Gynandromorphism in *Automeris io* (Lepidoptera: Saturniidae)

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Gynandromorphs fascinate people for a number of reasons, one of which may be that these aberrations concern gender identity, something every human can relate to. The number of gynandromorphic Lepidoptera described in the literature continues to increase but still remains relatively low. For instance, when Scriber and Evans (1988) reviewed the relevant literature, bilateral gynandromorphs formed a list under a page-long, with only three species of Saturniidae included. There are certainly many additional gynandromorphic specimens out there, published or unpublished, and the silk moths are no exception (e.g., Pariser 1927, Majerus 1986, Eitschberger 1994). Nevertheless, finding one continues to be an occasion for excitement.

Scriber and Evans (1988) hypothesized that hybridization may be causing an increase in the frequency in gynandromorphism in the Tiger Swallowtail complex. This finds support in known interspecific lab crosses of other species. For instance, Standfuss, in his experiments on *Saturnia*, found that in primary hybrids, gynandromorphs were rare (2 in 4000), but in secondary hybrids, they were numerous. A backcross of *Saturnia pavonia/pyri* hybrid male with *S. pavonia* female produced 12 gynandromorphs out of 54 offspring (Cockayne 1915). In butterflies that have different wing patterns but hybridize readily, such as *Heliconius* or certain *Papilio*, a bilateral gynandromorph in a hybrid can be especially striking (e.g., Emmel & Boender 1990; Blanchard & Descimon 1988).

One of the possible causes of sexual mosaics in hybrids is considered to be divergence of sex-determining systems rather than loss of chromosomes or double fertilization of binucleate oocytes (the latter is suggested by Blanchard & Descimon 1988) as the primary cause of bilateral gynandromorphism. Most gynandromorphic individuals, however, are likely to be intersexes (males or females in all of their tissues, with features of the opposite sex expressed in some of them) (see Narita et al. 2010 for a review of various causes of gynandromorphism). It is this kind of phenomenon observed by Richard Goldschmidt in his experiments crossing gypsy moth races from Europe and Asia (e.g., Goldschmidt 1934), which helped him postulate theories that later led to the development of modern physiological genetics (e.g., Golubovsky 2010).

In addition to hybridization, gynandromorphism can result from a variety of mutagenic factors. The Chernobyl nuclear disaster, for example, may very possibly have instigated, a year later in southern Russia, the appearance of a population of lycaenids *Meleageria daphnis*, with a high frequency of sexual mosaics and bilateral gynandromorphs (Dantchenko et al. 1995). The fallout from the Fukushima reactor prompted a detailed study of mutations in local populations of the pale grass blue butterfly *Zizeeria maha*. The authors “obtained a small number of gynandromorphs in which one of the forelegs was morphologically male and the other female in the Fukushima F1 generation, the EMS-mutagenesis experiment, and the internal exposure experiment…” (Hiyama et al. 2013, p. 17). Jahner et al. (2015, p.7) who found six gynandromorphs among over 7,000 *Plebejus anna* and *P. melissa* butterflies collected within 10 years across the United States, note that all “were captured or reared in the 16 mo following the Fukushima nuclear accident and none have been captured since.” Hessel (1964), who in 1962 collected a bilateral gynandromorph of *Automeris io*, mentions discoveries of two other saturniid gynandromorphs the same year in the northeastern US. He suggests (p. 31) that “some fundamental environmental change” may be the cause of this localized triple occurrence of gynandromorphs. In October 1961, as the parents of these gynandromorphs were developing, the largest ever nuclear weapon was being tested above ground, not to mention the 200 or so other atmospheric nuclear tests that had been conducted by both the US and the USSR in the preceding years. While correlation does not always imply causation, this relationship, together with the internal exposure experiments by Hiyama et al. (2013), suggests that larval feeding on plants with low-level of radioactivity can be one of the possible causes of gynandromorphism.

To date, there have been few reports of *A. io* gynandromorphs in the literature. The recent photo of a specimen in the News (Menaker 2014) taken by an anonymous student over 30 years ago has symmetrical male antennae in an otherwise perfect bilateral gynandromorph. Such gynandromorphs normally have one male and one female antenna (e.g., Figs. 1A, 9A). Among sexual mosaics, even almost entirely male individuals of *A. io* have asymmetrical antennae that are of intermediate pectination length (between that of a male and female) (Fig. 9). According to Traut et al. (2007, p. 341) “why in one case purely female and purely male patches arise and in others even single cells show intermediate characters, is not known. An investigation on the molecular level with a focus on the expression of sex-determining cascade genes in intersexes has not yet been performed.” In any case, the asymmetry of the antennae is found not only in sexual mosaics and gynandromorphs of silk moths, where the sexual dimorphism of the antennae...
is obvious, but in other Lepidoptera as well. For instance, even the partial gynandromorph of a butterfly *Dismorphia spio* had asymmetrical antennae (Sourakov 2011). Hence Menaker’s report, which is not supported by a voucher or even a collector’s name, requires confirmation.

For several decades, Thomas Manley reared and crossed *Automeris io* from around the US. He created some remarkable broods of aberrant specimens and studied the genetics and biology of *A. io* in great detail (Manley 1990, 1993). By the time he reported having reared two gynandromorphic specimens (Manley 1971), he had raised over 10,000 *A. io* specimens. Manley illustrated these sexual mosaics, referring to them as specimens 18-69 and 19-69 (here, I refer to their origins as broods 18-69 and 19-70, according to their labels (Fig. 1C-F)). Manley obtained additional gynandromorphic specimens in three other broods during 1970-1973. Two of these broods, 10-70 and 13-70,
produced a total of three mosaics (Fig. 2), while brood 9-73 yielded numerous sexual mosaics (Figs. 3-7) and a bilateral gynandromorph (Fig. 1A-B). I am indebted to Larry Gall of the Peabody Museum at Yale University for allowing me to access Manley’s collection.

This proliferation of gynandromorphic specimens, as well as a number of other heritable wing pattern aberrations among Manley’s broods, coincided with the explosion of mutations in disjunct wild populations of *Drosophila melanogaster* (Ivanov & Golubovsky 1977). For instance, in 1973 a 100-fold rise of mutability was detected at one of the fruit flies’ loci (Golubovsky 1980; Berg 1982). According to Berg, who supports her conclusions by numerous references (e.g., Hook 1978), the mutability rise occurred that year among humans as well. Golubovsky (1980) suggests that male recombination factors (MR) induced by viruses may be responsible for the simultaneous rise in mutations within wild *Drosophila* populations. He remarks that MR also produce chromosome breakage during meiosis: “In some cases one or more chromosomes can be completely pulverised” (p. 147).

Fig. 2. Sexual mosaics of *Automeris io* from the collection of laboratory broods created by Thomas R. Manley (donated to the Peabody Museum, Yale University), broods 10-70 (A-D) and 13-70 (E-F).
Fig. 3. Sexual mosaics of *Automeris io* from the collection of laboratory broods created by Thomas R. Manley (donated to the Peabody Museum, Yale University), brood 9-73.
Fig. 4. Sexual mosaics of *Automeris io* from the collection of laboratory broods created by Thomas R. Manley (donated to the Peabody Museum, Yale University), brood 9-73.
Fig. 5. Sexual mosaics of Automeris io from the collection of laboratory broods created by Thomas R. Manley (donated to the Peabody Museum, Yale University), brood 9-73.
Fig. 6. Sexual mosaics of *Automeris io* from the collection of laboratory broods created by Thomas R. Manley (donated to the Peabody Museum, Yale University), brood 9-73.
Fig. 7. Sexual mosaics of *Automeris io* from the collection of laboratory broods created by Thomas R. Manley (donated to the Peabody Museum, Yale University), brood 9-73.
The most recent, to my knowledge, gynandromorphic specimen of *A. io* illustrated in Fig. 8, was reared by me and deposited in the collection of the McGuire Center for Lepidoptera and Biodiversity. It was part of a brood of ca. 60 sibling individuals. The line originated from 19 wild-collected eggs in September 2013 in Gainesville, Florida, where two subspecies, *A. io* and *A. io. lilith* co-occur and are able to interbreed (Sourakov 2014; pers. obs.). Considering that the eggs were collected in an isolated patch of woods and on a plant on which *A. io* larvae are unable to feed (*Crotalaria pallida*), they may have represented an already mutated stock. Two consecutive sib-sib crossings produced two cousin lines with numerous offspring, some of which exhibited aberrations that will be reported at a later date. The gynandromorphic specimen was large – forewing (FW) length = 46.3mm (the average female FW in this line = 40.8±3.2mm (N=30), with a maximum of 47.2mm; males are much smaller, ca. 30mm). The egg, from which this specimen was reared, was laid on 1 May 2014; larvae were fed *Prunus serotina*; pupation occurred on 14 August; emergence followed on 28 September 2014, hence representing a non-diapausing individual, but with somewhat delayed emergence (a non-diapausing pupa normally develops in 25-30 days).

On the wing surfaces, the male characteristics in this otherwise female specimen are expressed on the left FW dorsum and right FW ventrum. On the former, a male-colored patch occupies interspace between veins R and M₁, and also extends basally along the cell (Fig. 8A). On the ventral surface of the opposite wing, the male patch is almost symmetrical to the dorsal one, but is wider, extending along the costal margin between veins Sc and M₁ (Fig. 8B). The sexually dimorphic discal 1 (DI) wing element is present on the left FW in both the male and female state (Fig. 8C). Antennae are asymmetrical (Fig. 9D), with the left antenna more pectinate than normal. Head, legs, palpi and abdomen (Fig. 8D-F) are covered with a mix of female and male-colored hairs with female ones being predominant and the male hairs distributed asymmetrically. Genitalia appear to be normal for a female, and the abdomen, when dissected, revealed a normal (in appearance) egg mass.

The male/female patches of coloration in all sexual mosaics illustrated here are distributed so that the contrasting characters are manifested on opposing sides of the body, as is the case in most sexual mosaics, reflecting the bilateral nature of differentiation in larvae and pupae (Scriber & Hagen 1990). These specimens could have resulted from damage to one of the genes that control sex-determination. Something as radical as the loss of the W chromosome in one of the embryonic cells could have led to male tissue development, but it is more likely that the development of male tissues is due to an error during sex-specific splicing of a sex-determining cascade gene (e. g., of an *A. io*-equivalent of *Bmdsx* gene - the system described for *Bombyx mori*) (see Traut et al. 2007 for details). Perhaps the mutation that made the sex-determining system unstable in these specimens occurred already in the wild populations and was expressed phenotypically via repeated inbreeding. Alternatively, intersexes could also have resulted from infection in larvae, as in studies conducted by Narita et al. (2007). This would make it easier to explain why sexual mosaics, such as in Manley’s brood 9-73, are not only asymmetrical but also so variable, as infection would spread randomly in different tissues and could affect individuals to different degrees and at various stages of sex-determination. If this is the case, inbreeding could be just another factor that reduced immune resistance to the infecting agent.

In total, we are now up to seven reliable reports of *A. io* broods producing gynandromorphic specimens, six of which represent inbred lines, and only two of which had more than a single gynandromorphic offspring. The absolute majority of these aberrant specimens originated from Manley’s broods. The gynandromorphic aberrations, like the ones reported here, may help elucidate genetic controls of embryonic development of wing and body coloration in various Lepidoptera species. Additional reports of gynandromorphic specimens of *A. io* and other Lepidoptera, as well as details concerning their origin, would be of great interest.

### References


Fig. 8. A sexual mosaic of *Automeris io* from a brood, representing F2 generation within an inbred laboratory line. Reared at the McGuire Center, Florida, 2014. (A) Dorsum; (B) Ventrum; (C) Close up of the left forewing; (D-F) Head, thorax, and abdomen. Yellow scales and hairs represent male phenotype.
Fig. 9. Antennae of *Automeris io*: (A) bilateral gynandromorph; (B-J) mosaic gynandromorphs; (K) normal male; (L) normal female. Corresponding complete specimens are figured as follows (A)-Fig.1A; (B)-Fig.1E; (C)-Fig.6E; (D)-Fig.3G; (E)-Fig.7A; (F)-Fig.7E; (G)-Fig.4E; (H)-Fig.4G; (I)-Fig.5G; (J)-Fig.8A.


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The Mailbag . . .

James Scott has reopened the discussion of the “subspecies problem” that has been going on since the Devil was a little boy (“Do subspecies exist?”, NEWS, Summer 2015). I have no intention of contributing at length to the chronic logorrhea associated with this issue, but since I devote two class periods to it in the “Principles of Systematics” course I teach at U.C. Davis, I think I’ve thought it through enough that I can make a few points very succinctly. So here goes:

1. Scott illustrates perfectly why the subspecies category is problematic when he distinguishes between “good” and “bad” subspecies. His subspecies are “good”; other people’s are “bad.” There is no objective “scientific” criterion for telling one from the other. It’s all a matter of subjective judgment or personal taste.

2. During the heyday of the Neo-Darwinian Synthesis, it made perfectly good sense to treat subspecies as species-in-the-making. With the molecular-genetic tools now available to us, we have learned -- painfully -- that there is no predictable relationship between phenotypic differentiation and genomic differentiation. Dramatic phenotypic differences can reflect the action of a handful of genes under strong selection, against a background of genomic uniformity. Things that look alike can be quite different at the genomic level. Each case has to be studied on its own merits. Nearly all naming decisions are based on phenotype anyway. (My research group has a paper coming out momentarily in which we address these issues for the Sandhill Skipper, Polites sabuleti, one of the most subspecies-burdened taxa in the North American butterfly fauna. Want a copy? E-mail me and ask for a pdf.)

3. Given that there are no objective criteria for delimiting subspecies, if we must have them -- and they do have their uses! -- the “75% rule” proposed by the ornithologist Dean Amadon in 1949, while never objectively justified, is probably as good a rule of thumb as one can get. Of course, different people focus on different things; that’s why we have “splitters” and “lumpers” (and Scott, who is both). So your 75% may not match mine!

4. I’ve developed a package of readings for use in my course. Many readers of the NEWS are too young to remember the discussions of several decades ago, and most readers do not have unlimited journal access and so cannot easily track down and read the classic papers on the subject. If you want to delve into this literature, e-mail me and I’ll send you a batch of pdfs.

5. Of course, if you’re a religious cladist, all of this is nonsense. All terminal taxa are species. Period.

Peace and joy,

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