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WHY NATURAL HYBRIDS ARE HARD TO DETECT AND VERIFY: EXEMPLIFIED BY A RARE PRIMARY HYBRIDIZATION EVENT BETWEEN TWO TIGER SWALLOWTAIL BUTTERFLY SPECIES IN NORTHERN MICHIGAN

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ABSTRACT. Phenotypically intermediate specimens are often attributed to interspecific hybridization. Such observations of putative interspecific hybridizations are sufficiently rare to warrant literature records. However, it is seldom that additional evidence is available to document such an event. To illustrate this process, we have examined several lines of evidence documenting such a rare natural interspecific ("Primary," F-1) hybridization event in northern Michigan (Charlevoix Co.) between a *P. canadensis* female and a *P. glaucus* male. We describe analyses using a historical framework of extensive spatial and temporal sampling of multiple traits to illustrate the complexity of approaches required for identifying hybrids. We emphasize the importance of thorough ecological as well as morphological trait analysis relative to parental types for documenting true natural hybrids.

Additional key words: *Papilio glaucus*, *Papilio canadensis*, diapause, morphological analysis, host plants.

Although rare, interspecific hybridization in natural populations has been reported from several Lepidoptera families (Remington 1968, Sperling 1990). However, extensive study is required to convincingly demonstrate or document such events. Use of multivariate morphometric analyses are often required even in large and distinct species such as the giant silkmoths of the *Hyalophora cecropia* group (Collins 1984) or butterflies such as the *Limenitis* species group (Platt 1983, Boyd et al. 1999). We used a multi-trait approach combined with an extensive historical data base for these diagnostic traits between *Papilio canadensis* and *P. glaucus*.

The swallowtail butterflies of the Papilionidae family are also a large, well-known showy group of more than 560 species with generally well-studied natural histories and host plant relationships (Scriber et al. 1995). The genus *Papilio* has historically included nearly half (>200) of the species in the family worldwide, although the precise phylogenetic relationships are still being clarified (Munroe 1960, Hancock 1983, Miller 1987, Sperling 1987, Scriber 1995, Reed & Sperling 1999). Hand-pairing of these *Papilio* has produced extensive interspecific hybridization with the production of viable offspring in laboratory research programs (see reviews in: Ae 1995, Brown et al. 1995, Clarke 1995, Scriber et al. 1990, 1995, 2003). Natural hybrid zones have also produced specimens "intermediate" in appearance that have been assumed to be interspecific hybrids. Several examples have been reported from the *Papilio machaon* species group in North America (between the *P. machaon, P. polyxenes,* or *P. zelicaon*; Sperling 1987) and in other areas between *P. machaon* and *P. hospiton* (Clarke & Larson 1986, Clarke 1995). Additional putative interspecific hybrids of *Papilio* have also been reported on other continents (Hancock 1983, Collins & Morris 1985, Johnson & Matusik 1987, Tyler et al. 1994).

We have also conducted extensive interspecific hybridization with tiger swallowtails of the North American *Papilio glaucus* group (reviewed in Scriber et al. 1995) and have used multivariate morphometric analyses of adult wing traits (Luebke et al. 1988, Scriber 1990, Scriber 2002a) and diagnostic larval characters (Hagen et al. 1991, Scriber 1998) and electrophoretically-detectable species-diagnostic allozymes to identify natural populations of introgressed interspecific hybrids in the field (Hagen & Scriber 1991, Scriber 1996a). Morphological traits from known lab-hybrids have been used to identify suspected intermediates between *P. glaucus* and *P. rutulus* (Scott & Shepard 1976, Clarke & Clarke 1983, Scriber et al. 1990); *P. rutulus* and *P. multicaudatus* (Brower 1959, Garth and Tilden 1986); *P. eurymedon* and *P. rutulus* (Wagner 1978, West & Clarke 1988, Scriber et al. 1995); *P. glaucus* and *P. multicaudatus* (Scriber et al. 1995, Rahn 2001); and *P. glaucus* and *P. canadensis* (Scriber 1982, Luebke et al. 1988, Scriber et al. 1996, 2002a).

Our studies of the natural hybrid zone between *Papilio glaucus* and *P. canadensis* that exists from Minnesota and Wisconsin through Michigan and central New York State to southern New England have demonstrated the existence of several historically-stable, geographically-defined, and ecologically-significant trait step clines that differ interspecifically in these two tiger swallowtail species (Scriber 2002b; Table 1). These trait differences include differential abilities to detoxify tulip tree leaves (*Liriodendron tulipifera*, and other species of Magnoliaceae) and quaking aspen leaves (*Populus tremuloides*, and other species of Salicaceae). These differences (Scriber 1986b, Lindroth et al. 1988, Scriber et al. 1991) have a genetic basis, and hybrids are able to detoxify and grow on plants in both families due to intermediate levels of autosomally controlled detoxification enzymes (Scriber 1986b, Scriber et al. 1989, 1999). In 3-choice oviposition bioassays (quaking aspen, black cherry, and tulip tree leaves) this female (#15116) laid a little more than 40% of her eggs on aspen, which closely fits the typical profile for a *P. canadensis* female (Scriber et al. 1991, Scriber 1994). Females of *P. glaucus* typically place fewer than 5% of their eggs on quaking aspen in such an arena as "mistakes" (Scriber 1993).

Another major interspecific ecological trait difference is that *P. glaucus* individuals have an environmentally-determined pupal diapause induction (they directly develop into adults at long photoperiods: Rockey et al. 1987a, Vallella & Scriber 2002) whereas *P. canadensis* has an "obligate diapause" that is photoperiod insensitive (Scriber 1988) and sex-linked on the X-chromosome (Rockey et al. 1987b). Hybrids are variable in this respect, depending on the direction of the cross (obligate diapause tendencies are inherited from the father's X-chromosome in female offspring since the females are the heterogametic sex in Lepidoptera). Therefore, a male *P. canadensis* parent in an interspecific hybrid will produce hybrid daughters that all diapause, even under long day photoperiods.

Below, we describe results of the first verification of a natural "primary" interspecific hybridization event for tiger swallowtail butterflies. Despite the occurrence of hybrid "morphotypes" in Wisconsin (Luebke et al. 1988) and elsewhere (Scriber 2002b) and evidence of extensive genetic introgression of species-diagnostic allozymes and Magnoliaceae host detoxification abilities in the last few years (Hagen 1990, Hagen et al. 1991, Hagen & Scriber 1991, Scriber 1996, 2002a, Ording 2001), we have never found a primary F-1 hybrid individual in the field. Of thousands of individuals collected and examined, none have been heterozygous for all of the diagnostic allozyme traits (PGD, LDH, and HK), despite wing traits, larval detoxification and oviposition behavior that would be considered diagnostically "intermediate" between parental species types (Ording 2001, Scriber 2002b). The LDH-100 allele is apparently quickly (and totally) selected out of the hybrid populations in Wisconsin, Michigan, eastern New York, and southern Vermont (Scriber 2002a, b), and other than in this family from our Charlevoix female we have never seen LDH-100 north of Clinton Co. in southern Michigan *P. glaucus* territory (Fig. 1; Nielsen 1999).

METHODS

Female butterflies collected in the field were brought to the lab for 3-choice oviposition preference assays (tulip tree, which is toxic to *P. canadensis*; quaking aspen, which is toxic to *P. glaucus*; and black cherry, which is mutually and naturally acceptable to both species for larval survival and growth). These leaves were arranged along the sides of round clear plastic arenas that rotate in front of lights 10 times per hour (see methodology details in Scriber 1993). Eggs were counted and collected daily while females are fed 20% honey water solution. Neonate larval eclosion from the eggs occurred at approximately 5–7 days in controlled environment chambers at 25°C and 16:8 photoperiods. These fresh neonate (first instar) larvae were distributed evenly across the three host plants and reared in controlled environment chambers with conditions set as described below. Leaves of tulip tree, quaking aspen and black cherry from Ingham County were changed each 48 hours and survival noted for all individual larvae in all treatments.

The results of our odd brood #15116 were discovered in the process of conducting a larger study of the impacts on larval/pupal offspring fitness of interspecific hybrids relative to parental types (Donovan 2001). The pure parental genotypes (*Papilio glaucus* and *P. canadensis*) were reared simultaneously with hybrid larvae of reciprocal pairing types (*canadensis* females mated to *glaucus* males; and *glaucus* females mated to *canadensis* males) on three host plants (tulip tree, quaking aspen, and black cherry) at three different temperatures (15°C, 23°C, and 31°C). For each family of all 4 genotypes during 1999 and 2000, 36 larvae were randomly distributed to the 9 treatments (two larvae per treatment) with 2 growth chamber replicates of each. For the purposes of comparison here, the temperature treatments and chamber replications were lumped to highlight the 3 host plant effects (Table 2). In 1999, 5 *canadensis* families (180 larvae), 2 *glaucus* families (72 larvae), 6 Pc × Pg families (216 larvae) and 4 families of Pg × Pc (144 larvae) were bioassayed. In 2000, there were 5 *canadensis* families (180 larvae), 4 (144 larvae) of Pc × Pc, 4 families (144 larvae) of Pg × Pc, with 5 *glaucus* families (180 larvae).

Pupae were weighed and set up in small cylindrical screen cages for eclosion as adults and wing expansion. After 6 weeks, remaining pupae were presumed to be in diapause and were moved from chambers to storage in dark coolers maintained at 4–5°C until the following Spring when they were again set up in screen cylinders for adult eclosion. Adult specimens were scored for morphological wing traits as in Luebke et al. (1988), sometimes mated for livestock rearing, and then frozen alive at –80°C for subsequent electrophoresis. Electrophoresis techniques using cellulose acetate plates for the diagnostic allozymes (LDH = lactate dehydrogenase; PGD = 6-phosphoglucose dehydrogenase; and HK = hexokinase) were basically conducted as in Hagen and Scriber (1991) modified slightly as in Stump (2000).

TABLE 1. Summary of physiological, biochemical, and behavioral differences between *P. glaucus* and *P. canadensis*, and their modes of inheritance, if known. See text for additional explanation.

Character	*P. glaucus*	*P. canadensis*	Inheritance	Reference
Environmental determination of pupal diapause	YES	NO	X-linked	1, 2
Oviposition preference	tuliptree	aspen	X-linked	3
Larval survival (Aspen)	very low	high	polygenic	4, 5, 6
Larval survival (Tuliptree)	high	very low	polygenic	4, 5,16
Hexokinase (Hk) alleles	100	110	autosomal	8
Lactate dehydrogenase (Ldh) alleles	100	80, 40	X-linked	1,7, 8
6-Phosphogluconate dehydrogenase (Pgd)	100, 50	125, 80, and 150	X-linked	1, 7, 8
Adult hindwing width black on anal cell	10–40%	55–90%	autosomal	9, 10

1. Hagen and Scriber 1989; 2. Rockey et al. 1987a; 3. Scriber 1994; 4. Scriber 1986b; 5. Scriber 1988; 6. Scriber et al. 1989; 7. Hagen 1990; 8. Hagen et al. 1991; 9. Luebke et al. 1988; 10. Scriber 1982; 16. Scriber 2002a.

RESULTS

Detection and verification of hybridization. Among female butterflies of *Papilio canadensis* collected in northern Michigan (Charlevoix County 1999) we obtained offspring (from a single family derived from a field captured female) that were clearly primary hybrids. Neonate larval survival, and even survival through the final instar, of some offspring on tulip tree leaves (*Liriodendron tulipifera* of the Magnoliaceae) was our first clue. In addition, morphological traits of larvae were intermediate, and pupae exhibited direct development (non-diapause) resulting in eclosion of adults within a 1–3 weeks, including females. This lack of diapause was puzzling since basically all pupae of *P. canadensis* usually enter an obligate diapause, controlled by an X-linked trait. Adult offspring that emerged also had wing patterns that clearly looked to be similar to our lab-paired interspecific ("reference") hybrid specimens. Electrophoresis using allozymes "diagnostic" (with nearly fixed differences) for the 2 tiger swallowtail species (*P. canadensis* and *P. glaucus*) confirmed the identity of the field-captured female as "canadensis" and confirmed all of the sons and daughters as primary F-1 hybrid offspring (indicating that the unseen father was a *P. glaucus*).

We have examined this Charlevoix population and others near the hybrid zone for many years, yet have never found any evidence of a primary F-1 hybrid (heterozygous for all diagnostic allozymes). The results we report here (having occurred at a distance considerably north of the center of the Michigan hybrid zone; >150 km), may be partly explained due to several warmer than normal years and recently documented general northward movement of several typical *P. glaucus* traits, from the south (Scriber 2002a, b). Extensive interspecific genetic introgression from the southern species ("glaucus"-type traits) was known to have occurred northward along seasonal isoclines of total degree day accumulations of 2600–2300 (above a base 50°F), especially along the warm Lake Michigan shoreline and since 1998 (Ording 2001, Scriber 2002a).

Our observations began with the comparative study of multiple families of larvae from lab-paired hybrids (both reciprocal types) for comparison of fitness with parental species as part of another study (Donovan 2001). One of the first "odd" characteristics of family #15116 that we observed in offspring of this female *P. canadensis* collected in Charlevoix County, Michigan in 1999 was that many (92%; Table 2) of the neonate larvae survived the entire first instar feeding on tulip tree (*Liriodendron tulipifera*). Exceedingly few neonate larvae of *P. canadensis* have ever survived the first instar on tulip tree (less than 1% of 446 individuals from dozens of families; Scriber et al. 1995), but these offspring from family #15116 were also surviving into the later instars and pupae as well (18 of 36; Table 2). When in the final instar, it was clear that the superanal (dorsal) stripe was only weakly (faintly colored) yellow, instead or sharply yellow with pointed protuberances as observed typically with "canadensis." These larval "tail" patterns looked much more like those seen in the hybrids or "glaucus" larvae (JMS, pers. obs.).

After pupation, many individuals of this family developed directly into adults within 10–16 days (non-diapausing), including females. This was also a very atypical character for *P. canadensis*, which have an environmentally non-sensitive (obligate) diapause (see Table 1). These results led us to suspect the possibility of interspecific hybridization as a possible explanation. Careful checks of our rearing records and data charts

TABLE 2. The 10-day and full larval survival of *Papilio canadensis*, *P. glaucus*, and their primary (both reciprocal) hybrids as a function of host plant (TT = tulip tree; BC = black cherry; QA = quaking aspen). The number of larvae for each plant is indicated. The overall percent of direct developing (i.e., non-diapausing) pupae is also presented for each genotype. This study was part of a larger hybrid vigor study in 1999 and 2000 (Donovan 2001). The odd brood we discovered and report here (#15116 from Charlevoix Co. Michigan) is included for comparison.

		10-day survival (%)			Survival to pupa (%)			Direct development	
Genotype & Year	(N)	TT	BC	QA	TT	BC	QA	%	(n = pupae)
P. canadensis									
1999*	(60)	45.0	93.3	83.3	3.3	45.0	36.7	0.0	(52)
2000*	(60)	36.7	78.3	75.0	15.0	53.3	36.7	0.0	(64)
2-yr. mean		40.8	85.8	79.2	9.1	49.2	36.7	0.0	
Pc × Pg									
1999	(72)	75.0	84.7	84.7	59.7	58.3	26.4	33.3	(156)
2000	(72)	77.1	83.3	79.2	45.8	52.1	30.9	34.0	(100)
2-yr. mean		76.1	84.0	82.3	52.8	55.2	28.6	33.6	
Pg × Pc									
1999	(48)	83.3	91.7	83.3	56.3	54.2	29.2	32.6	(92)
2000	(48)	89.6	85.4	81.3	35.4	29.2	8.3	18.6	(43)
2-yr. mean		86.5	88.6	82.3	45.9	41.7	18.8	25.6	
P. glaucus									
1999	(24)	58.3	50.0	0.0	37.5	45.8	0.0	33.3	(30)
2000	(60)	85.0	80.0	15.0	25.0	37.5	0.0	17.2	(29)
2-yr. mean		71.7	65.0	7.5	31.3	41.7	0.0	25.3	
Family #15116	(12)	91.7	83.3	75.0	50.0	66.7	16.7	48.8	(16)

* Populations selected for this hybrid fitness study (Donovan 2001) as *P. canadensis* (from Emmet, Cheboygan, Charlevoix, and Isabella Counties in Michigan and Clark Co. Wisconsin) exhibited some introgression from *P. glaucus*, especially with regard to tulip tree detoxification abilities since the regional climatic warming that began in 1998. In the years from 1980–1997, larval survival on tulip tree from the same populations in Clark Co. Wisconsin and those in Michigan north of Clare was essentially non-existent (Scriber 1982, 2002a).

convinced us that we were dealing with the offspring of a field-collected *P. canadensis* female that had somehow naturally mated with a *P. glaucus* male before we captured her. We conducted electrophoresis on the offspring and the mother to confirm this hypothesis.

Our electrophoresis analyses produced allozyme profiles that basically confirmed the assessment that this was a "pure" *P. canadensis* female that had mated to a *P. glaucus* male before we captured her in Charlevoix County. The mother was clearly a typical *P. canadensis* (hemizygous as LDH-80 & PGD-125; homozygous HK-110; Hagen & Scriber 1989). The hindwing black band width of the anal cell was 70% of the distance to the origin of the Cu2 vein, and also was clearly "*canadensis*" ("*glaucus*" bands are generally less than 40%; see Scriber 1982, Luebke et al. 1988). In addition, the submarginal yellow forewing band on the ventral side was solid as in "*canadensis*" (not a series of yellow spots as in "*glaucus*:" Luebke et al. 1988).

The 2 male offspring were both heterozygous at the diagnostic PGD locus (100/125) and also heterozygous at the LDH locus (100/80) as would be expected for primary hybrids (bottom of table 3). Their HK alleles were not resolved clearly. The 6 daughters tested were also all as expected for a primary hybridization event (as we hypothesized), exhibiting the hemizygous sex-linked PGD 100 and the LDH 100 alleles which had to have been inherited from their putative *P. glaucus* father. The autosomal HK were heterozygous, as would be expected, for the 4 daughters that had clearly visualizable bands on the gel.

Individual specimens collected from this Charlevoix County population from 1998–2001 were scored and analyzed for trends of differences in the individual black band widths of hindwings for females and males (Figs. 1, 2). The parental *P. glaucus* typically has band widths that are 40%–10%, while *P. canadensis* typically shows 55%–90%. Reference hybrids from lab pairings of 29 different families (with more than 500 lab-reared adult offspring) range from 35%–60% (Scriber 1982, 2002a). It seems from the scoring indices (Figs. 1, 2) that 1998 and 1999 populations were characteristically *P. canadensis* in nature. However, both 2000 and 2001 males and 2001 females have individuals with significantly narrower band widths, which are likely to represent interspecifically intermediate traits. Analyses using *t*-tests show significant mean differences of the 2001 females from both 1998 and 1999 females ($p < 0.027$ and $p < 0.020$, respectively; Fig. 1). Females from 2000 were intermediate and not statistically narrower than those from 1998 and 1999. Males from the

TABLE 3. Summary of male allozyme frequencies (the most common alleles)for *Papilio* populations in Michigan compared to Charlevoix Co. and the odd family (#15116). Some data pre-1992 from Hagen et al. (1991), and some 1998–2000 (from Stump 2000; Ording 2001; Scriber et al. unpubl.). (* = diagnostic for the species *P. glaucus*)

	LDH				PGD						HK		
Latitude & Counties	(n)	100*	80	40	(n)	100*	50*	125	80	150	(n)	100*	110
45.8–45.3N (Northern Lower Peninsula)													
Charlevoix													
1992	(50)	0	92	8	(50)	0	0	94	4	2	(3)	0	100
1999	(8)	0	87	13	(8)	0	0	100	0	0	(8)	13	87
2000	(20)	0	90	10	(33)	0	0	95	5	0	(33)	0	100
Cheboygan	(47)	0	94	6	(47)	7	0	89	2	2	(32)	0	100
Emmet													
1992	(28)	0	100	0	(28)	0	0	80	14	6	(26)	0	100
1999	(24)	0	91	9	(24)	0	0	88	4	8	(7)	14	86
Presque Isle	(50)	0	100	0	(50)	13	0	82	4	1	(36)	0	100
42.8–42.0N (Southern Michigan)													
Allegan	(24)	92	8	0	(24)	96	0	4	0	0	(0)	–	–
Clinton	(3)	100	0	0	(3)	100	0	0	0	0	(3)	100	0
Ingham													
Pre-1992	(61)	85	13	2	(61)	93	1	6	0	0	(39)	95	5
1992	(12)	100	0	0	(12)	84	8	8	0	0	(12)	100	0
Jackson	(4)	100	0	0	(4)	100	0	0	0	0	(4)	100	0
Lenawee	(34)	100	0	0	(35)	94	3	3	0	0	(28)	93	7
St. Joseph	(29)	94	6	0	(29)	97	0	3	0	0	(5)	100	0
Washtenaw	(29)	93	7	0	(29)	100	0	0	0	0	(28)	73	27
** Offspring of Hybrid Family #15116													
Males	(2)	50	50	0	(2)	50	0	50	0	0	(2)	50	50
Females	(6)	100	0	0	(6)	100	0	0	0	0	(3)	50	50

2000 population have narrower bands than those from both 1998 ($p < 0.001$) and 1999 ($p < 0.019$). While the 2001 males exhibit hybrid-like (narrower) bands in some individuals, the mean 55.8% appeared slightly shifted back toward the "canadensis" type. Nonetheless, 2001 males were still significantly narrower than both 1998 ($p < 0.002$) and 1999 ($p < 0.012$; Fig. 2).

DISCUSSION

Natural hybrids have always been exceedingly difficult to document in the field for many reasons. Primary among these reasons is that hybrids do not differ greatly in appearance from the parental types in various morphological characters (Platt 1983, Arnold 1997, Porter et al. 1997). Multivariate analyses with known parental types and known hybrids (lab-paired as "reference groups") are usually needed to identify the relatively rare hybrids and introgressed individuals from all of the field-collected "unknowns" (Collins 1984, Sperling 1987, Luebke et al. 1988, Scriber 1990, Boyd et al. 1999). Physiological (e.g., diapause regulation or host plant use abilities) or biochemical differences (allozymes or mitochondrial DNA) can help with the taxonomic diagnoses, but such analyses are seldom undertaken.

We conclude from our laboratory documentation that the female *P. canadensis* collected from northern Michigan (Charlevoix County) in June 1999 must have mated to a male *P. glaucus* to produce "primary" interspecific hybrids observed in family #15116. Populations of tiger swallowtail butterflies collected at this same location (approximately 45 degrees North latitude) have always exhibited strict "*canadensis*" traits in the past, prior to 1999 (Scriber 2002a, b). The larvae have never survived to pupation, and extremely few have even survived the neonate (first instar) stage on tulip tree leaves. Other individuals collected at this Charlevoix County site but reared on acceptable natural hosts such as black cherry (*Prunus serotina*) and quaking aspen (*Populus tremuloides*) all exhibited the diapause trait, even under long day photoperiods (16–18 hours; Table 2). Individuals of this population have historically had "*canadensis*" type allozymes (PDG, LDH, and HK; Donovan 2001, Ording 2001; Table 1) and have possessed morphological (black bands in hind wing) traits that were typically "*canadensis*" in nature (>55% of the distance of anal cell to origin of the Cu-2 vein), not "*glaucus*-like" (<40%, Scriber 1982; Fig. 1).

However, our postulated 1999 primary hybridization event would have been possible only with the presence of a male *P. glaucus* at considerably greater distance North than ever previously reported from the center of the hybrid zone in Michigan (Scriber 1996a). We do know that 1998 and 1999 were exceptionally

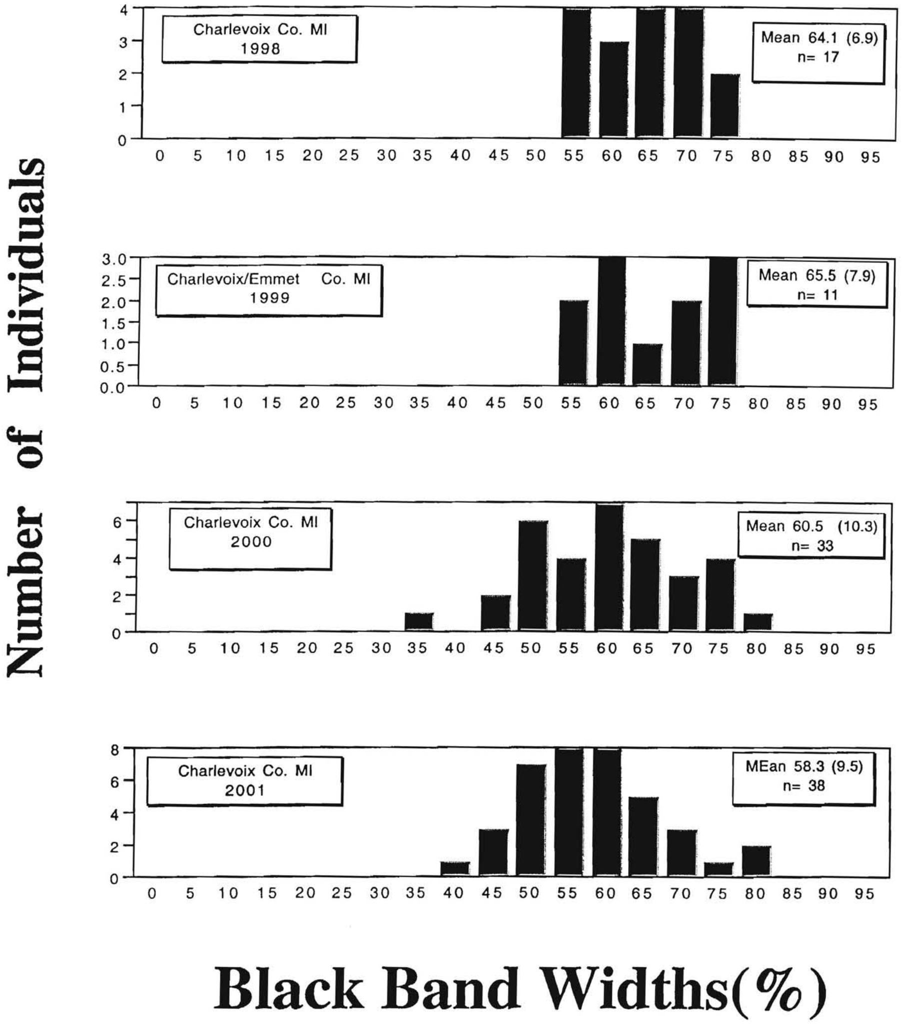

FIG. 1. Population black band widths (hind wing) trait frequencies for individual females collected in 1998, 1999, 2000, 2001 in Charlevoix County, Michigan. Our 1999 odd brood (#15116) female scored as a 70%. Immediately adjacent (6–12 km) Emmet Co. females were included in 1999, since we only collected 3 females from the Charlevoix Co. population that year. A significant trend toward narrower band widths seems to have occurred during this period, perhaps reflecting more extensive interspecific hybridization than our single documented family might suggest alone. *P. glaucus* individuals typically have 10%–40%, *P. canadensis* 55–90%, and hybrids 35–60% (see text).

warm years in total seasonal degree day accumulations and that there is clear evidence of extensive northward introgression of "*glaucus*" traits has been occurring in central Wisconsin, west central Michigan, New York, and Vermont since 1998 (Scriber 2002a, b). However, we have never seen a pure *P. glaucus* male as far north as Charlevoix County (Table 3) or anywhere from Minnesota to Massachusetts north of the hybrid zone as delineated by seasonal degree day accumulations of less than 2700 F DD (based on wing characters and allozyme electrophoresis; Hagen et al. 1991, Scriber 1996, Stump 2000, Ording 2001).

In 2000, intensive sampling of adults from this Thumb Lake site in Charlevoix County (n = 83 male captures) yielded about a dozen males with "glaucus-like" hindwing bands (20%–40%; Fig. 2). However, no adult primary hybrids were seen in those we were able to run for the 3 diagnostic allozymes (Table 3). We hoped to capture individuals of primary hybrids derived from offspring oviposited before the 1999 field-capture of this particular female (that produced brood #15116). It is also noteworthy that only one or two clearly "glaucus-type" males or females were detected in those captured in 1998 and 1999 based on band widths alone (Figs. 1, 2).

Long distance dispersal of *P. glaucus* individuals may occur with strong storms, as was reported in 1997 for a dark female that was collected even further north in Dickinson County of the Upper Peninsula of Michigan (Scriber et al. 1998). Perhaps this wild male parent

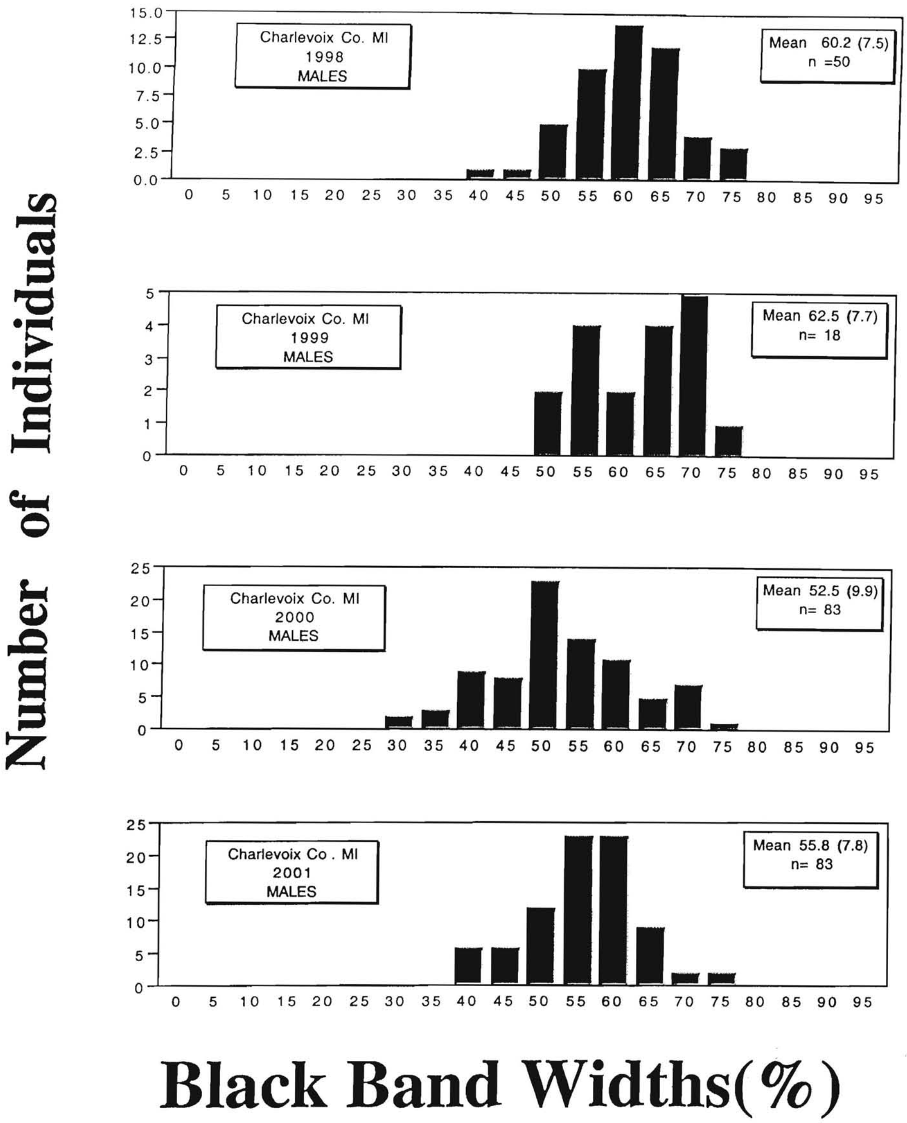

FIG. 2. Population relative black band widths for individual males captured in Charlevoix County, Michigan during 1998, 1999, 2000, and 2001. A significant shift toward narrower bands was clear after 1999.

of brood #15116 was an isolated 1999 "blow-in." However, we do know that considerable northward gene flow has been happening along the west coast of Michigan and the Islands off the Leelanau peninsula with the warm lake effects and "season extensions" northward beyond the expected latitudinal limits (Scriber & Gage 1995, Ording 2001, Scriber 2002a, b).

Natural hybridization (e.g., of our female *P. canadensis* with a *P. glaucus* male postulated as an explanation for brood #15116) would not have been suprising based upon the results of experimental field mating preference studies with tethered, size-matched virgin females of each tiger swallowtail species. In Charlevoix County during 1997 in 2-choice interspecific field mating preference bioassays of free-flying *P. canadensis* males, 82% of the 476 copulations observed were with the heterospecific *P. glaucus* female, rather than with the conspecific (*P. canadensis*) female (Deering 1998, Deering & Scriber 2002). In contrast, free-flying *P. glaucus* males in Florida had preferred their conspecific *P. glaucus* females in 98% of all copulations observed for the 1997 and 1998 field seasons (Deering & Scriber 2002). Without *P. glaucus* females in the area to select from, the mating with our *P. canadensis* female (#15116, or others) seems more probable for any *P. glaucus* males recently flown in or blown in (Scriber et al. 1998).

We did observe directly developing adults, both

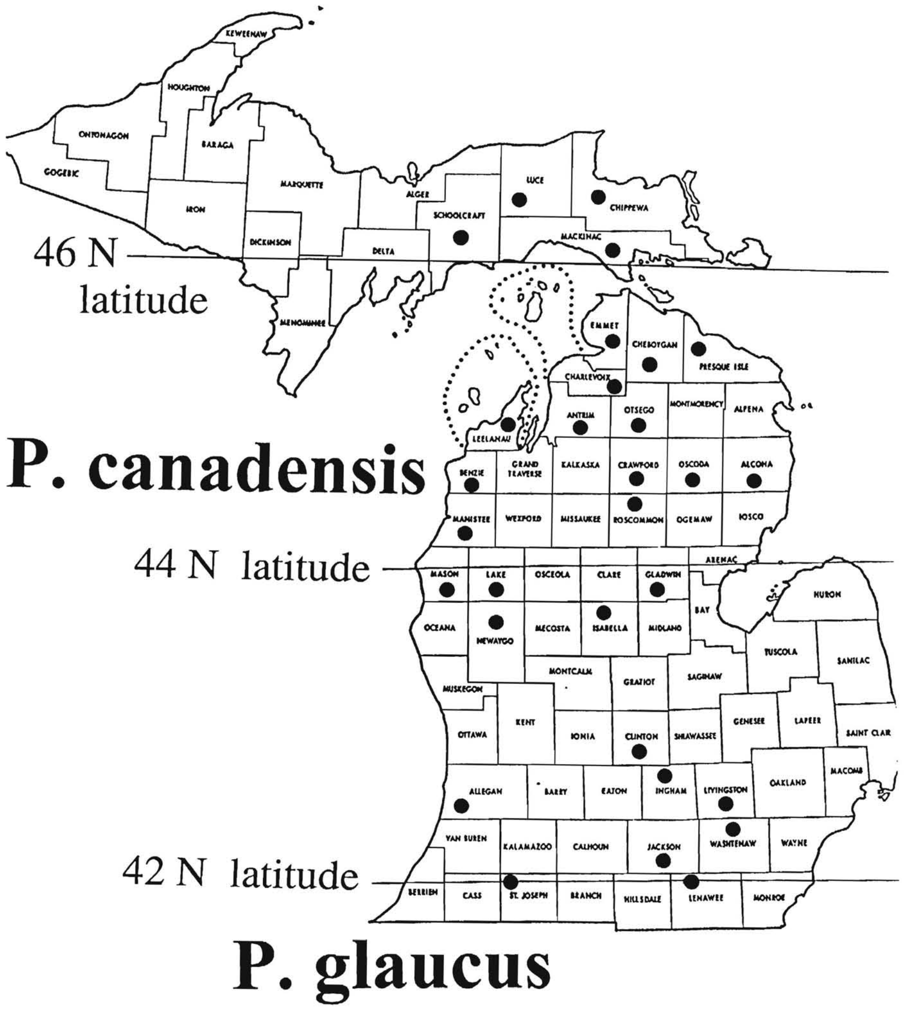

FIG. 3. Michigan map of counties. Historically, the hybrid zone separation between the two tiger swallowtail species, *Papilio canadensis* and *P. glaucus*, centered approximately across the 43 degree North latitude band (Scriber 1996a, 2002a, Nielsen 1999). Charlevoix county is in upper northwest of lower Michigan peninsula.

males (n = 2) and females (n = 6) from offspring of this female (brood #15116) that survived to pupation. However, other offspring of this female that may have grown and pupated in the field at Charlevoix County (i.e., from eggs laid before her capture) would certainly not have led to a successful second generation this far north, even if they had found mates. Insufficient growing degree-day accumulations (DDs) existed to permit larval growth and pupation of a second generation before the Fall freezes and leaf abscission (Scriber & Gage 1995, Scriber 1996b, Tesar & Scriber 2002). The 20-year average was less than 2200 F DD's, and even in these very warmest years, 1998, and 1999 there were still less than 2500 F DD's (Scriber & Lederhouse 1992, Scriber 2002b). It seems clear that selection against these or any similar non-diapausing hybrid genotypes (*P. canadensis* females and *P. glaucus* males) would be severe, whereas those of reciprocal parental crosses (*P. glaucus* females and *P. canadensis* males) would be able to survive Fall and Winter as dispausing pupae (Rockey et al. 1987a, b, Scriber 2002b).

In fact, this brood mortality of direct developing adults in areas with insufficient thermal unit accumulations to complete the (second) generation, may explain the very strong selection gradient for univoltinism at the hybrid zone. One sex-linked allozyme disjunction (step-cline) for lactate dehydrogenase is evident along a northward cline across the hybrid zone in New England and here in Michigan (LDH-100 is also "diagnostic" for the *glaucus* species whereas LDH-80 and LDH-40 are diagnostic for "*canadensis*"; Hagen 1990, Ording 2001, Scriber 2002b). Other sex-linked allozymes and autosomal traits such as tulip tree detoxification abilities have moved northward extensively, but not LDH-100 (Scriber 2002a). The "true" second generation capability also stops short of those latitudinal distances observed for the species-diagnostic PGD-100, HK-100, tulip tree detoxification abilities, and the narrow black hindwing bands (Ording 2001, Scriber 2002a, b).

It is important to realize that not all hybrids are evolutionary "dead-ends" (Arnold & Hodges 1995, Futuyma & Shapiro 1995, Arnold 1997). The interspecific hybridization of *P. glaucus* and *P. canadensis* that occurs across the Great Lakes region hybrid zone does not always result in maladapted offspring. In fact, the larval growth rates and pupal sizes of interspecific hybrids are sometimes greater than either parental species type (Scriber et al. 2003). In no case were performances of reciprocal hybrid genotypes less than either parent when reared on combinations of three hosts (tulip tree, quaking aspen, and black cherry) at three different temperatures (15°C, 23°C, and 31°C; Donovan 2001). We suspect that such genetic introgression from interspecific hybridization may contribute significantly to the different genetic combinations that may be locally suited to islands in the Great Lakes islands such as South Manitou and North Manitou Islands of The Sleeping Bears Dunes National Park (Ording 2001) or to local climatic "cold pockets" such as the area just east of this Charlevoix County site (Scriber 1996b), or in latitudinal/altitudinal zones where seasonal thermal unit resources for completing a generation are "constrained" (Collins 1984, Scriber 2002b). It will be interesting to follow the genetic changes in these populations and the extent of interspecific hybridization if the Great Lakes regional climate continues to warm as seen globally (Parmesan & Yohe 2003).

Since some hybrids have been unique enough to have incorrectly been assigned species status (Tyler et al. 1994), and since morphological traits alone are often insufficient to confirm hybrid status, we have provided a multi-trait analysis as a mini-review. Spatially and temporally extensive trait analyses may be the only way to assess the extent genetic introgression across hybrid zones and for identifying parental versus hybrid status. We have recently (since 1998) seen extensive introgression of tulip tree detoxification abilities (from *P. glaucus*) to locations more than 200 miles North of the 1980–1997 hybrid zone center (Scriber 2002a). These most recent observations were based upon more than 3080 larvae of 136 families and for 800–900 field-captured adults (for morphometric introgression of diagnostic adult wing traits) in Michigan alone. Since 1999, in this Charlevoix population, neonate survival on tulip tree has increased from 10% in 2000, to 35% and 33% in 2001 and 2002. This hybrid family (#15116) from Charlevoix was apparently an early forerunner of additionally extensive hybridization and backcross introgression that has been documented in the Great Lakes and New England region since 1998. It also supports the general lack of pre-zygotic reproductive isolation observed between these species in the field (Deering & Scriber 2002).

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