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NOT ALL BILATERAL GYNANDROMORPH BUTTERFLIES ARE INTERSPECIFIC HYBRIDS: NEW *PAPILIO* SPECIMENS FROM FIELD POPULATIONS

MARK SCRIBER
Department of Entomology, Michigan State University, E. Lansing, MI 48824; research associate McGuire Center for Lepidoptera and Diversity, University of Florida, Gainesville, FL 32611-2710; email: scriber@pilot.msu.edu

RODRIGO MERCADER
Department of Entomology, Michigan State University, E. Lansing, MI 48824

HOWARD ROMACK
28 Irish Lane, Cambridge, NY 12816,

AND

MARK DEERING
Lepidoptera Curator, Sophia Sachs Butterfly House, 15193 Olive Blvd., Chesterfield, MO 63017

ABSTRACT. Bilateral gynandromorphs (half male, half female) have previously been reported in interspecific hybrids. However, we report 6 new nearly perfect bilateral gynandromorphs, three (one *Papilio canadensis*, and two *P. glaucus*) of which were collected from areas not near hybrid zones. New interspecific gynandromorphs also are described for apparent *P. rutulus* x *P. multicaudatus* hybrids. By using hand-paired interspecific hybrids we have generated both diapausing and direct developing (non-diapausing) gynandromorphs. While X (or Z)-linked regulation of diapause in these *Papilio* may be similar throughout the male and female halves of the individual, the dark melanic color [Y (or W)-linked] is distinctly different. Wing color regulation is differentially implemented on dorsal and ventral surfaces as seen in sexual mosaics described here. The developmental irregularities resulting in bilateral gynandromorphs and sexual mosaics are not solely due to interspecific genetic incompatibilities. However, genetic factors seem to play a major role in their expression. The existence of dark melanic female patchy patterns supports the suggestion that control of the W (=Y)-linked b^+ melanic gene is both cell-specific and sex-specific in its ability to suppress the BAS enzyme activity that normally shunts dopamine to the yellow papiliochrome pathway. Interspecific hybrids involving dark mothers (with the Y-linked b^+ gene) mated to *P. multicaudatus*, *P. rutulus*, and *P. canadensis* males (all species lacking the dark morph) have also produced hybrid daughters with "intermediate" suppression of melanism giving a dusty colored appearance (differing from color mosaics). This uniform "dusty" coloration often has been referred to as "cinnamon," and may reflect incomplete genetic penetrance.

Additional key words: melanic wing color, sex chromosomes, *Papilio glaucus*, *P. canadensis*, *P. rutulus*, *P. multicaudatus*, *P. appalachiensis*

Lepidoptera that have developed abnormally to produce mosaic color patterns and a mixture of other traits, often representing intersexes (part female and part male) have frequently been described (Clarke & Ford 1980; Ayala & Kiger 1984; McCafferty & Bloodgood 1986). If such developmental errors occur early in development (perhaps from fertilization of a binucleate oocyte; Blanchard & Descimon 1988) the result might be a perfect (or near-perfect) bilateral gynandromorph with one half being male and one half female (Clarke & Clarke, 1983).

A large number of gynandromorphs have been reported in Lepidoptera from at least 10 different species of Papilionidae, eight species of Lycaenidae, four Hesperiidae, ten Pieridae, four Nymphalidae, and several families of moths including Saturniidae, Pyralidae, Geometridae, and Lymantriidae (reviewed in Scriber & Evans 1988b; see also Sibatani 1980, 1983a, b; Shuey & Peacock 1985). Cockayne (1935) provides numerous additional records, and the Milwaukee Public Museum's James R. Neidhoefer Collection has several hundred mosaics and at least 150 bilateral gynandromorphs. David and Marc Perlman in Illinois have also compiled a large collection of mosaics and gynandromorphs.

A number of suggestions have been advanced to potentially explain these developmental abnormalities including: extreme temperatures (Standfuss 1900; Drescher & Rothenbuhler 1963; Riotte 1978), ultraviolet light (Nekrutenko 1965), viral infections (Gardiner 1972; Blau 1978; Sevastopulo 1973), translocations of parts of the sex-chromosomes and/or autosomes (Blanchard & Descimon 1988; Scriber & Evans 1987; Marec et al. 2001), and background uranium radiation-induced somatic mutations and the Chernobyl nuclear power plant disaster (Dantchenko et al. 1995). Interspecific hybrid crosses have also been suspected as a potential cause of mosaic/gynandromorphs (Whicher 1915; Cockayne 1935; Clarke & Sheppard 1953; Clarke *et al*. 1977; Platt 1983; Blanchard & Descimon 1988; Scriber & Evans 1988a, b). In *Papilio* such gynandromorphs have been found to be most often the result of interspecific (or intersubspecific) crosses (Scriber & Evans 1988b). Asymmetrical color mosaics may also result from such interspecific crosses, but are more often seen from the central parts of a species geographic range, or from intraspecific pairings, which suggests that mosaic gynandromorphs could be the result of more complex and basically different developmental processes than bilateral gynandromorphs (Clarke & Clarke 1983; Blanchard & Descimon 1988).

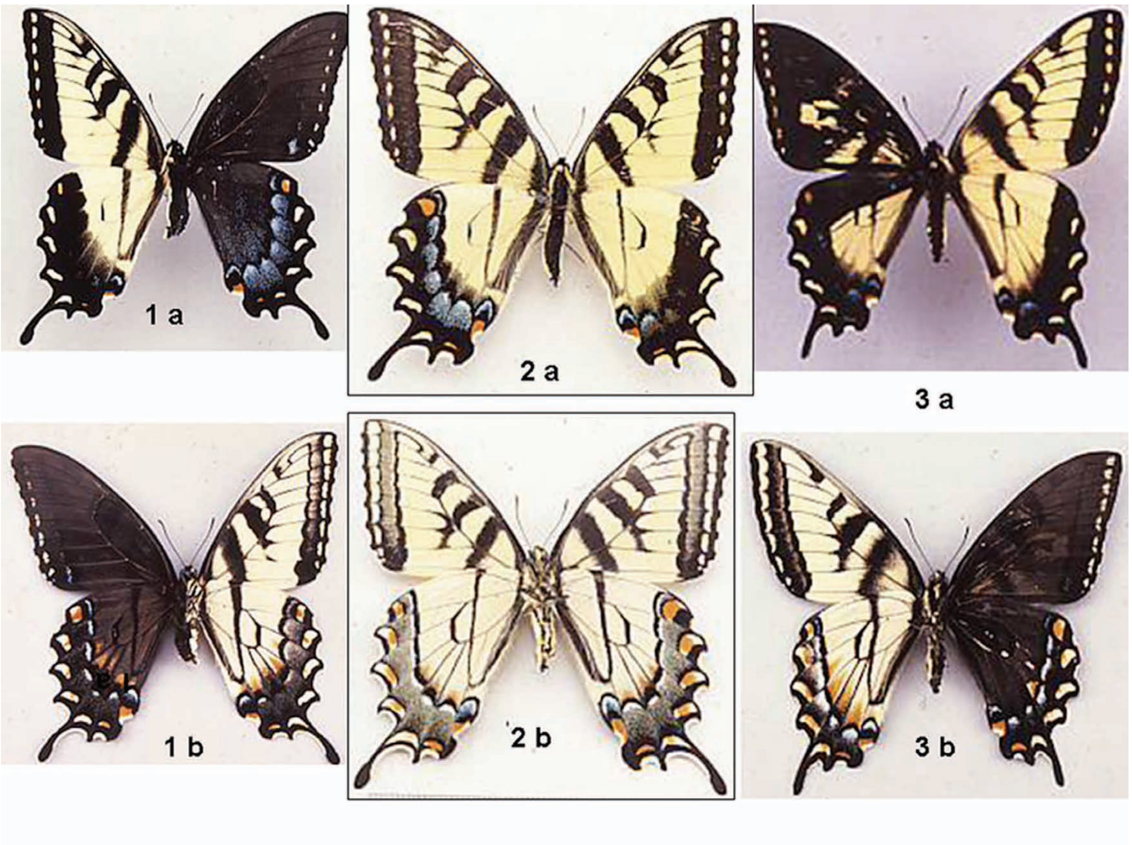
The evidence we had collected from 1979–1986 with the Papilionidae in eastern North America suggested that perhaps only interspecific hybridization would generate bilateral gynandromorphs, and at that time we had never found a "natural" (i.e. field-collected) bilateral gynandromorph (Scriber & Evans 1988b). However, we have subsequently collected interesting mosaics and near-perfect bilateral gynandromorphs from field populations, some of which were not clearly near a hybrid zone where introgression from different species was obvious. We report on some of these interesting aberrations here.

Tiger Swallowtail Gynandromorphs and Color/Sexual Mosaics. For an earlier study (Scriber & Evans 1988b), we reared nearly 30,000 butterflies from more than 4,000 families of *Papilio glaucus* and *P. canadensis* (previously considered subspecies *P. glaucus canadensis*; Hagen *et al*. 1991). Of these individuals we observed 6 of 34 color mosaic adults, all of which possessed basic dark (mimetic) wing patterns of females interrupted by yellow patches or blotches on one to three of the individual wings. These yellow patches represent either the non-mimetic female, or male type pattern. Such color mosaics may represent intersexes or gynandromorphs with part male and part female asymmetric traits. They may be either gynandromorphs or sexual mosaics (Blanchard & Descimon 1988; Cockayne 1935; Ford 1955; Sevastopulo 1973). In *Papilio glaucus* and *P. alexiares* Hopffer, both polymorphic yellow and black forms are found (Scriber *et al*. 1996). Only these two *Papilio* species have the polymorphic dark and yellow morph females, clearly showing the color contrast in gyndromorphism/mosaicism. One such *P. glaucus* female (with one yellow half and one mostly dark half) has recently been figured and described by Bradshaw (2000).

In addition to these 34 color mosaics, we also observed three perfect bilateral gynandromorphs (including one that is half dark female and half yellow male) and two "nearly" perfect bilateral gynandromorphs basically half dark and half yellow (Scriber & Evans 1988a, b). These perfect bilateral gynandromorphs were all offspring from interspecific hybrid crosses. Furthermore, while the dark color of the female half was expressed in the pairing of a dark Ohio female with a *P. alexiares* Mexican male (Fig. 1), the female half of the second gynandromorph [a dark female *P. glaucus* from South Carolina (with the b^+ gene) mated with a *P. canadensis* male from Marinette Co., Wisconsin] had the dark potential suppressed (Fig. 2; Scriber *et al*. 1996). The third gynandromorph (a yellow *P. glaucus* from Pennsylvania mated with a *P. canadensis* from Juneau Co., Wisconsin) had the female half yellow because it likely lacked the Y-linked dark gene from the mother and also probably received an X-linked suppressor gene from the *P. canadensis* father (Scriber *et al*. 1996).

Such gynandromorphs are not only fascinating in appearance, but also with regard to physiology. Both of these *P. glaucus* × *P. canadensis* bilateral gynandromorphs emerged in the second year and did not develop directly as did many of their "normal-looking" brothers. This reflects an X-linked obligate diapause that is hemizygous in the female hybrids (and the female half of these gynandromorphs; XY or ZW), but heterozygous in its sibling male hybrids (XX or WW), which allows direct development and adult male eclosion without diapause. Apparently the two halves of both gynandromorphs were cued to eclose as adults in the next season rather than directly (at the 16:8 L:D photophase, 23°C rearing conditions; Scriber & Evans 1988b).

Of the 9,000 or more field collected specimens during this study (1979–1987; Scriber & Evans 1988a, b) we never collected an obvious bilateral or nearly bilateral gynandromorph. The closest was one reared from a field-collected female from Jasper Co. Texas (Fig. 4). However, we collected many color mosaics (dark and yellow patterns being the most obvious). In fact, 33 of the 39 color mosaics were field-captured individuals (6 were lab-reared from hand-paired hybrids). While most of these field-collected color mosaics were from Georgia, Ohio, Illinois, and Texas, one family from a female captured in Dane Co., Wisconsin produced 17 of the color mosaics. It is possible that some introgression from *P. canadensis*



FIGS. 1–3. **1**. A bilateral gynandromorph (from brood #4210) from a lab cross of a dark morph *P. glaucus* female (Adams Co., Ohio) mated to a wild collected male *P. alexiares garcia* from Nuevo Leon, Mexico. (a) dorsal, (b) ventral. **2.** A bilateral gynandromorph (from brood #628) from a lab cross of a dark morph *P. glaucus* female (Pickens Co., South Carolina) mated to wild collected male (*P. canadensis* from Marinette Co., Wisconsin (collected 1–5 July 1983 by Don Caine). (a) dorsal, (b) ventral. **3.** A gynandromorph, or sexual mosaic, which appears to be more than 60% male (from brood #2830) from a dark morph *P. glaucus* female (reared from eggs obtained from a dark morph female wild collected in Clay Co., West Virginia) bred to a wild collected *P. rutulus* male (reared from eggs obtained from a wild female collected in Sacramento Co., California). (a) dorsal, (b) ventral.

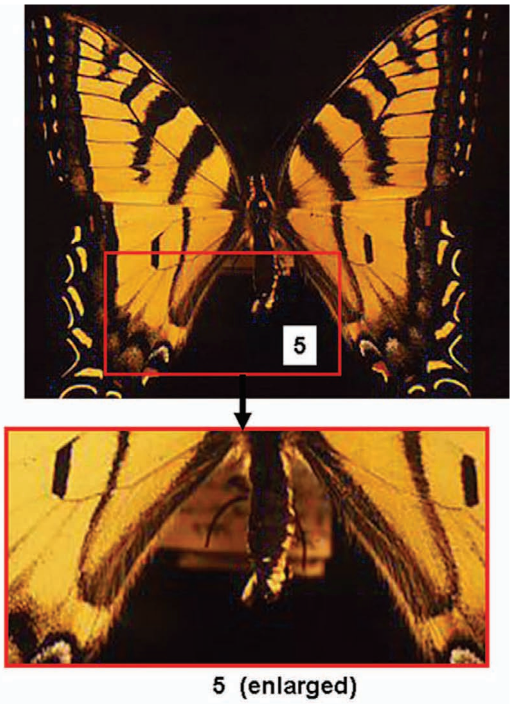
could have occurred in this geographic area since it is very near the hybrid zone (Luebke *et al*. 1988). In fact, the famous 19th century Strecker collection (Strecker 1878; Walsten 1977; Ehle 1981; Shapiro 1981a, b; Clarke & Clarke 1983) of *P. glaucus* color (or sexual) mosaics (which had been on loan from the Chicago Field Museum to the Allyn Museum) may reflect such introgression in those mosaics near the *Papilio glaucus*/*P. canadensis* hybrid zone in the Midwest and Pennsylvania (Scriber *et al*. 2003). Here we review our hypothesis in light of new information.

We reared these hybrid larvae only through to pupation on black cherry (*Prunus serotina*) leaves, since this host is used by both *P. canadensis* and *P. glaucus* (and by all four other species in the *P. glaucus* group; Scriber 1996). If we had attempted to rear these gynandromorphs on tulip tree, *Liriodendron tulipfera* (this *P. glaucus* favorite is toxic to *P. canadensis*; Scriber 1982) or on quaking aspen, *Populus tremuloides* (this *P. canadensis* favorite is toxic to *P. glaucus*, Lindroth *et al*. 1988), these insects might have might have survived to become adults, as normal hybrids, since detoxification enzymes are inherited autosomally, and are used inside the gut, they can be expressed throughout the insect regardless of whether they are produced by one or both halves (male or female) of the gynandromorphs (Scriber 1986; Scriber *et al*. 1989).

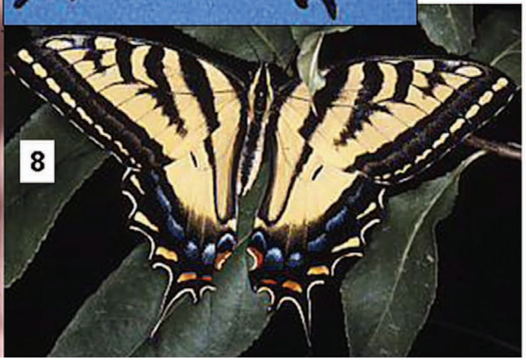
RESULTS

We collected a female *Papilio canadensis* from Cheboygan Co., Michigan during the period 9–12 June 1997. Larvae were reared on black cherry. One of the resulting adults was a nearly perfect bilateral gynandromorph with blue hindwing color and female genitalia on the right, and with male genitalia and coloration on the left (Fig. 5).

Another near-perfect bilateral gynandromorph was actually collected in the field on 15 June 1996 in southwestern Vermont near the New York border (H.R.). This specimen has basically female blue



FIGS. 4–5. **4.** A nearly perfect gynandromorph or sexual mosaic (from brood #1091) from a wild collected dark morph (*P. glaucus* female from Jasper Co., Texas. (a) dorsal, (b) ventral. **5.** A bilateral gynandromorph lab-reared from a family that was derived from a female *P. canadensis* collected in Charlevoix Co., Michigan, 9-12 June 1997 (a) dorsal, (b) close-up (In collection of Mark Deering).



FIGS. 6–8. **6.** A bilateral gynandromorph of *P. canadensis* collected (H.R.) in Bennington Co., Vermont on 15 June 1996 (a) dorsal, (b) ventral. **7.** A bilateral gynandromorph collected (H.R.) on 3 June 1989 in Lincoln Co., Wyoming. Possibly *P. rutulus* female (left half) and *P. multicaudatus* male (right half). **8.** A hybrid (lab-paired, lab-reared) of a *P. rutulus* female x *P. multicaudatus* male (brood #7806).

coloration on the right hindwing and slightly heavier (wider) black bands (as in females) on the right forewings (Fig. 6; this specimen appears to possess 2 male claspers and no ovipositor).

The third nearly perfect bilateral gynandromorph is one collected from Lincoln Co. (Wyoming) on 3 June 1989. This individual appears to be half female *P. rutulus* and half male *P. multicaudatus* (Fig. 7). Both species lack the dark morph females and probably the Y-linked b^+ gene (Scriber *et al*. 1990, 1996). The right hindwing has the extra "tail" and the right forewing appears somewhat similar to *P. multicaudatus* in banding. The left female half looks *rutulus*-like. For comparison, we also present a female from an interspecific lab pairing of *P. rutulus* female × *P. multicaudatus* male (Fig. 8, brood #7806). In this specimen, the two species traits appear somewhat intermediate throughout and did not segregate into the two halves (one female and one male, as in the interspecific gynandromorph; Fig. 7).

A field-captured, nearly-perfect bilateral gynandromorph between the nymphalids *Limenitis arthemis arthemis* (Drury) and *L. a. astyanax* (F.) was captured in the same area of the southwestern Vermont/New York border as the *P. canadensis* gynandromorph (not figured) and may be part of an active hybrid zone. These *Papilio* and *Limenitis* species (sub-species) complexes have significant introgression and exhibit centers within their hybrid zones in New England, as they do across the Midwest in Michigan and Wisconsin (Platt & Brower 1968; Scriber 1996).

We have also lab-reared a dark nearly perfect bilateral gynandromorph from a dark, east Texas *P. glaucus* mother (brood #8858, Janice Bossart). A specimen photo sent to us by Loretta Lutman of Ashboro, North Carolina (Randolf Co.) also appears to be a wild, bilateral gynandromorph of *P. glaucus*, with the female half dark (Fig 9).

Perfect bilateral gynandromorphs, with half expressing male traits and the other female traits, are extremely rare. Color/sexual mosaics with asymmetrical blotches or patterns of color (or other mixed gender traits) are somewhat more common than bilateral gynandromorphs among these swallowtails, but they are still quite rare. Earlier studies with nearly 30,000 lab-reared North American *Papilio* resulted in 30–40 color mosaics (approximately 0.001 % of reared adults was an obvious color and/or sexual mosaic; Scriber & Evans 1988b). It is easiest to find color (or sexual) mosaics in *P. glaucus* when the female is of the dark morph, but the hindwing blue color of females is also helpful in spotting them (Fig. 10). One thing that is clearly characteristic of color mosaics is that the dorsal and ventral patterns are determined independently; a few examples are illustrated in Figs. 11–16. Another mosaic from Illinois shows asymmetry (Fig. 17). It is also possible to have bilateral differentiation as in size or color for the same sex and single species individual larvae, pupae, and adults (see Scriber & Hagen 1990).

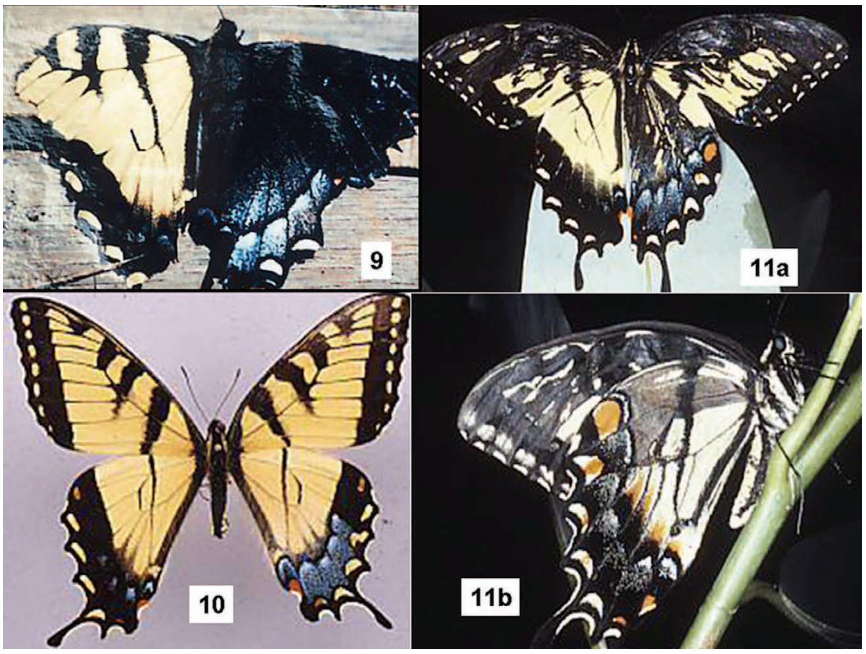
DISCUSSION

We describe six new and nearly perfect bilateral gynandromorphs from field collections in northern Michigan, southwestern Vermont, Montana and Texas. Four of these are likely to represent interspecific or intersubspecific hybrids (Figs. 5–7, and the *Limenitis* from Bennington Co. Vermont, collected by HR). While perfect and near-perfect bilateral gynandromorphs appear to be often generated by interspecific hybridization, we report two gynandromorph specimens from Texas and North Carolina (Fig. 9) and East Texas (J. Bossart and JMS, not figured here) that were not near the *P. canadensis/glaucus* hybrid zone.

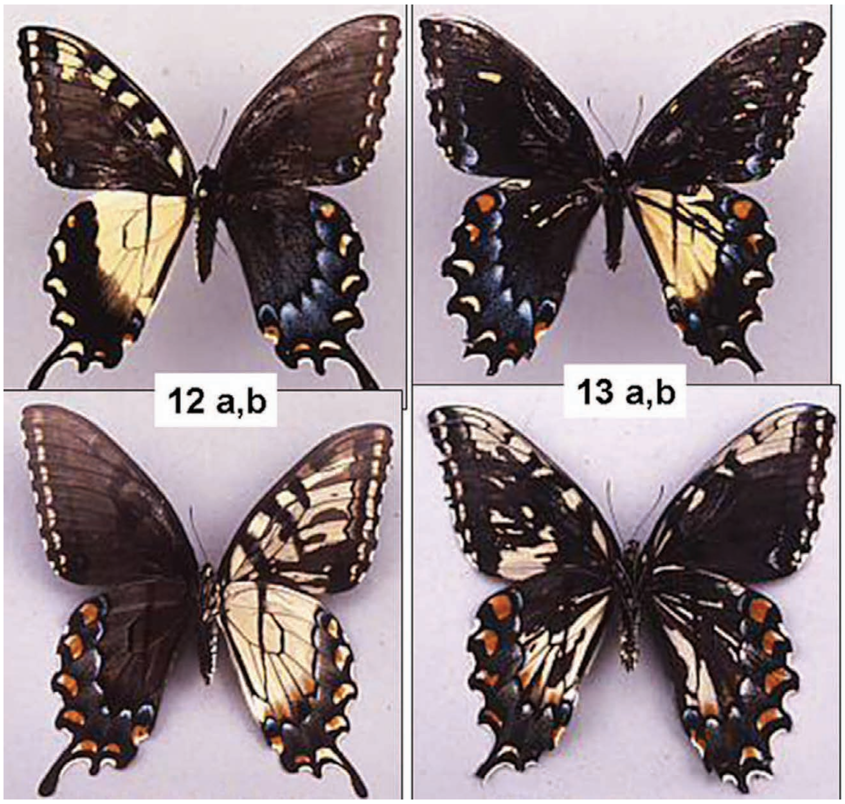
The somewhat unique ability of individual butterflies with one half male and one half female to develop as larvae, pupae, and live adults capable of flight (e.g. Scriber *et al*. 1995; Conners 2000) is fascinating, but still poorly studied and understood. The dramatic differences in wing color with partial dark morph females and yellow-patterned males enables us to easily detect individual bilateral gynandromorphs or asymmetrical sexual mosaics, but the yellow morph female gynandromorphs of tiger swallowtail butterflies are no less fascinating.

Here we have presented one example of interspecific bilateral gynandromorphs that were bred from *Papilio glaucus* female and *P. canadensis* male parents (Fig. 2; see also Scriber *et al*. 1995). This interspecific pairing united genes in the resulting gynandromorph that have segregated into the two gender-differentiated halves with the heterogametic female [XY (=ZW)] possessing the Y-linked black (b^+) gene of dark morph females plus the X-linked suppressor (s^+; Scriber *et al*. 1996), resulting in the yellow (suppressed) tiger pattern for the female half (Fig. 2). This contrasts with the interspecific (*P. glaucus* × *P. alexiares*) gynandromorph with the dark melanic (b^+) gene and no suppressor (s^-, an enabler), where the female half is black (Fig 1).

The biochemical pathways (temporally and spatially regulated by enzymes, including dopa carboxylase) are apparently coordinated by multiple enzyme activities of the melanin (black) and papiliochrome (yellow) specific pathways. These enzyme regulations must be conducted simultaneously in the same gynandromorphs (Koch *et al*. 2000a), all of which is coordinated by the single major Y-linked factor (Scriber *et al*. 1996) on the female



FIGS. 9–11. **9.** A natural bilateral gynandromorph of *P. glaucus* from Randolph Co., North Carolina (photo from Loretta Lutman). **10.** A sexual mosaic (gynandromorph) with mostly male characters (except the blue coloration in the left hindwing) brood 2025, from a wild-collected yellow female, Clark Co., Georgia. **11.** A color/sexual mosaic (lab-reared from a female collected in Ohio, 1989 (a) dorsal, (b) ventral.



FIGS. 12–13. **12.** A gynandromorph or sexual mosaic, which appears to be more than 60% female (from brood #3622) from a pure *P. glaucus* lineage (in which the mother was the daughter of a dark morph female collected in Hocking Co., Ohio and mated to a male from Adams Co., Ohio). (a) dorsal, (b) ventral. **13.** A gynandromorph or sexual mosaic, which appears to be more than 75% female but with male claspers (from brood #3935) from a wild collected dark morph *P. glaucus* female (Scioto Co., Ohio). (a) dorsal, (b) ventral.

side of the individual; but these genes are either not expressed, or not present, on the male half of the individual. The activity of other sex-linked traits such as obligate diapause regulators would explain the direct development (non-diapause) of both halves of the *P. glaucus/P. alexiares* gynandromorph and the one year delay (diapause) of the *P. glaucus/P. canadensis* gynandromorph (with both the hemizygous female and the heterozygous male having the X-linked obligate diapause gene, od$^+$). Other sex-linked traits such as oviposition preferences could not be analyzed, but in the future researchers could examine the sex-linked diagnostic allozymes and mitochondrial DNA variation with regard to distribution in the two halves of such gynandromorphs.

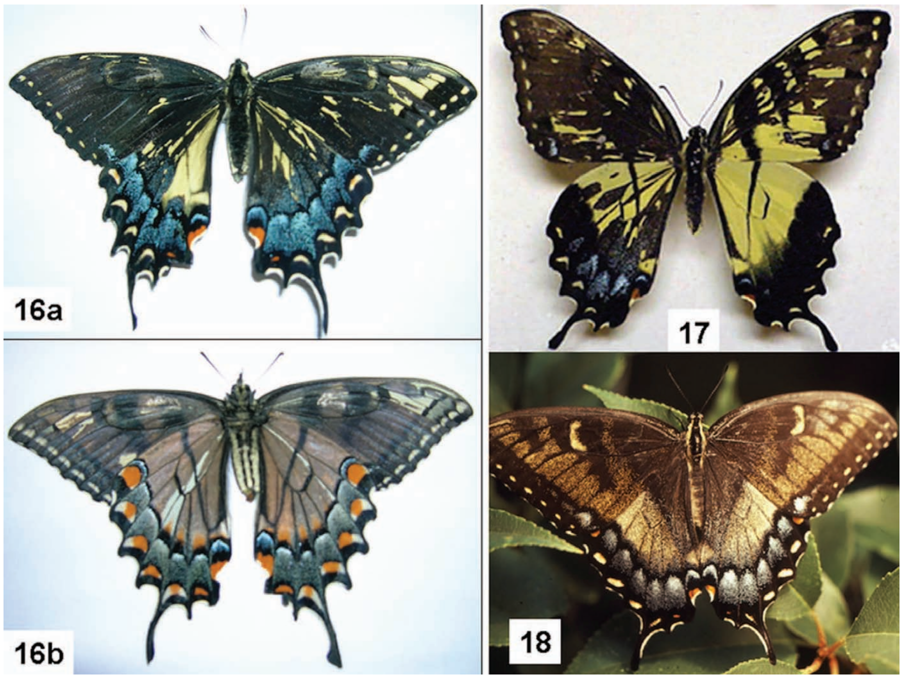
In melanic females, black melanin replaces the background yellow papiliochrome (Koch *et al*. 1998). The key enzyme involved is BAS (n –β- alanyl-dopamine-synthase), which shunts (suppresses) dopamine from the melanin pathway into the production of the yellow papiliochrome pigment (probably in response to dosage of the Z (=Y)-linked b$^+$ gene products; Scriber *et al*. 1996). The mixed mosaics we figure here appear to reflect the fact that melanism is both sex-specific and pattern-specific (cell autonomous), and therefore melanic specimens of *P. glaucus* are not just null mutants as in some ebony *Drosophila* strains (Koch *et al*. 2000b). The suppressor/enabler gene (s$^-$/s$^+$) that permits dark female expression of the b$^+$ melanin gene has been identified on the Z (=X) chromosome (Scriber *et al* 1996), and may involve tyrosine hydroxylase, or linked factors that are involved in production of dopa (common to both melanin and papiliochrome pathways) (Koch *et al*. 2000a, b). The late incorporation of tyrosine into melanin may also be involved in the incomplete penetrance (where yellow scales are brownish) suggesting that sex-linked control genes and their sex-linked modifiers exert their effects by differential interpretation of the patterning signal (e.g. the delay in development of melanic scales is female-specific) (Ffrench-Constant & Koch 2003).

The specific melanin (b$^+$) modifier genes (s$^+$/s$^-$) on the Z-chromosome, remain to be identified, but we have isolated the tyrosine hydroxylase gene from the Z-chromosome of *P. glaucus* (Putnam *et al*. 2007). The Z-chromosome also carries other genes of major significance in this species, including oviposition preferences (Scriber 1993; Mercader & Scriber 2007; Mercader *et al*. 2009), diapause regulation (Rockey *et al* 1987a, b), post-diapause emergence delays (Scriber *et al*. 2008), and allozymes (lactate dehydrogenase (Ldh), and phosphogluconate dehydrogenase (Pgd)), which appear to be species-diagnostic for *P. glaucus*, *P. canadensis*, and *P. appalachiensis* (Hagen *et al*. 1991; Scriber & Ording 2005; Scriber *et al*. 2008). The genetic incompatibilities of some recombinant hybrid genotypes appear to involve the Haldane effect in the heterogametic female pupae (Hagen & Scriber 1995). The delayed emergence of recombinant hybrid swarms (*P. appalachiensis*: Pavulaan & Wright 2002; Scriber & Ording 2005) may be controlled by the Z-linked obligate diapause gene (od$^+$; Scriber *et al*. 2008) or a closely linked post-diapause developmental rate controlling gene on this Z-chromosome, such as identified for the delayed mid-July flight of European corn borer (Dopman *et al*. 2005). The tpi allozyme locus appears to correspond closely with this factor in the corn borer, and we have determined that it is also Z-linked in *P. glaucus/P. canadensis* (Hagen & Scriber 1989; Scriber 1994).

Females of *P. glaucus* that are intermediate in their expression of melanism (with different degrees of "dusted" or "cinnamon" appearance; Fig 18) can be a result of rearing and/or pupal eclosion at higher temperatures (Ritland 1986). Such intermediate melanism may reflect variable penetrance, suggesting that the black melanin is superimposed over the yellow papiliochrome (Koch *et al*. 1998), which may appear brown in color (Ffrench-Constant & Koch 2003). However, we have also seen such intermediate melanism in hybrid daughters of dark females mated to *P. multicaudatus* (Fig 9), to *P. rutulus* (Scriber *et al*. 1995), and to *P. canadensis* (from Dane Co. WI, not figured here). Most *P. canadensis* males have X-linked suppressors (s$^+$) that completely prevent the expression of melanism in hybrid daughters of dark mothers (Scriber *et al*. 1996). Here we report the partial expression of melanism ("cinnamon" with striations) in daughters of a primary F-1 hybrid pairing between a dark *P. glaucus* mother mated with a male *P. canadensis* from central Michigan (in Isabella Co. on the cooler side of the historical hybrid zone; see below). These are not color mosaics as described earlier. These "cinnamon" intermediates of this Michigan *P. canadensis* father and dark *P. glaucus* mother, reared and emerged at 22° C, were also not due to high temperatures (28–30° C) as in Fig. 18 (see Ritland 1986).

This result suggests that the wild Isabella Co. male parent of this pairing (#20011) had at least one Z-linked enabler (s$^-$), which permitted expression of the Y-linked dark gene (b$^+$). The introgression of the X-linked enabler has also been documented in daughters of another Isabella Co. *P. canadensis* male mated to a dark *P. glaucus* mother (#20015; Scriber *et al*. in prep.) and

FIGS. 14–15. **14.** A gynandromorph or sexual mosaic which appears to be more than 50% female (from brood #4196) from a lab cross of a dark morph *P. glaucus* female (Champaign Co., Illinois) mated to a hybrid male (mother of which was the daughter of a dark morph *P. glaucus* female collected in Georgia and whose father was a *P. canadensis* collected in Lincoln Co., Wisconsin. (a) dorsal, (b) ventral. **15.** A female color mosaic (from brood #3770) from a dark morph *P. glaucus* female (the daughter of a wild dark morph *P. glaucus* female, #3104, collected in Athens Co., Georgia) mated to a wild *P. canadensis* male (collected in Tompkins Co., New York). (a) dorsal, (b) ventral.



FIGS. 16–18. **16.** A field-reared *P. glaucus* from Lancaster Co. PA 2004 (pupa from Wm. Houtz). **17.** A specimen from David and Marc Perlman (photo provided, pers. comm. to JMS). **18.** An intermediate "dusty" or "cinnamon" type reared from Dane Co., Wisconsin at high temperatures (28–30° C).

also in daughters of several males of the hybrid swarm late flight at the New York/Vermont border where no dark females (with the W-linked b^+ gene for melanism; Scriber *et al* 1996) have ever been collected (the nearest collection of a dark female is roughly 150 miles south; Scriber *et al*. in prep). The Z-linked enabler has also been detected in males captured from the hybrid swarm of late flight individuals in *P. appalachiensis* populations from West Virginia, but here, on the warmer side of the hybrid zone (Scriber *et al*. 2009b), the W-linked gene for melanism (b^+) does also occur as evidenced by recombinant hybrid-like dark females (Pavulaan & Wright 2004; Scriber & Ording 2005; Scriber *et al*. 2009a, b).

Individual species diagnostic traits moving northward across the historical hybrid zone include: 1) autosomal tulip tree detoxification (Scriber 1986, 2002; Scriber *et al*. 1999), 2) several wing color traits (Luebke *et al*. 1988), 3) some X-linked traits such as the allozyme allele Pgd-100 (Hagen & Scriber 1989; Hagen *et al*. 1991; Scriber *et al*. 2008), and 4) oviposition preferences (Scriber *et al*. 1991; Mercader & Scriber 2007; Mercader *et al*. 2009). However, other X-linked species diagnostic traits (including Ldh-100 allozyme alleles and bivoltine potential (od^-); Rockey *et al*. 1987) as well as the Y-linked dark gene (b^+) and certain mitochondrial DNA alleles have not moved northward across the historical hybrid zone (Stump *et al*. 2003; Scriber *et al*. 2008). The divergent selection pressures on different parts of the X-chromosome (and differential movement of these parts) in the genomes of both *P. glaucus* and *P. canadensis* may be historically old (Putnam *et al*. 2007). However, recent origins of interspecific introgression and recombination combined with strong divergent selection on different regions of the X-chromosome may have been catalyzed by recent climate warming, possibly leading to the Mountain Swallowtail (potential hybrid species), *P. appalachiensis* (Pavulaan & Wright 2002; Scriber & Ording 2005). We will continue to investigate the linkage relationships of the dark morph enabler gene (s-) with other parts of the X-chromosome and how it may be involved in the easily detected sexual color mosaics.

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