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#### INTERSPECIFIC COPULATION OF A DARK MORPH *PAPILIO GLAUCUS* FEMALE AND A MALE *P. POLYXENES* (PAPILIONIDAE): OBSERVATION AND SIGNIFICANCE

**Additional key words:** heterospecific mating, swallowtail butterflies, pre-zygotic reproductive isolating mechanisms, sexual selection.

Pre-zygotic reproductive isolation separating species of swallowtail butterflies involves spatial (allopatric/parapatric); morphological, temporal, behavioral, physiological, and other mechanisms (such as female choice or "cryptic sexual selection" of conspecific rather than heterospecific sperm in multiply-mating species; Eberhardt 1996, Stump 2000). Post-zygotic failure of hybrid embryos, larvae, pupae or adults to survive and reproduce has been observed for laboratory hand-pairings of interspecific *Papilio* hybrids, in some cases following "Haldane's Rule," which may increase in negative impacts with increased genetic distances between the hybridized species (Haldane 1922, Hagen & Scriber 1995).

Despite the various natural reproductive isolating mechanisms maintaining species integrity among *Papilio* butterflies, there is a large amount of evidence from various laboratory interspecific hybridizations that suggests post-zygotic barriers are minimal (Ae 1995, Brown et al. 1995, Scriber et al. 1991, 1995). Natural interspecific hybridization (or any matings) among *Papilio* individuals are rarely seen in the field, however it has been estimated that more than 6% of the 200+ species of *Papilio* hybridize naturally (Sperling 1990). This suggests that the populations of these species are maintained primarily by ecological factors

rather than by strong prezygotic reproductive isolating mechanisms.

In an attempt to determine the actual field mating preferences of free-flying tiger swallowtail butterflies at critical transects of the natural hybrid zone between *P. glaucus* and *P. canadensis* (Scriber 1996), we used fresh virgin females of both species in size-matched tethered pairs at natural field sites for *P. canadensis* males in northern Michigan and *P. glaucus* males in Florida. While the free-flying Florida *P. glaucus* males selected and copulated the conspecific females in 98% of the cases, the converse was not observed. In northern Michigan, *P. canadensis* males strongly preferred the heterospecific females (*P. glaucus*; yellow morphs) rather than females of their own species in 83% of all copulations (Deering & Scriber 2002). However, in preliminary studies it was noticed that the mimetic dark morph females of *P. glaucus* (Scriber et al. 1996) were basically ignored by *P. canadensis* males in field tethering trials when paired with *P. canadensis* females (JMS et al. unpublished). This apparent failure of *P. canadensis* males to recognize or select dark morph *P. glaucus* females is part of a larger project on interspecific hybridization that led to an unexpected observation in northern Michigan involving the notable encounter with *P. polyxenes*.

In a separate study evaluating *P. canadensis* male mating preferences for *P. glaucus* yellow versus dark morph females (HH & JMS unpublished), we tethered pairs of size-matched virgin females at various sites in northern Michigan (including the Upper Peninsula) using techniques described by Lederhouse (1995) and Deering and Scriber (2002). In the process of evaluating these behavioral interactions between males of the recently diverged *P. canadensis* species and the two female color forms of the ancestral *P. glaucus*, an unusual interspecific mating-attempt (and eventually a copulation) was observed between the dark morph of the Eastern Tiger Swallowtail female and a wild Eastern Black Swallowtail (*Papilio polyxenes asterias* Stoll) male. This single event is what we report here. The results of the 2-choice *P. glaucus* dark and yellow females by males of *P. canadensis* are the subject of a larger continuing study.

This interspecific copulation of a wild male *P. polyxenes* and a thread-tethered dark morph female *Papilio glaucus* in the field has ecological and phylogenetic significance. Incidentally, this observation also apparently represents the first capture of a *P. polyxenes* from Delta County in Michigan's Upper Peninsula. The tethered pair of dark and yellow morph *P. glaucus* females was set out (at 0.5 m apart, 1.5 m above the ground) on a Russian (or Autumn) Olive bush (*Eleagnus* spp.) on 12 June 2001 at about 3:00 PM on a sunny, calm day when the temperature was about 80°F (27°C). The experimental regime was intended to test male mating preferences for *P. canadensis* (not *P. polyxenes*). The location of the bush was at the edge of a cow pasture off "J" road, east of 535 near Bark River, Michigan 15 miles West of Escanaba (Delta Co.). After several "hits" by the *P. polyxenes* male on the dark female, he was successful at copulating. The pair was separated after one minute (as usual with our experimental tethered matings of swallowtail males; Deering & Scriber 2002), and the male was returned to Michigan State University as a voucher specimen for analysis (currently stored at -80°C). The *polyxenes* male had a forewing length of 38 mm while the *glaucus* female had forewings 54 mm in length.

The two species involved in this heterospecific copulation event in the field are phylogenetically separated by a large distance (Munroe 1961). In fact, *P. polyxenes* is in an entirely different section (II) of the Papilionidae than *P. glaucus* (Section III, Munroe 1960, Miller 1987). Natural hybrids have been reported within the *P. glaucus* species group (Brower 1959, Scott & Sheppard 1976, Wagner 1978, Rahn 2001). While natural hybrids within the *P. machaon* group includes hybrids with *P. polyxenes* and *P. machaon*, and *P. polyxenes* and *P. zelicaon* (Sperling

1987), the taxonomic difference between *P. polyxenes* and *P. glaucus* represents the greatest genetic distance between species in a copulation ever reported for the 560 species in this family (Papilionidae), with the exception of a female *Battus philenor* (L.) with a male *Eurytides marcellus* (Cramer) in Texas (Rausher & Berenbaum 1978). An interspecific copulation between *P. canadensis* and *P. palamedes* was also recently reported (Deering & Scriber 1998).

The copulation of this *P. polyxenes* male and our *P. glaucus* female is especially surprising because *P. polyxenes* males are usually territorial, live in open fields, and aggressively defend "leks" (Lederhouse 1982, Lederhouse & Scriber 1996). This mating system contrasts dramatically with the "patrolling" type behavior observed for males throughout the *P. glaucus* species group (including *canadensis*) along woodland streams or roads in forested areas, or along hedgerows and woodlot edges (Lederhouse 1995). The *polyxenes* male may have been initially attracted to the blossoms on the *Eleagnus* bushes and secondarily encountered the dark female *glaucus*.

The dark morph female of *P. glaucus* in this tethered pair appears visually similar to the typical female of *P. polyxenes* in size and black/blue colors, presumably due to convergent evolution related to the common mimicry system and the model species *Battus philenor* (the pipevine swallowtail butterfly; see Brower 1958). However, even after the visual attraction to the female, the male *P. polyxenes* persisted in the grappling and copulation. It has been suggested that the ultraviolet wing reflections from dark and yellow morph individuals of *P. glaucus* females are very similar, and serve as species-specific cues for mate recognition for conspecific males (Platt et al. 1984). Apparently males of *P. canadensis* (and males of western tiger swallowtail species; Brower 1959) do not successfully use these ultraviolet cues, perhaps since they have only yellow-striped monomorphic females to select from in their species (Clarke & Sheppard 1962). Similarly, males of *P. polyxenes* have basically monomorphic dark morph females to select from in their species. Consequently, as close mimics of the pipevine swallowtail, dark morph females of *P. glaucus* could visually be mistaken for a female *P. polyxenes*.

This encounter event with our experimental dark morph female and wild male *P. polyxenes* in the Upper Peninsula of Michigan is rare but not entirely unnatural. In fact, in 1997 a dark morph female of *P. glaucus* was collected in Dickinson Co., not more than 50 miles away (Scriber et al. 1998), and *P. polyxenes* have been collected in several other counties even further north (Fig. 1; Nielsen 1999). This black swallowtail capture near Es-

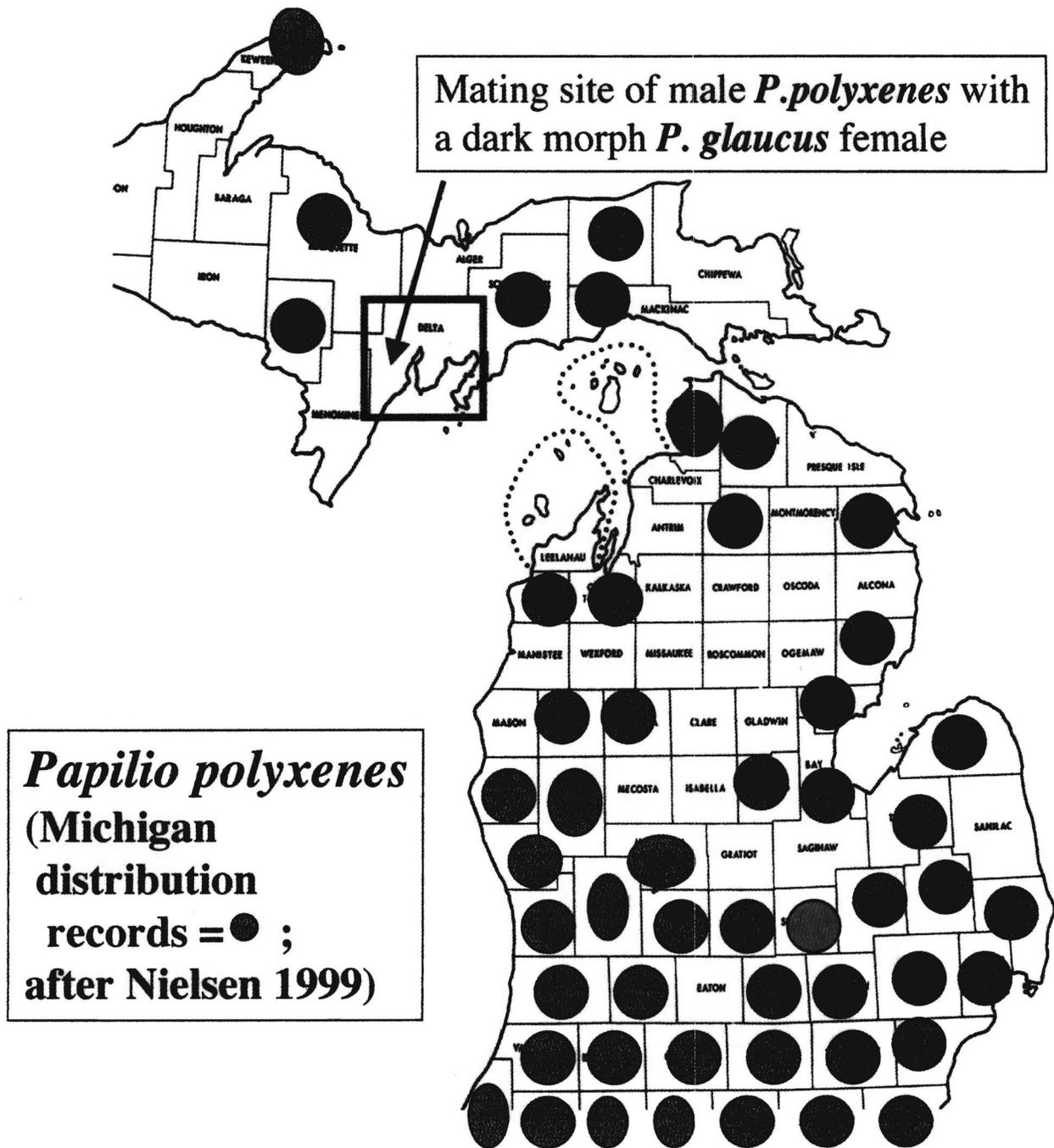


FIG. 1. The site in Delta County, Michigan where the *Papilio polyxenes* male mated with our dark morph *Papilio glaucus* female. It is a new Michigan county record for *P. polyxenes* (Nielsen 1999).

canaba, Michigan is however the first ever reported from this particular area (Delta County; see Nielsen 1999).

Such observations near species range borders and distribution records moving northward during the past decade apparently relate to the regional warming trends that have occurred in this Great Lakes area (Scriber & Gage 1995, Scriber 2002). It is possible that

the sparseness of individual *P. polyxenes* in that county, combined with the experimental presence of a dark morph female of *P. glaucus* (which is historically very rare; Scriber et al. 1996) resulted in an interspecific "mistake" in copulation. Mimetic coloration in female mimics that is good enough to dupe potential predators has been described (Brower 1958, Codella &

Lederhouse 1989). However, duping males of another species as we have observed here is even more impressive. Such a mating has never been reported from anywhere in the eastern USA where these two species are extensively sympatric (Opler & Krizek 1984).

Of 40 laboratory hand-pairings of *P. glaucus* group species (Section III) with *Papilio* species from section II of the Papilionidae (Munroe 1961), only 12 produced any eggs, only 5 produced fertile eggs, and only a single individual ever reached the pupal stage (where it died; Ae 1979). These results suggest that pre-zygotic and post-zygotic reproductive isolation between these two sections of the genus is strong. However no attempts to hand-pair *P. polyxenes* with *P. glaucus* are reported in the literature.

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