus* may be an example of an appearance of a concealed hereditary character through a change in gene background by a hybridization. If a hybrid lacks the ability to diapause, as the data suggest, this phenomenon would be an important factor in preventing the establishment of a hybrid population in nature.

Although the characters of the hybrids between P. bianor and P. maackii were clarified extensively by the two hybrid males obtained, the data are still too small to discuss the compatibility relation between the parental species.

Since *P. protenor* and *P. helenus* are sympatric in Japan, as are *P. bianor* and *P. maackii*, the existence of natural hybrids must be considered, although a hand-pairing by-passes normal courtship behavior completely, as previously stated. The writer sent photographs of the two kinds of hybrids to Dr. T. SHIROZU, Kyushu University, and he wrote to the writer that he has never seen a specimen which resembles the writer's hybrid between *P. protenor* and *P. helenus*, but he has occasionally seen specimens which resemble the hybrid between *P. bianor* and *P. maackii*. Therefore, the difficulty in distinguishing wild *P. bianor* and *P. maackii* may be due partially to natural hybridization and resulting introgression.

SUMMARY

1. Four fertile matings between *P. protenor* female and *P. helenus* male, one fertile mating by a reciprocal cross, and one fertile mating between *P. bianor* female and *P. maackii* male were obtained, using a technique of hand-pairing.

2. The durations of copulation in the hybrid matings were recorded, and those of the fertile matings lasted about one to two hours.

3. The average egg fertility in the five crosses between *P. protenor* and *P. helenus* was 36.5% and the average egg hatchability was 29.6%. The highest hybrid egg fertility was 98.2% and the highest egg hatchability was 82.3%.

4. The egg hatchability of the cross between *P. bianor* and *P. maackii* was 10.5%.

5. The developmental rates of the hybrids do not seem to differ extensively from the developmental rates of the parental species in the above two kinds of hybrid. Differences of developmental rate and larval survival due to foodplant differences were prominent in P. helenus and in the hybrids between P. protenor and P. helenus.

6. Characteristics of the hybrids between P. protenor and P. helenus and between P. bianor and P. maackii were compared to those of the parental species.

7. Haldane's Rule fits both hybrid combinations. All 15 hybrid P. helenus \times P. protenor were males. The 2 hybrid P. bianor \times P. maackii were also males.

8. The pupe of the hybrid between P. protenor and P. helenus seem to lack the ability of diapause.

Three back-cross hand-pairings using the F_1 hybrids between P. 9. protenor and P. helenus and two using a F_1 hybrid between P. bianor and P. maackii were obtained, but no eggs hatched and no fertilization could be found.

10. Existence of natural hybrids between P. protenor and P. helenus and especially between P. bianor and P. maackii must be considered.

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