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EXPERIMENTAL HYBRIDIZATION BETWEEN *PHYCIODES THAROS* AND *P. PHAON* (NYMPHALIDAE)

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ABSTRACT. *Phyciodes tharos* and *P. phaon* are common species that occur sympatrically over much of the southern United States. The species differ in larval, pupal, and adult phenotypic appearance, habitat preference, and larval foodplant. F_1 hybrids and backcrosses between the species showed an unusual pattern of incompatibility, with relatively slight hybrid breakdown in one direction of the cross and total inviability in the reciprocal cross. The results differ strongly from those obtained in crosses between *P. tharos* and other *Phyciodes* species.

In the southern part of the southeastern United States *Phyciodes tharos* Drury and *P. phaon* Edwards are the dominant *Phyciodes* species. They are closely related taxonomically, but while *P. tharos* ranges northward into southern Canada, *P. phaon* is confined to the South and Southwest and ranges into Central America. Except for *P. phaon,* all of the half-dozen or so members of the *P. tharos* species group feed on asters in the larval stage. The larval foodplant of *P. phaon* is *Lippia* (Verbenaceae), an herbaceous perennial found commonly along sandy roadsides in the Deep South.

In northern Florida (e.g., Alachua, Bradford, and Levy Cos.) *P. tharos* and *P. phaon* are often sympatric along roadsides and in other open, sandy areas, but while *P. phaon* seems fairly closely restricted to this habitat, *P. tharos* is common also in moist grassy fields, lawns, and pine-palmetto savannah. Laboratory experiments indicate that *P. tharos* larvae feed readily on a wide array of *Aster* species, and that each habitat contains suitable foodplants.

Both species are multivoltine, with the spring emergence beginning in mid-March in northern Florida. First generation adults of both species are of the extreme "spring" phenotype ("marcia" in *P. tharos*, "hiemalis" in *P. phaon*) with much more extensive dark markings on the ventral hindwing than in the "summer" phenotype ("morpheus" and "phaon," respectively). Although fairly similar in appearance, adults of the two species are easily distinguished by differences in color pattern (Fig. 1, Table 1). The larvae also differ in appearance (Table 1), although in general that of *P. phaon* is more similar in appearance to *P. tharos* than are those of *P. campestris* Behr (Oliver, 1978) or *P. batesii* Reakirt (Oliver, 1979a). In addition first instar larvae of both *P. campestris* and *P. batesii* form rudimentary communal webs, whereas *P. tharos* and *P. phaon* do not. The haploid chromosome numbers of both latter species is 31 (Maeki and Remington, 1960). There are apparently no records of natural hybrids. The rela-

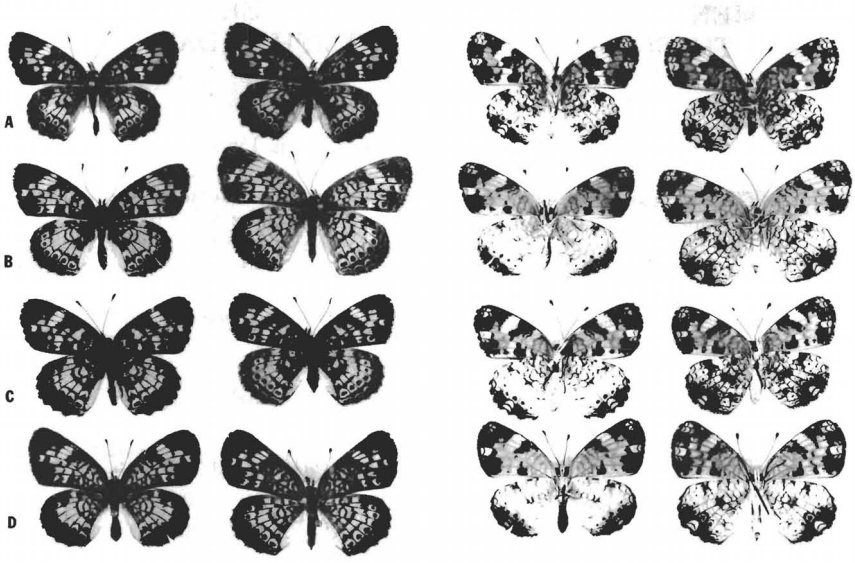

FIG. 1. Parental-type, F_1 hybrid, and backcross adults: **Row A,** *P. phaon*; **B,** *P. tharos*; **C,** F_1 hybrids *P. phaon* ♀ × *P. tharos* ♂; **D,** backcrosses (*P. phaon* ♀ × *P. tharos* ♂) ♀ × *P. tharos* ♂. Specimens show, left to right: male dorsal, female dorsal, male ventral, female ventral.

tively close taxonomic relationship of *P. tharos* and *P. phaon,* together with the unusual foodplant of *P. phaon,* made it seem especially desirable to me to investigate the relationship between these two species as part of my ongoing study of the evolutionary genetics of the *P. tharos* group.

METHODS AND MATERIALS

Laboratory stock was derived from one female of *P. tharos* taken 17 March 1979 in Gainesville, Alachua Co., Florida; two females of *P. tharos* and one male and two females of *P. phaon* taken 16 and 21 March 1979 four mi. west of Otter Creek, Levy Co., Florida; and from one male and four females of *P. tharos* and three females of *P. phaon* taken 17, 18, and 24 March 1979 three mi. north of Waldo, Bradford Co., Florida. Cultures at the University of Florida were maintained at 25°C and under approximately natural photoperiod conditions (for April at latitude 29°30'N). On 19–21 April 1979 the cultures were transferred to my laboratory in Pennsylvania, where they were maintained at 27°C days, 24°C nights, and given 16 h light/24 h using rows of fluorescent tubes.

TABLE 1. Differences in phenotypic appearance of mature larva, pupa, and adult of *P. tharos*, *P. phaon*, and their F_1 hybrid (*P. phaon* ♀ × *P. tharos* ♂).

Character	*P. tharos*	*P. phaon*	F_1 hybrid
I. Mature larva			
a. Proleg color	Brown	White	Very light brown
b. Color tubercle bases	Brown	White	Brown
c. Color dorsal dark stripes	Chocolate brown w. small white flecks	Mottled, 50% white, 50% brown	Mottled, more than 50% brown
d. Width, dorsal light stripes	Narrower than width of tubercles	About as wide as tubercles	Lower: solid white, same width as *P. tharos*; upper: mottled to extent of *P. phaon*
e. Width, lateral light stripes	Narrower than width of tubercle	About 4 times as wide as tubercle	Same as (d.)
f. Color, head capsule	Brown w. dorsal white patch, very small lateral-ventral patch	Dorsally like *tharos* but with large ventral white patch	Dorsally like *P. tharos*, ventral patch reduced to ½ size of *P. phaon*
II. Pupa			
a. Color	Light tan to dark wood brown	Usually light tan	Light tan to dark brown
b. Overall shape	More angular, projections more pronounced	More rounded, projections less pronounced	Intermediate
III. Adult.			III.
a. Dorsal body vestiture	W. large proportion of tawny hairs	Very few tawny hairs	Like *P. tharos*
b. Color, ventral antennal club	Almost always very dark	Light tawny gray	Variable, light to dark
	Relatively light	Relatively heavy	Intermediate
c. Intensity, black pattern elements	Tawny areas nearly even; median black band often broken into sep. patches	Median tawny band, marginal lunule, submedian spot contrastingly pale; black band heavy and continuous	Intermediate
d. Color, dorsal and ventral forewing			
e. Color, ventral hindwing	Ground even straw yellow; discal and basal dark markings tan, outer marginal patch chocolate brown; dark markings less crisp	Ground cream w. a large contrasting central tawny spot; dark markings all chocolate brown, usually very crisp	Ground intermediate; often w. central tawny spot; both *P. tharos* and *P. phaon* discal and basal dark markings present, not quite superimposed; markings crisp

Matings were made by the hand-pairing method (Clarke, 1952) or in small cages. Mated females were housed in 10 × 20 cm glass cylinders and given potted plants or cut sprigs of various asters or *Lippia nodiflora* (L.) Michx. for oviposition. Eggs were removed daily and counted, and the substrate leaf kept fresh until the eggs hatched. Larvae of *P. tharos* were reared on cut sprigs of *Aster* in 10 × 20 cm glass cylinders with screening over the tops; whereas, *P. phaon* were reared on cut sprigs of *Lippia* in 7 × 10 cm closed plastic boxes, since the *Lippia* plants tended to desiccate if exposed to the open air. After transferral of the cultures to Pennsylvania all hybrid larvae were reared on *Aster* because of the unavailability of *Lippia*. For this same reason no control broods of *P. phaon* were reared in Pennsylvania after early May.

F_1 progeny of wild-collected wild-mated females or (in two cases) unmated, wild-collected females were used for the hybrid pairings and as parental-type stock for backcrosses. No stock used was inbred. Observations were made on parental population, F_1 hybrid, and backcross phenotypic appearance and larval foodplant acceptance, interspecific courtship behavior, development times and adult eclosion patterns, fertility, adult sex ratios, and embryonic, pupal, and eclosing adult viability. Single species controls were reared simultaneously for comparison with hybrid and backcross broods.

Data on egg fertility, viability, and sex ratios were treated statistically using the Wilcoxon Two-Sample Test. Adult fertility was measured by a count of the number of visibly developing eggs divided by the total number of eggs laid after a single mating. Development times from hatching of the egg to eclosion of the adult were estimated by calculating the 99% confidence intervals for the medians of the distributions (Owen, 1962). Distributions of development times within broods or series of broods have been represented by adult eclosion graphs showing the number of adults emerging from pupae each day.

RESULTS

Interspecific Courtship Behavior

Males of both species show vigorous courtship behavior when caged with females of their own or of the other species. Females apparently rarely accept males of other species, however, and only one interspecific pairing, *P. phaon* ♀ × *P. tharos* ♂, was made without forced pairing.

Phenotypic Appearance

Differences in phenotypic appearance of the fifth instar larvae, pupae, and adults of the parental species and their F_1 hybrid (*P. phaon*

♀ × *P. tharos* ♂) are summarized in Table 1. Adults are shown in Fig. 1. The backcross (*P. phaon* ♀ × *P. tharos* ♂) ♀ × *P. tharos* ♂ generally resembled *P. tharos* except for the following characters: 1) larva—width of lateral light stripes slightly wider than on P. tharos; 2) adult—color of ventral antennal club dark, sometimes with a light tip; 3) color of dorsal and ventral forewing intermediate between *P. tharos* and *P. phaon*; 4) color of ventral hindwing with ground lighter than *P. tharos*, often with a slight, diffused central tawny spot; discal and ventral markings more like *P. tharos* but with some *P. phaon* influence present; dark markings more sharply defined than on *P. tharos*. The larva and pupa of *P. phaon* and the larva of *P. tharos* have been figured by Emmel and Emmel (1973) and the pupa of *P. tharos* by Holland (1931).

In nature both *P. tharos* and *P. phaon* show seasonal polyphenism regulated by photoperiod (Oliver, 1976, unpubl. data). In both species the short-day forms, which fly in fall and spring, have the underside of the hindwings suffused with dark brown or violet-brown, which obscures the other dark markings. The character of this suffusion is closely similar in both species. Under the laboratory conditions described above only light-colored long-day forms were present in the parental cultures. The males in the F_1 hybrid broods were only of the long-day form; varying proportions of the females were of the short-day form (Brood No. 79-6: 23.3%; 79-17: 0%; 79-18: 13.1%; 79-34: 0%; 79-35: 17.6%; 79-36: 71.1% [see Table 4 for numbers of females involved]). A few backcross females showed moderate expression of the short-day phenotype.

Foodplant Acceptance

In laboratory oviposition tests *P. phaon* accepted only *Lippia*, *P. tharos* only *Aster*. F_1 hybrid females (*P. phaon* ♀ × *P. tharos* ♂) accepted either plant readily.

Newly hatched larvae without feeding experience given the foodplant of the other species fed to a very limited extent and died without increase in size. F_1 hybrid larvae of the cross *P. phaon* ♀ × *P. tharos* ♂ fed readily on either foodplant. One early brood of this cross was split into *Lippia*- and *Aster*-feeding groups. Growth was markedly slower on *Lippia*, and the *Lippia*-feeding group was changed to *Aster* when the cultures were moved to Pennsylvania. Part of one brood of the backcross (*P. phaon* ♀ × *P. tharos* ♂) ♀ × *P. tharos* ♂ was started on *Lippia* and grew until the second instar, when all of the larvae (N = 40) died.

Viability, Fertility, and Sex Ratio

Embryonic viability in the parental control broods was extremely high. In the F_1 hybrid (*P. phaon* ♀ × *P. tharos* ♂) there was significant

reduction in viability (Table 2). In the reciprocal hybrid there was either complete very early embryonic inviability or a complete lack of egg fertilization following insemination. Females mated in this cross oviposited normally (in large egg patches), rather than in the pattern demonstrated by uninseminated females (very few eggs laid, widely scattered over the foodplant). Since in other *Phyciodes* crosses this has proved to be an infallible indicator of mating success, these matings were assumed to be successful even though the presence of spermatophores was not verified. The backcross (*P. phaon* ♀ × *P. tharos* ♂) ♀ × *P. tharos* ♂ showed a significant reduction in visible egg fertility and reduced embryonic viability. The reciprocal backcross *P. tharos* ♀ × (*P. phaon* ♀ × *P. tharos* ♂) ♂ showed massive reductions in egg fertility and complete embryonic inviability. The reduction in visible egg fertility in these crosses may have been due to very early embryonic inviability or to reduced parental fertility. No stock of *P. phaon* could be maintained in Pennsylvania for backcrosses because of a lack of *Lippia* for foodplant.

Post-larval (i.e. prepupal, pupal, and ecdysing adult) viability was significantly lower for the *P. phaon* parental broods than for *P. tharos* (P = .005) (Table 3). This may have been due to the different larval rearing containers for *P. phaon*, since humidity in these containers was very high. F_1 hybrid and backcross broods were reared in the same manner as *P. tharos*; post-larval viabilities of these two series of broods were significantly lower than for *P. tharos* (P = .005 for both values) but not lower than both *P. tharos* and *P. phaon* considered together. It is possible that neither parental foodplant was ideal for the hybrid larvae, and that some reduction in viability was due to this.

Adult sex ratios in the F_1 hybrid broods and in the backcross broods showed no change from those of the parental control broods (Table 3).

Development Times and Eclosion Graphs

Development times from hatching of the egg to eclosion of the adult were about the same for the two parental species, although some broods of *P. phaon* averaged a day or so faster than *P. tharos* (Table 4). Development times of the *P. phaon* broods varied more than did those of *P. tharos*.

In F_1 hybrid broods of the cross (*P. phaon* ♀ × *P. tharos* ♂) reared on *Aster*, males showed development times similar to those of the control broods, but those of the F_1 females averaged at least several days longer than those of the controls. Development times of both males and females of this F_1 hybrid tended to vary more than those of the controls. In addition, the eclosion graphs of both sexes showed

TABLE 2. Mean egg fertility and embryonic viability (with standard deviation) of *Phyciodes tharos* and *P. phaon* control, F_1 hybrid, and backcross broods. P values refer to comparison for differences of hybrid or backcross broods with controls.

Species/cross	No. of broods	No. of eggs	Fertile/laid	Hatched/laid	Hatched/fertile
			Parental Controls		
P. tharos	9	2399	0.995 ± 0.005	0.995 ± 0.005	1.000 ± 0.000
P. phaon	4	697	1.000 ± 0.000	1.000 ± 0.000	1.000 ± 0.000
			F_1 Hybrids		
th ♀ × ph ♂	9	3955	0.000 ± 0.000 (P < 0.001)	0.000 ± 0.000 (P < 0.001)	—
ph ♀ × th ♂	7	2285	0.991 ± 0.019 (N.S.)	0.945 ± 0.039 (P < 0.005)	0.944 ± 0.032 (P < 0.005)
			Backcrosses		
(ph ♀ × th ♂) ♀ × th ♂	11	2910	0.905 ± 0.117 (P < 0.001)	0.747 ± 0.239 (P < 0.001)	0.806 ± 0.192 (P < 0.001)
th ♀ × (ph ♀ × th ♂) ♂	7	2576	0.012 ± 0.014 (P < 0.001)	0.000 ± 0.000 (P < 0.005)	0.000 ± 0.000 (P < 0.005)

TABLE 3. Mean incidence (percentages) of prepupal through eclosing adult inviability and adult viability of *Phyciodes tharos* (th), *P. phaon* (ph), F_1 hybrids, and backcrosses. Tests of significance refer to comparison with combined parental controls (sex ratio) or *P. tharos* alone (viability).

Species/cross	No. of broods	Dead prepupae	Dead pupae	Total no. eclosing	Sex ratio (mean % ♂♂)	Eclosion incomplete	Viable adults
th controls	8	0.0	3.6	541	54.2	2.0	94.6
ph controls	4	5.8 (N.S.)	28.2 (P = 0.01)	194	49.1	1.8 (N.S.)	66.8 (P = 0.005)
ph ♀ × th ♂	6	0.3 (N.S.)	9.2 (P < 0.05)	575	46.0 (N.S.)	2.2 (P = 0.025)	87.6 (P = 0.005)
(ph ♀ × th ♂) ♀ × th ♂	6	1.3 (P = 0.025)	13.2 (P = 0.10)	435	49.9 (N.S.)	4.4 (P = 0.025)	81.2 (P = 0.025)

TABLE 4. Development times in days from hatching of egg until eclosion of adult for *P. tharos* and *P. phaon* control broods, F_1 hybrid (*P. phaon* ♀ × *P. tharos* ♂) and backcrosses ([*P. phaon* ♀ × *P. tharos* ♂] ♀ × *P. tharos* ♂). "Medians" show 99% confidence limits. See text for rearing conditions. Mr = March, A = April, My = May, J = June.

		Males			Females		
Brood no.	Date hatched	N	Min–Max	Median	N	Min–Max	Median
			P. tharos				
79-3	29 Mr	37	25–27	26	43	24–30	26–27
79-5	29 Mr–3 A	16	25–29	26–28	18	24–31	26–28
79-9	27–29 Mr	111	24–30	26	125	25–32	27
79-10	30 Mr–1 A	37	25–26	25–26	27	25–28	25–26
79-24	4 My	47	18–20	19	30	19–24	19
79-25	1–4 My	54	18–21	19–21	32	19–21	19–20
79-26	4 My	33	18–19	18	20	18–19	18–19
			P. phaon				
79-7	29 Mr–1 A	30	23–30	24–26	34	25–29	26–27
79-12	31 Mr	23	23–24	23	34	23–25	23–24
79-13	1–2 A	35	23–26	24	30	24–31	24–26
			F_1 Hybrids				
79-6	29 Mr–1 A	44	25–35	29–30	29	37–52	40–43
79-17	1–6 My	35	19–26	20	65	21–31	23–24
79-18	30 A–5 My	64	17–24	19–21	63	21–29	23–25
79-34	8–11 My	10	19–24	19–24	17	22–29	22–26
79-35	7–11 My	56	18–27	19–20	93	20–30	23–26
79-36	6–10 My	58	18–44	19–22	45	22–33	25–28
			Backcrosses				
79-47	5 J	9	17–20	17–20	16	19–24	19–24
79-49	4–5 J	50	16–20	17–18	49	17–26	18–19
79-50	5 J	54	15–20	17–18	83	17–22	19–20
79-51	4–5 J	28	16–23	17	23	18–23	19–21
79-55	7–8 J	67	17–25	19–20	61	18–24	20–21
79-58	10 J	22	15–20	17–19	20	18–21	19–20

a tailing-off effect. This was more marked in the females (Fig. 2). Because of slightly different rearing conditions, broods hatched during March and early April cannot be compared with those hatching during May and June. Development times of the backcrosses to *P. tharos* were significantly shorter than those of *P. tharos* or of the F_1 hybrids.

DISCUSSION

At first glance the results seem to give a somewhat contradictory picture of incompatibility between *Phyciodes tharos* and *P. phaon*. In the F_1 hybrid (*P. phaon* ♀ × *P. tharos* ♂) and its backcross to *P. tharos* ♂, hybrid sex ratios are normal and development times only slightly affected; whereas, the reciprocal hybrid is totally inviable,

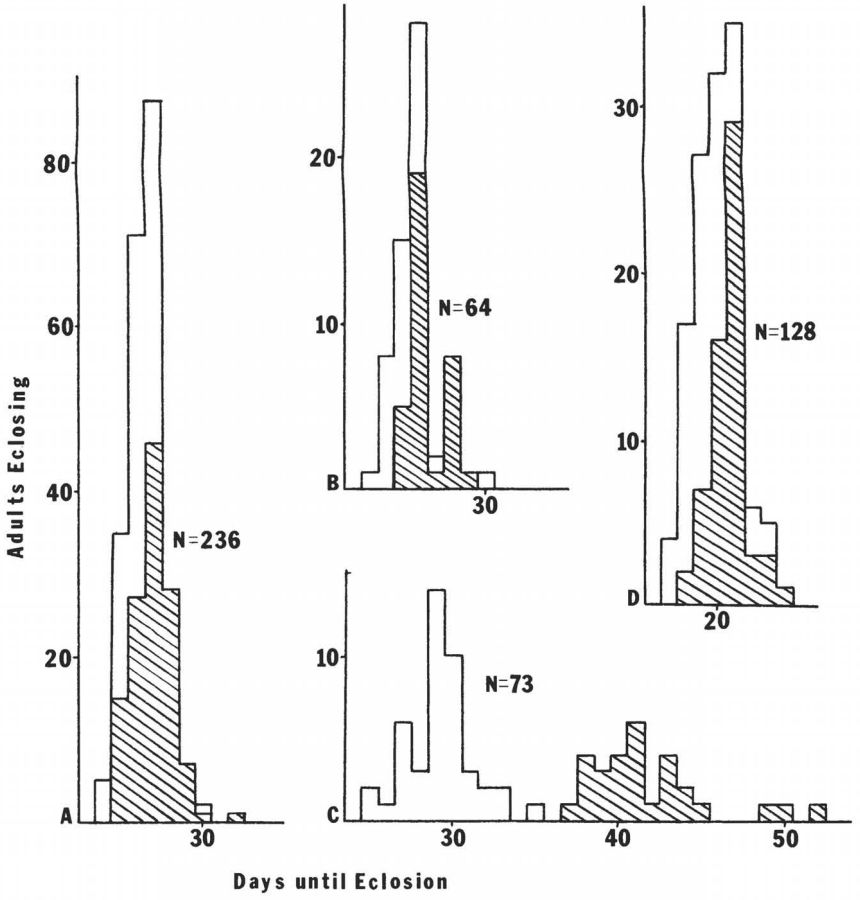

FIG. 2. Distributions of times required for development of typical *P. tharos*, *P. phaon*, F_1 hybrid, and backcross broods from hatching of eggs until eclosion of adults. **A,** *P. tharos*, Brood 79-9; **B,** *P. phaon*, Brood 79-7; **C,** F_1 hybrid *P. phaon* ♀ × *P. tharos* ♂, Brood 79-6; **D,** backcross (*P. phaon* ♀ × *P. tharos* ♂) ♀ × *P. tharos* ♂, Brood 79-55. (See Table 4 and text for rearing conditions and dates.)

and the backcross *P. tharos* ♀ × (*P. phaon* ♀ × *P. tharos* ♂) ♂ almost so. It would appear from these results that, while the nuclear materials of *P. tharos* and *P. phaon* are quite compatible (i.e. can cooperate to direct harmonious growth and development) and while *P. tharos* nuclear material is relatively compatible with *P. phaon* or hybrid cytoplasm, *P. phaon* nuclear material is highly incompatible with *P. tharos* cytoplasm. This incompatibility may involve crucial differences in one or more of the many factors that determine the composition of the cytoplasmic environment in which foreign nuclear material must

function to produce a viable hybrid individual organism. An incompatibility of this sort may be ultimately attributable to a relatively slight degree of differentiation in gene regulation (see Oliver, 1979b, for a fuller discussion) and does not contradict the other results presented here. The conclusion is, then, that *P. phaon* and *P. tharos* show relatively slight overall genetic differentiation. Hybridization in nature is probably prevented by barriers involving courtship behavior.

The pattern of hybrid incompatibility in the present series of crosses is quite different from that shown in hybridization experiments using *P. tharos* and *P. campestris* (Oliver, 1978), *P. batesii* (Oliver, 1979a), or the entity I have referred to as *P. "tharos* Type B" (Oliver, 1979a, 1980). These combinations show a homogeneous pattern of effects, which involves mainly slight to moderate (but not total) reduction in viability in the F_1 hybrids and backcrosses, skewed F_1 adult sex ratios, and abnormal F_1 hybrid development times. In the F_1 hybrid females eclosion occurs before that of the males when *P. tharos* is the male parent. In the reciprocal crosses female eclosion is delayed, and male and female curves usually do not overlap. In the (*P. phaon* ♀ × *P. tharos* ♂) F_1 hybrid broods, however, females show slightly delayed rather than speeded up eclosion times. These abnormalities in development time probably are in some way related to parental species differences in larval diapause induction thresholds (Oliver, unpubl. data).

Expression of the "marcia"-"hiemalis" phenotypes in the female F_1 hybrids may indicate that this form results unless the "morpheus"-"phaon" form is induced. One possible explanation for failure of this induction in female hybrids is that the "switch" gene and/or modifiers are linked with both a diapause induction-development rate gene complex and with sex. Since it is the females that are heterogametic in Lepidoptera, in this cross the female "morpheus"-"phaon" form must be induced in *P. phaon* cytoplasm using regulation by *P. tharos* genetic material, this induction fails in a high percentage of individuals. This case seems to be analogous to that in the cricket genus *Pteronemobius* (Masaki, 1978), where the F_1 hybrid males (the heterogametic sex in Orthoptera) show abnormal growth rates and photoperiodic responses.

The pattern of hybrid incompatibility between *P. tharos* and *P. phaon* differs also from that in butterfly hybrids outside the genus (reviewed in Lorković, 1978, and Oliver, 1979b). In general these latter show incompatibility similar to that in the other *Phyciodes* crosses discussed above. I know of no case in which the reciprocal F_1 hybrids differ so drastically in viability as do those between *P. tharos* and *P. phaon,* although recent crosses between *Pieris callidice*

Hübner and *P. occidentalis* Reakirt (Pieridae) (Shapiro, 1980) show a basic similarity that may be due to the same genetic effects.

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