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DIAPAUSE DYNAMICS AND HOST PLANT UTILIZATION OF *COLIAS*
PHILODICE, *COLIAS INTERIOR* AND THEIR HYBRIDS
(LEPIDOPTERA: PIERIDAE)

David N. Karowe¹

ABSTRACT

Abnormal diapause dynamics and, to a lesser extent, reduced efficiency of host utilization by hybrid larvae constitute potential post-zygotic barriers to gene flow between a multivoltine legume-feeder, *Colias philodice* (Lepidoptera: Pieridae) and a univoltine *Vaccinium*-feeder, *C. interior*. At the time when *C. interior* larvae enter diapause, approximately 50% of hybrid larvae ceased feeding but did not enter diapause, and subsequently starved. Hybrid larvae readily accepted the host plants of both parental species. However, relative to *C. philodice*, hybrid larvae displayed a significantly reduced fifth instar relative growth rate (RGR) when fed the primary legume host, *Medicago sativa*. Reduced growth of hybrid larvae was attributable to both reduced relative consumption rate (RCR) and reduced efficiency of conversion of digested food (ECD), but not to reduced digestive efficiency (AD). Relative to *C. interior*, hybrid larvae fed *Vaccinium myrtilloides* displayed reduced pupal weight and reduced ECD. These results may explain in part why all known naturally-occurring hybrids among North American *Colias* arise from parental species with similar host plant ranges and diapause strategies.

Reproductive isolation is sometimes believed to exist because it maintains what Mayr (1963) terms "harmonious, well-integrated gene complexes." For herbivorous insects, diapause dynamics and the utilization of host plant tissue may constitute such coadapted gene complexes, and adverse effects associated with their dissolution (i.e. reduced hybrid fitness) may provide formidable barriers to gene flow between sympatric species. The genus *Colias* (Lepidoptera: Pieridae) contains several pairs of sympatric species that differ strikingly in patterns of voltinism and larval host plant range, yet can be made to hybridize in the laboratory (Ae 1956, 1959). As such, *Colias* presents an unusual opportunity to evaluate the importance of differences in larval host plant range and diapause strategy as post-zygotic barriers to gene flow.

Although the taxonomy of the 14 North American species of *Colias* (Sulphur) butterflies is problematical (Burns 1975, Scott 1986), three apparently natural groups are easily distinguished: multivoltine legume-feeders (4 species), univoltine legume-feeders (5 species), and univoltine *Vaccinium*- or *Salix*-feeders (5 species). All known naturally-occurring hybrids among North American *Colias* arise from parental species with similar host plant ranges and diapause strategies. Hybridization occurs in nature between *C. eurytheme*

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Boisduval and *C. philodice* Latreille (both multivoltine legume-feeders), *C. eurydice* Boisduval and *C. cesonia* Stoll (both multivoltine legume-feeders), *C. alexandra* Edwards and *C. occidentalis* Scudder (both univoltine legume-feeders), *C. nastes* Boisduval and *C. helca* LeFebre (both univoltine legume feeders), and *C. interior* Scudder, *C. palaeno* L., and *C. pelidne* LeConte (all univoltine *Vaccinium*-feeders; Scott 1986). Despite considerable spatial and temporal sympatry, no hybrids are known to arise from species with different voltinism patterns or larval host plant ranges.

Between-group hybridizations are particularly interesting because they represent the potential immediate dissociation of coadapted gene complexes. In this study, I describe the diapause dynamics and food utilization abilities of hybrid larvae produced from matings between *Colias philodice* Latreille, a multivoltine legume-feeding species, and *Colias interior* Scudder, a univoltine *Vaccinium*-feeding species. Specifically, I ask whether hybrids between *C. philodice* and *C. interior* exhibit reduced fitness and, if so, whether reduced fitness is attributable to decreased fertility, host acceptance, early instar survivorship, efficiency of host utilization, and/or developmental abnormalities.

MATERIALS AND METHODS

Study Organisms. Adults of *C. philodice*, the Common or Clouded Sulphur butterfly, and *C. interior*, the Pink-edged Sulphur butterfly, are spatially and temporally sympatric throughout much of northern Michigan. Although morphologically very similar, *C. philodice* and *C. interior* differ in several important respects. *C. philodice* is multivoltine, with a distinct spring brood from early to late May, and a virtually continuous flight period from early June through October. In contrast, *C. interior* is univoltine, with a single flight period from early July to early August. Larvae of *C. philodice* feed on a variety of herbaceous legumes (Fabaceae) and, in areas sampled in Michigan, use *Medicago sativa* (alfalfa) and *Trifolium pratense* (red clover) as primary host plants, and *Melilotus alba* (white sweet clover) as a secondary host plant. The leguminous hosts of *C. philodice* produce young foliage from May to October and contain a diversity of secondary compounds, including cyanogenic glycosides, saponins, oestrogens, phenolics, and alkaloids (Harborne et al. 1971, Conn 1981, Arora 1983). In contrast, in the areas studied, *C. interior* larvae feed exclusively on *Vaccinium myrtilloides* and *V. angustifolium* (Ericaceae). Both species of *Vaccinium* produce young leaves only during a single synchronous flush in May.

Matings. Virgin *C. philodice* females were obtained by rearing several hundred offspring from 14 females caught near Ann Arbor, Michigan. Rearing was completed at The University of Michigan Biological Station (UMBS) in Pellston, Michigan. To synchronize the eclosure of *C. philodice* females with the flight period of *C. interior* males, *C. philodice* pupae were allowed to develop until they could be sexed (approximately 2-3 days before eclosure), at which time they were placed at 3-5°C. Females and males maintained under refrigeration for up to three weeks eclosed normally, generally within 24 hours after being returned to room temperature.

Matings were obtained by introducing 243 newly eclosed virgin *C. philodice* females into a cages containing either 262 newly eclosed *C. philodice* males or 87 *C. interior* males collected near Trout Lake, Michigan. Cages were inspected every 30 minutes; successful copulation lasts 1-3 hours (inability to rear *C. interior* larvae through diapause precluded *C. interior* × *C. philodice* matings). Mating pairs were immediately removed and placed in a 25-cm diameter clay pot containing fresh *M. sativa*. Eggs were collected each day,

and were judged to be fertile if they turned from white to red within 96 hours. Twice each day, all adults were fed to satiation on a 10% solution of clover honey in water. Eggs were obtained in the same manner from *C. philodice* females mated as described above and from *C. interior* females collected near Trout Lake.

Host preference of neonate larvae. To determine the feeding preferences of neonate *C. philodice*, *C. interior*, and *C. philodice* × *C. interior* larvae (hereafter "hybrid larvae"), 20 paired trials were conducted using the following two treatments: in one 5.5-cm plastic Petri dish, five larvae were placed on a *M. sativa* leaf adjacent to a *V. myrtilloides* leaf, and in the other five larvae were placed on a *V. myrtilloides* leaf adjacent to a *M. sativa* leaf. After 0.5, 1, 2, 4, 8, and 24 hr, each pair of dishes was inspected to determine the location and feeding activity of each larva. If a larva remained on the original leaf and initiated feeding, it was considered to have accepted that host plant; if not, it was considered to have rejected that host plant.

Early instar survivorship. The survivorship of 100 *C. philodice*, *C. interior*, and hybrid larvae on each of *M. sativa* and *V. myrtilloides* (total = 600 larvae) was monitored through the end of the second instar. Larval density was no greater than 10 per 5.5-cm plastic Petri dish. Fresh food was provided every second day.

Diapause dynamics. To determine whether the different "diapause strategies" (sensu Tauber and Tauber 1981) of *C. philodice* and *C. interior* constitute a potential post-zygotic barrier to gene flow, larvae of each species and their hybrids were reared in 11-cm Petri dishes (five larvae per dish) under either a 18:6 L:D cycle with a corresponding 29:23°C temperature cycle, or under ambient conditions in laboratories at UMBS and subsequently at the University of Michigan, Ann Arbor. Laboratory temperatures varied between 20–28°C over the entire rearing period. Photoperiod averaged approximately 16 hr of light per day.

C. philodice larvae were reared on *M. sativa* (n = 55 dishes), *C. interior* larvae on *V. myrtilloides* (n = 33 dishes), and hybrid larvae on both *M. sativa* (n = 51 dishes) and *V. myrtilloides* (n = 50 dishes). Fresh food was provided every second day or more often if necessary, and feeding activity of each larva was monitored until it entered diapause, pupated, or died of starvation. A number of late instar larvae died of viral infection (see below) and are not included. A larva was determined to have entered diapause if it ceased feeding for three days without becoming visibly smaller, adopted the characteristic swollen appearance (Hayes 1982), and became relatively insensitive to environmental stimuli such as touch.

Larval nutritional ecology. Since hybrid larvae survived when fed either *M. sativa* or *V. myrtilloides*, it was possible to compare the feeding efficiencies of hybrid and *C. philodice* larvae on *M. sativa*, and hybrid and *C. interior* larvae on *V. myrtilloides*. Quantitative nutritional indices were calculated during the fifth instar for 15 hybrid and eight *philodice* larvae fed *M. sativa*, and for five hybrid and five *C. interior* larvae fed *V. myrtilloides*. Methods were as described in Waldbauer (1968), with the following three modifications. First, because very few hybrid and *C. interior* larvae survived to the fifth instar, a wet weight/dry weight ratio established for *C. philodice* larvae was used to estimate the initial dry weight of all larvae used in feeding trials. Second, a wet weight/dry weight ratio established previously for *C. philodice* pupae was used to estimate the dry weight of all individuals at pupation. Hybrid pupae were allowed to eclose in order to obtain adult specimens. *C. philodice* and *C.*

Table 1. Feeding preferences of newborn *C. philodice*, *C. interior*, and hybrid larvae. Larvae were considered to have accepted a host if they initiated feeding within 24 h, and to have rejected it if they did not.

Species	Placed on <i>M. sativa</i>		Placed on <i>V. myrtilloides</i>	
	Accepted	Rejected	Accepted	Rejected
<i>C. philodice</i>	97	3	0	100
Hybrid	88	12	86	14
<i>C. interior</i>	0	100	95	5

interior pupae were allowed to eclose to determine whether all hybrid adults larvae used in nutritional ecology trials were male (Ae 1959, Bowden 1966); because this in fact was the case, female *C. philodice* and *C. interior* larvae were excluded from all statistical comparisons.

Statistical analysis. Chi-square analysis was used to determine whether the proportion of non-feeding larvae differed between broods, host plants, or rearing conditions, and whether the overall proportion differed from 50:50. Mean values of relative growth rate (RGR), relative consumption rate (RCR), approximate digestibility (AD), efficiency of conversion of digested food (ECD), pupal dry weight, and instar duration were compared using Students' t-tests or, when variances were unequal between treatments, Mann-Whitney U-tests (Sokal and Rohlf 1981).

RESULTS

Matings. As expected, *C. philodice* females more readily mate with *C. philodice* than with *C. interior* males. Sixty-two conspecific matings occurred, compared to only 2 between *C. philodice* females and *C. interior* males. Although males of both species actively courted *C. philodice* females, females nearly always adopted their "refusal posture" (Silberglied and Taylor 1978) when approached by *C. interior* males.

Fecundity and egg fertility. Relative to intraspecific matings, interspecific matings did not result in decreased fecundity or egg fertility. *C. philodice* females mated by *C. interior* males laid slightly more eggs (361 ± 8 ; $n = 2$) than females mated by *C. philodice* males (295 ± 123 ; $n = 13$), though this difference was not significant ($t = 0.75$, $d.f. = 13$, $p > 0.4$). Fertility of hybrid eggs (95.3%) was nearly significantly higher than that of *C. philodice* (94.6%) and *C. interior* (93.6%) eggs ($\chi^2 = 5.77$, $d.f. = 2$, $0.10 > p > 0.05$).

Feeding preferences of neonate larvae. Both *C. philodice* and *C. interior* larvae displayed marked preferences for their own host plant. Newly hatched *C. philodice* larvae readily accepted *M. sativa* and categorically rejected *V. myrtilloides*, while the opposite was true for newly hatched *C. interior* larvae (Table 1). Hybrid larvae readily accepted the host plants of both parental species. Of the hybrid larvae placed initially on *M. sativa*, 88% initiated feeding on *M. sativa*, 11% initiated feeding on *V. myrtilloides*, and 1% remained on *M. sativa* but did not feed. Of the larvae placed initially on *V. myrtilloides*, 86% initiated feeding on *V. myrtilloides*, 12% initiated feeding on *M. sativa*, and 2% remained on *V. myrtilloides* but did not feed.

Early instar survivorship. Larvae of each parental species were able to survive only on their own host. All *C. philodice* larvae given *V. myrtilloides*

Table 2. Number of continuously feeding (F) and non-feeding (Non-F) *C. philodice*, *C. interior*, and hybrid larvae reared under each of two light and temperature regimes (see text for details of rearing conditions).

Species	Host plant	Ambient Conditions		Environmental Chamber	
		F	Non-F	F	Non-F
<i>C. philodice</i>	<i>M. sativa</i>	194	0	117	0
Hybrid					
Female 1	<i>M. sativa</i>	22	18	17	24
	<i>V. myrtilloides</i>	24	20	17	21
Female 2	<i>M. sativa</i>	50	42	43	51
	<i>V. myrtilloides</i>	35	43	46	35
<i>C. interior</i>	<i>V. myrtilloides</i>	0	61	15	82

and all *C. interior* larvae given *M. sativa* failed to initiate feeding and starved within 96 hr. However, survivorship of hybrid larvae through the second instar did not differ significantly from that of the corresponding parental species on either *M. sativa* (94% vs. 98%) or *V. myrtilloides* (97% vs. 96%).

Diapause dynamics. As expected, no *C. philodice* larvae entered diapause between 1 and 7 August when maintained under either light and temperature regime (Table 2). However, all second instar *C. interior* larvae maintained on a laboratory bench and 85% of those maintained at 18:6 L:D and 29:23°C entered diapause at this time. Unlike the parental species, hybrid larvae were distinctly dimorphic in their diapause behavior (Table 2). Fifty-three percent of 510 hybrid larvae fed actively through the third instar, and continued feeding until they either pupated or died of viral infection. The remaining 47% ceased feeding during the second or third instar, but did not appear to enter true diapause. Unlike *C. interior* larvae, non-feeding hybrid larvae did not display a swollen appearance, nor did they become insensitive to touch. Within seven days, all hybrid larvae shriveled and died, apparently of starvation. The proportion of non-feeding larvae did not differ between the two families ($\chi^2 = .01$, $df = 1$, $p > .9$), between the two host plants ($\chi^2 = .43$, $df = 1$, $p > .5$), or between the two light and temperature regimes ($\chi^2 = .01$, $df = 1$, $p > .9$).

Larval mortality due to viral infection. At the time when *C. philodice* larvae were in the fourth instar, *C. interior* larvae had entered diapause, and hybrid larvae had either ceased feeding and died in the second instar or had fed continuously and were in the late third or fourth instar, larvae began to display symptoms typical of infection by a polyhedrosis virus (Ae 1959). Nutritional indices are reported only for fifth instar larvae that produced healthy adults.

Larval nutritional ecology. A second potential barrier to interspecific hybridization is apparent in the reduced ability of fifth instar hybrid larvae to utilize *M. sativa* tissue for growth. When fed *M. sativa*, hybrid larvae performed significantly worse than *C. philodice* larvae (Table 3). Relative to *C. philodice* larvae, hybrid larvae showed a 24% decrease in pupal weight and a 31% decrease in relative growth rate (RGR). Reduced RGR of hybrid larvae was attributable to reduced relative consumption rate (RCR), reduced efficiency of conversion of digested food (ECD), and increased instar duration.

Table 3. Performance of fifth instar *C. philodice* and hybrid larvae on *M. sativa*. Means are given, with standard deviations in parentheses. Means followed by different letters are significantly different at $p < .05$ by Student's t-test or, if variances among strata are unequal, by Mann-Whitney U-test.

Species	n	RGR	RCR	AD	ECD	ECI	Instar Duration (h)	Pupal Dry Weight (gm)
<i>C. philodice</i>	8	.415 a (.063)	1.76 a (.22)	.400 a (.076)	.613 a (.153)	.236 a (.025)	74.8 a (14.7)	.0582 a (.0045)
Hybrids	15	.285 b (.044)	1.34 b (.26)	.445 a (.035)	.494 b (.084)	.216 b (.024)	122.9 b (27.6)	.0445 b (.0067)

However, hybrid larvae digested *M. sativa* as efficiently (AD) as did *C. philodice*.

Similarly, hybrid larvae performed significantly worse than *C. interior* larvae on *V. myrtilloides* (Table 4). Relative to *C. interior* larvae, hybrid larvae displayed an 11% decrease in pupal weight and a 31% decrease in ECD. However, hybrid larvae consumed *V. myrtilloides* at a higher rate and therefore did not display a reduced RGR.

DISCUSSION

The results of this study must be interpreted with caution, since all larvae were obtained from only two *C. philodice* × *C. interior* matings. Nonetheless, the results of this study suggest that abnormal diapause dynamics and, to a lesser extent, reduced host utilization abilities constitute potential post-zygotic barriers to gene flow between the multivoltine legume-feeder, *C. philodice*, and the univoltine *Vaccinium*-feeder, *C. interior*.

As suggested by Oliver (1979), hybrid inviability appears to arise from "disruption of the integration of growth and developmental processes", particularly at a time when "crucial hormonally regulated tissue changes" occur. At the developmental stage when *C. interior* larvae normally enter diapause, approximately 50% of hybrid offspring cease feeding and subsequently starve. Hybrid larvae also suffer reduced ability to utilize *M. sativa* and *V. myrtilloides* for growth. In contrast, neither maternal fecundity, egg fertility, host acceptance, nor early instar survivorship contributed to reduced hybrid fitness.

Genetic control of diapause in insects frequently involves both sex-linked and autosomal loci (Tauber and Tauber 1981, Taylor and Karban 1986). The

Table 4. Performance of fifth instar *C. interior* and hybrid larvae on *V. myrtilloides*. Means are given, with standard deviations in parentheses. Means followed by different letters are significantly different at $p < .05$ by Student's t-test or, if variances among strata are unequal, by Mann-Whitney U-test.

Species	n	RGR	RCR	AD	ECD	ECI	Instar Duration (h)	Pupal Dry Weight (gm)
<i>C. interior</i>	5	.139 a (.032)	1.55 a (.27)	.251 a (.030)	.366 a (.078)	.090 a (.009)	217.2 a (43.3)	.0313 a (.0094)
Hybrids	5	.154 a (.028)	2.10 b (.27)	.287 a (.025)	.253 b (.022)	.073 b (.009)	228.8 a (65.9)	.0279 b (.0017)

results of this study are consistent with the hypothesis that in *Colias* the initiation of diapause is controlled by genes located on the X-chromosome but that successful entrance into diapause requires the concomittant expression of autosomal loci. It is plausible that, because they possess only a *C. interior* X-chromosome, female hybrid larvae initiate diapause at the same time as *C. interior*, but that epistatic interactions between their *C. interior* X-chromosome and *C. philodice* autosomes prevent female hybrid larvae from successfully entering diapause (Coyne and Orr 1989). If *C. philodice* alleles controlling the initiation of diapause are dominant, then male hybrid larvae would not initiate diapause in August. This would explain why 50% of hybrid larvae die attempting to enter diapause and 50% develop continuously. X-chromosome "supergenes" in *C. philodice* are known to influence both the production of male sex pheromones and mate selection by females (Gruha and Taylor 1979, 1980a,b), and it may be that selection has favored linkage between loci controlling mate choice and those controlling diapause. As noted by Scriber and Lederhouse (1992), sex linkage could facilitate adaptive divergence in diapause strategies.

Host acceptance and utilization by hybrid larvae. Unlike either parental species, hybrid larvae initiated feeding on both *M. sativa* (Fabaceae) and *V. myrtilloides* (Ericaceae). This result is consistent with Scriber's (1982, 1987) observations that F₁ hybrids between either *Papilio canadensis* or *P. rutulus* (both Salicaceae-feeders) and either *P. glaucus* or *P. australis* (both Magnoliaceae-feeders) display high survivorship and growth on both the Salicaceae and Magnoliaceae.

Although they accepted both parental host plants, hybrid larvae did not perform as well as the corresponding parental species on either host plant. The reduced efficiency with which fifth instar hybrid larvae utilize *M. sativa* for growth constitutes a second, but lesser, potential barrier to interspecific gene flow. In matings involving *C. philodice* females, all hybrid eggs would be laid on *M. sativa* or other legumes. Relative to *C. philodice* larvae, hybrid larvae fed *M. sativa* displayed significant reductions in relative growth rate (RGR) and pupal weight, both of which are positively correlated with fecundity in *C. philodice* (Karowe 1990). Similarly, both ECD and pupal weight of hybrid offspring were reduced on *V. myrtilloides*. However, since no hybrid larvae successfully entered diapause, it was not possible to compare performance on early season *Vaccinium* foliage, on which *C. interior* larvae normally feed.

The performance of fifth instar hybrid larvae suggests that adaptation to *M. sativa* and *V. myrtilloides* has not required major genetic changes in digestive physiology, since hybrid larvae digested each host as efficiently as the respective parental species. However, adaptation to these two hosts appears to have involved divergence in loci controlling the post-digestive utilization of plant tissue, since hybrid larvae displayed significantly reduced ECD on both *M. sativa* and *V. myrtilloides*. This conclusion is again consistent with Scriber's (1987) observation that reduced growth of *P. canadensis* × *P. glaucus* hybrids was generally attributable to reduced ECD rather than reduced AD or RCR.

Hybridization in nature. The morphological similarity between *C. philodice* and *C. interior* precludes the use of visual techniques to determine the frequency of interspecific matings in nature. However, the reluctance of *C. philodice* females to mate with *C. interior* males suggests that hybridization in nature may be rare. In the laboratory, interspecific matings always involved recently eclosed *C. philodice* females (Ae 1956, this study) presumably because, before their wings harden, females are not able to adopt the refusal posture (Silberglied and Taylor 1978).

This and previous studies (Ae 1956, 1959) suggest that *C. philodice* females are more discriminating than males. Indeed, mate choice by *C. philodice* females is mediated both by precise chemical cues (Grula and Taylor 1979, Grula et al. 1980) and by species-specific visual cues (the wings of *C. interior* males, like those of *C. philodice* males, do not reflect ultraviolet light [personal observation]). In contrast, *Colias* males rely on inherently more ambiguous visual cues, such as wing coloration (Silberglind and Taylor 1978). This difference may have resulted from intense competition for females, which are mated on average within 18 minutes of eclosion (Taylor 1972). Selection may therefore have favored the use of fairly general visual cues by males because they minimize the probability of failing to detect a receptive female.

Evolution of reproductive isolation between *C. philodice* and *C. interior*. Despite considerable spatial and temporal sympatry, no hybrids are known to arise among North American *Colias* species with different voltinism patterns or different larval host plant ranges (Scott 1986). The data presented here are consistent with the hypothesis that behavioral pre-mating isolating mechanisms are maintained because they prevent investment in less fit hybrid offspring. It is therefore tempting to infer that reproductive isolation evolved in part as a response to selection against less fit hybrids (the concept of "reinforcement" [Paterson 1978]). However, Templeton (1981) and Butlin (1987) have argued that reinforcement is rare because gene flow between hybridizing species generally leads to the rapid extinction of those genetic differences that favor reproductive isolation. Reproductive isolation, they argue, is more likely to evolve in allopatry as a pleiotropic effect of selection for other attributes, such as increased accuracy of mate recognition. Under such circumstances, reduced fitness of *C. philodice* × *C. interior* hybrid larvae would not drive the evolution of reproductive isolation.

Alternatively, it is possible that *C. philodice* and *C. interior* came into secondary contact at a time when reproductive isolation was not complete, and hybrid fitness was (as it now appears to be) low but not zero. Under such circumstances, reinforcement is more likely (Templeton 1981, Butlin 1987), and reduced hybrid fitness could have been directly responsible for the evolution of reproductive isolation between *C. philodice* and *C. interior*.

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