# Hybridization Studies of Genomic Compatibility and Phenotypic Expression in the Greater Fritillary Butterflies (Nymphalidae: Argynnini)

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# HYBRIDIZATION STUDIES OF GENOMIC COMPATIBILITY AND PHENOTYPIC EXPRESSION IN THE GREATER FRITILLARY BUTTERFLIES (NYMPHALIDAE: ARGYNNINI)

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**ABSTRACT.** The genetic compatibility of the greater fritillary butterflies was tested through hybrid crosses made between the North American genus *Speyeria* and the Eurasian genera *Argynnis* and *Mesoacidalia*. Numerous hybrid crosses made between *A. paphia* and *Speyeria* failed to produce any viable progeny, but a hybrid male was successfully produced between *M. aglaja* and *S. nokomis*. The genetic compatibility and divergence of *Speyeria* species was tested through hybrid crosses and back-crosses among the various species. All species of *Speyeria* appear to be inter-fertile in hybrid crosses with the exceptions of the two most divergent species, *S. idalia* and *S. diana*, that produce non-viable hybrid females and sterile hybrid males. As a consequence, it is postulated that inter-species gene flow through hybridization accidents in nature has been important in the past evolutionary history of this genus, and is partly responsible for the absence of consistent, non-overlapping diagnostic taxonomic characters among most species of the genus.

**Additional key words:** *Argynnis, Fabriciana, Mesoacidalia, Speyeria,* Mendelian traits

The greater fritillary butterflies belong to the tribe Argynnini, and consist of approximately 41 species that are widely distributed in temperate regions of Eurasia and North America, extending south to Mexico, Australia, India, and North Africa. Larvae feed almost exclusively on violets in the genus *Viola* (Violales: Violaceae). The adaptive radiation of these butterflies is entirely based upon this single larval foodplant. In the present paper, we report on the results of extensive inter-species hybridization studies with these butterflies, both within the genus *Speyeria* of North America and between *Speyeria* and the Eurasian genera *Argynnis* and *Mesoacidalia*.

## Taxonomy

The taxonomy of these butterflies is controversial regarding the delineation of both genera and species. Simonsen et al. (2006) have prepared the most recent cladistic analysis of this group based on a combination of both morphological and DNA sequence data that shows four major clades within the group. These clades may be classified into four genera or subgenera (Tuzov 2003) including *Argynnis*, *Fabriciana*, *Mesoacidalia*, and *Speyeria* based on a combination of wing pattern and genitalic characters. The first three genera are endemic to Eurasia and North Africa, while the latter genus is endemic to North America. These genera are defined as follows.

The genus *Fabriciana* is characterized by having black crescent-shaped or indented submarginal spots on the dorsal wings. On the ventral hindwing, the median spots are broad and square in shape, forming a somewhat continuous median band, and distinct dark postmedian spots are present. The valve of the male genitalia has a slender, finger-like process that is free above the valve. The uncus has a prominent dorsal tooth. This genus is comprised of 11 species as outlined by Tuzov (2003), with *F. kamala* (Moore) transitional in wing pattern towards *Argynnis*. *Fabriciana niobe* (Linnaeus) exhibits the most primitive characters in wing pattern and genitalic structure among the greater fritillaries that are shared with the lesser fritillaries of the genera *Issoria, Brenthis,* and *Boloria* (see cladogram in Simonsen et al. 2006). In general, species of *Fabriciana* are relatively conservative in these characters with the greatest divergence seen in such species as *F. argyrospilata* (Kotzsch) and *F. nerippe* (Felder & Felder).

The genus *Argynnis* is characterized by having round black submarginal spots on the dorsal wings. On the ventral hindwing, the median band is reduced to a thin median stripe, and dark postmedian spots are present. The male genitalia are similar to those of *Fabriciana*, but are highly variable among different species in ornamentation of the process and valve. The uncus is

also highly variable, and may have multiple dorsal teeth or no teeth. This genus is comprised of 10 species as outlined by Tuzov (2003), except that we recognize *A. castetsi* Oberthur on the Indian subcontinent as a species fully distinct from the more common and widespread *A. hyperbius* (Linnaeus). The species of *Argynnis* are all extremely divergent in wing color pattern and genitalic structure. On the basis of this divergence, the genus has been taxonomically divided into a number of small or monotypic genera in the past, including *Pandoriana, Damora, Nephargynnis, Argyronome, Childrena,* and *Argyreus* (Tuzov 2003). Despite this great divergence among the various species, they appear to be closely related in a phylogenetic sense as shown by the cladogram of Simonsen et al. (2006).

The genus *Mesoacidalia* has a wing pattern identical to that of *Fabriciana* on the dorsal wings. However on the ventral hindwing, the median band is broken into discrete, separate median spots, and dark postmedian spots are mostly absent except for faint shadows. In the male genitalia, the dorsal process of the valve is pressed down on top of the valve, instead of being free above the valve as in *Fabriciana* and *Argynnis*. In addition, the uncus lacks the dorsal tooth of *Fabriciana*. *Mesoacidalia* is comprised of 4 species in Eurasia including *M. vitatha* (Moore), *M. clara* (Blanchard), *M. aglaja* (Linnaeus), and *M. alexandra* (Menetries). Of these species, *M. vitatha* has thin or narrow black median bars on the forewings, and elongate or pointed median spots and sharp, triangular submarginal spots on the ventral hindwing. By contrast, *M. aglaja* has variable narrow to wide black median bars, short or rounded median spots, and rounded or flat submarginal spots. *Mesoacidalia clara* has a more extreme wing pattern similar to that of *M. vitatha*, while *M. alexandra* has a more extreme wing pattern similar to *M. aglaja*.

The genus *Speyeria* has a wing pattern virtually identical to that of *Mesoacidalia*. However, the process of the male valve is relocated along the side of the valve, and is broadly expanded into a club-like structure. This contrasts with the narrow, dorsal process of *Mesoacidalia*. Of these, *S. mormonia* (Boisduval) of the Rocky Mountains has a wing color pattern virtually identical to that of *M. vitatha*, and only differs in smaller size. However, *S. mormonia* has the *Speyeria*-type of male genitalia, while *M. vitatha* retains the more primitive *Mesoacidalia*-type of genitalia. As shown in the cladogram of Simonsen et al. (2006), *Mesoacidalia* and *Speyeria* are closely related sister genera. On this combined basis of genitalia and wing color pattern, the genus *Speyeria* in North America appears to be a monophyletic group that was probably derived from the Eurasian *M. vitatha*.

In sharp contrast to the species of *Argynnis*, most species of *Speyeria* are extremely conservative in both wing pattern and genitalic structure, and there are often no consistent diagnostic characters for separating the various species. Only two species exhibit highly divergent wing color patterns that allow for easy taxonomic identification, *S. idalia* and *S. diana*. All other species have overlapping characters of phenotype at least to some extent, and most exhibit a tremendous amount of geographic variation in wing color pattern (Hammond 1991). This is why Grey (1961) was not able to provide a diagnostic key for the identification of most species in the genus.

We presently recognize 16 reproductively isolated species in the genus *Speyeria* that maintain strongly distinct identities in sympatry, and exhibit strong ecological segregation in habitat and violet resource partitioning (Hammond 1981). These may be divided into two species groups based upon differences in the male and female genitalia as outlined by Grey (1961). The *callippe* group including *S. mormonia* has a slender claw-like uncus in the male and a single bursal chamber in the female. Other species of this group include *S. atlantis* (Edwards), *S. hollandi* (Chermock & Chermock), *S. sorocko* Scott, Kondla, & Spomer, *S. callippe* (Boisduval), *S. edwardsii* (Reakirt), *S. egleis* (Behr), *S. zerene* (Boisduval), *S. coronis* (Behr), *S. hydaspe* (Boisduval), and *S. adiaste* (Edwards). By contrast, the *cybele* group has a thick hook-like uncus in the male and a double bursal chamber in the female, although *S. nokomis* does retain the more primitive single bursal chamber. Species of this group include *S. aphrodite* (Fabricius), *S. cybele* (Fabricius), *S. nokomis* (Edwards), *S. idalia* (Drury), and *S. diana* (Cramer).

We should note that a major taxonomic disagreement presently exists regarding the delineation of species boundaries among taxa closely related to *S. atlantis*. Scott, Kondla, & Spomer (1998) separated the western subspecies of *S. atlantis* as a distinct species, *S. hesperis* (Edwards), an approach recently followed by Dunford (2009). However, Grey (1951) pointed out that a broad zone of clinal intergradation exists across Canada between the eastern *S. atlantis atlantis* and western members of the *hesperis* group, beginning in the Great Lakes region and extending westward across central Manitoba and northern Saskatchewan to the Rocky Mountains of Alberta, and then south to Colorado and New Mexico. Within these regions, both *S. hollandi* and *S. sorocko* maintain separate identities as distinct, reproductively isolated species. Based upon the reproductive relationships, we suggest that the western *hesperis* group must be treated as geographic subspecies of *S. atlantis* as originally classified by dos

Passos & Grey (1947), despite the superficial similarities of *S. hollandi* and *S. sorocko* to the eastern *S. a. atlantis.*

### Hybridization Studies

Over the past ten years, we have conducted extensive hybridization experiments with most of the species within the genus *Speyeria*. An earlier unpublished study attempted hybrid crosses between *Speyeria* and some of the European species, and we report on the results of this work in the present paper. Compatibility between the genomes of different taxa may be measured at six stages of divergence as discussed by Robinson (1971).

Stage 1 shows no genetic incompatibility with completely normal male and female hybrids.

Stage 2 shows some minor Haldane effects where the heterogametic sex (females) shows some minor abnormalities due to incompatible sex chromosomes and autosomes.

Stage 3 shows major Haldane effects where the hybrid females show major abnormalities or are completely non-viable, resulting in unequal sex ratios.

Stage 4 shows normal hybrid male development, but the males are completely sterile due to a failure of disparate chromosomes to pair up during the process of meiosis.

Stage 5 shows major abnormalities in male hybrids.

Stage 6 shows no hybrid viability at all. Either fertilized eggs die in early embryonic development or no fertilization takes place.

Reproductive isolation among the various species of *Speyeria* appears to result from species-specific sex pheromones in both males and females that are used during courtship and mating (personal observations). Interspecies courtship is frequently observed in the field, but this almost never results in hybrid matings. In the laboratory, we have by-passed this isolating mechanism by using the hand-pairing technique of Clarke (1952) for swallowtail butterflies. Male and female genitalia are mechanically joined together, forcing the butterflies to mate whether they are inclined to do so or not. In this way, every species of *Speyeria* can be hybridized with every other species no matter how divergent or distantly related they might be.

### Hybridization of *Speyeria* with European Genera.

An earlier study was designed to conduct hybridization experiments between *Speyeria* and the three European genera, and these results are discussed as follows. For this work, three European species were selected to represent each of these genera including *Fabriciana adippe* (Denis & Schiffermuller), *Argynnis paphia* (Linnaeus), and *Mesoacidalia aglaja* (Linnaeus). Unfortunately, the breeding stock of *F. adippe* was not particularly viable and no hybrid crosses could be made. However, an extensive effort was made to hybridize *A.*

*paphia* with various *Speyeria* species. A total of six hybrid crosses were successfully completed with *A. paphia*, but none of these crosses resulted in any viable progeny. This suggests a Stage 6 level of genomic divergence between *Argynnis* and *Speyeria* with virtually no genetic compatibility between these genera. This also corroborates the wide divergence between these genera shown in the cladistic study of Simonsen et al. (2006).

By contrast, two hybrid crosses were made between *Speyeria* and *M. aglaja*, and one of these made with *S. nokomis nitocris* (Edwards) from Arizona successfully produced a few viable male hybrids. These results indicate that some genetic compatibility still remains between the genomes of *Speyeria* and *Mesoacidalia*, and corroborates the cladistic study of Simonsen et al. (2006) that shows these genera as closely related sister genera. Unfortunately, it was not possible to backcross this hybrid to test for other measures of genetic compatibility such as fertility. The hybrid is shown in Figure 1 (1–2), and is intermediate in size and color pattern phenotype between the two parental species. On the ventral hindwing, it shows the green disc of the *M. aglaja* parent, and the wide yellow submarginal band and small silver median and submarginal spots of both parents. Thus in this hybrid, the green disc color of the *M. aglaja* parent was strongly dominant in phenotypic expression over the red-brown disc color of the *S. nokomis* parent.

### Hybridization of *Speyeria mormonia.*

As noted above, *S. mormonia* is the one North American species that is most similar to the Eurasian genus *Mesoacidalia*, and may be considered the most primitive for the genus *Speyeria*. We tested the genomic divergence of *S. mormonia* by crossing it with *S. aphrodite*. Specifically, we mated a male of *S. m. erinna* (Edwards) from the Oregon Cascades with a female of *S. a. alcestis* (Edwards) from Michigan. This hybridization combines a small species with a large species that are divergent in both male and female genitalic structure, although the adult color patterns are relatively similar.

Figure 1 (3-4) shows the phenotype of the hybrid male obtained from this cross. It was relatively small in size, only slightly larger than a normal *S. mormonia*, and it retained the narrow yellow submarginal band typical of *S. m. erinna*. Female hybrids showed a major Haldane effect and died as eggs in early embryonic development. However, male hybrids proved to be fertile in back-crosses, indicating a Stage 3 level of genomic divergence between *S. mormonia* and *S. aphrodite*.

The male hybrid was back-crossed to *S. a. alcestis*, and the genetic recombinants are illustrated in Figure 1

FIG. 1. Hybrid crosses of *Mesoacidalia* and *Speyeria* species. Scale-bar equals 20 mm. (**1**) *M. aglaja* ✕ *S. nokomis* male dorsal, (**2**) *M. aglaja* ✕ *S. nokomis* male ventral, (**3**) *S. mormonia erinna* ✕ *S. aphrodite alcestis* male dorsal, (**4**) *S. m. erinna* ✕ *S. a. alcestis* male ventral, (**5**) back-cross (*S. m. erinna* ✕ *S. a. alcestis*) ✕ *S. a. alcestis* male dorsal, (**6**) back-cross (*S. m. erinna* ✕ *S. a. alcestis*) ✕ *S. a. alcestis* male ventral, (**7**) back-cross (*S. m. erinna* ✕ *S. a. alcestis*) ✕ *S. a. alcestis* male ventral, (**8**) back-cross (*S. m. erinna* ✕ *S. a. alcestis*) ✕ *S. a. alcestis* female dorsal, (**9**) *S. aphrodite* ✕ *S. c. cybele* male dorsal, (**10**) *S. aphrodite* ✕ *S. c. cybele* male ventral, (**11**) *S. aphrodite* ✕ *S. c. cybele* female dorsal, (**12**) back-cross (*S. zerene picta* ✕ *S. c. cybele*) ✕ *S. c. cybele* male ventral, (**13**) *S. z. picta* ✕ *S. c. cybele* male dorsal, (**14**) *S. z. picta* ✕ *S. c. cybele* male ventral, (**15**) *S. z. picta* ✕ *S. c. cybele* female dorsal, (**16**) back-cross (*S. z. picta* ✕ *S. cybele*) ✕ *S. c. cybele* female ventral, (**17**) *S. edwardsii* ✕ *S. aphrodite ethne* male dorsal, (**18**) *S. edwardsii* ✕ *S. a. ethne* male ventral, (19) *S. edwardsii* ✕ *S. a. ethne* female dorsal, (20) back-cross (*S. z. picta* ✕ *S. c. cybele*) ✕ *S. c. cybele* female ventral.

FIG. 2. Hybrid crosses of *Speyeria* species. Scale-bar equals 20mm. (**1**) *S. zerene picta* ✕ *S. callippe semivirida* male dorsal, (**2**) *S. z. picta* ✕ *S. c. semivirida* male ventral, (**3**) *S. a. atlantis* ✕ *S. nokomis nitocris* male dorsal, (**4**) *S. a. atlantis* ✕ *S. n. nitocris* male ventral, (**5**) *S. c. cybele* ✕ *S. n. nitocris* male dorsal, (**6**) *S. c. cybele* ✕ *S. n. nitocris* male ventral, (**7**) *S. c. cybele* ✕ *S. n. nitocris* female dorsal, (**8**) *S. c. cybele* ✕ *S. n. nitocris* female ventral, (**9**) *S. c. cybele* ✕ *S. n. apacheana* male dorsal, (**10**) *S. c. cybele* ✕ *S. n. apacheana* male ventral, (**11**) *S. c. cybele* ✕ *S. n. apacheana* female dorsal, (**12**) *S. c. cybele* ✕ *S. n. apacheana* female ventral, (**13**) *S. n. nitocris* ✕ *S. idalia* male dorsal, (**14**) *S. n. nitocris* ✕ *S. idalia* male ventral, (**15**) *S. edwardsii* ✕ *S. idalia* male dorsal, (**16**) *S. edwardsii* ✕ *S. idalia* male ventral, (**17**) *S. a. atlantis* ✕ *S. idalia* male dorsal, (**18**) *S. a. atlantis* ✕ *S. idalia* male ventral, (**19**) *S. z. picta* ✕ *S. idalia* male dorsal, (**20**) *S. z. picta* ✕ *S. idalia* male ventral.

(5–8). Normal female hybrids were obtained in this back-cross (8), and butterflies segregated in phenotype between large individuals that exhibited red suffusion over the submarginal band on the ventral hindwing as in the parental *S. a. alcestis* (5–6), and small individuals that retained a narrow yellow submarginal band as in the parental *S. m. erinna* (7). Thus, the small size and yellow submarginal band of *S. mormonia* was somewhat dominant in phenotypic expression over the large size and red suffused submarginal band of *S. aphrodite*.

## Hybridization within the Central Radiation of *Speyeria*

Most species within the *callippe* species group have no constant diagnostic characters in either wing color pattern or genitalic structure as noted by Grey (1961), while *S. cybele* and *S. aphrodite* are slightly more divergent with genitalic differences in both males and females as discussed above. We conducted many hybrid crosses between various species, both within the *callippe* group and with both *S. cybele* and *S. aphrodite*. No adverse Haldane effects were observed in hybrid females, and all hybrids appeared to be fertile. Thus, the species of the *callippe* group plus *S. cybele* and *S. aphrodite* exhibited a Stage 1 level of genomic divergence among the various species with no indications of any genetic incompatibility.

These hybrid crosses provided an indication of the Mendelian phenotypic expression of the diagnostic characters that distinguish the various *Speyeria* taxa. A cross between a male of *S. zerene picta* (McDunnough) and a female of *S. callippe semivirida* (McDunnough) from the Washington Cascades was particularly informative as shown in Figure 2 (1–2). On the forewings, *S. callippe* usually has narrow black median bars, while *S. zerene* usually has wide median bars. On the ventral hindwing, *S. callippe* has a greenish brown disc, sharp pointed or elongate median spots, and sharp triangular submarginal spots. By contrast, *S. zerene* has a reddish brown disc, and rounded median and submarginal spots. In the hybrid, the wide median bars of *S. zerene* exhibited a strong dominant expression over the narrow median bars of *S. callippe*. However, the sharp, pointed median and submarginal spots of *S. callippe* showed a strong dominant expression over the rounded spots of *S. zerene*. Incomplete expression was exhibited by the disc colors, with some individuals having a reddish brown disc and others having green and red colors mixed together.

Figure 1 illustrates the phenotypic expression of these characters among various hybrid crosses. The primary cross between a male *S. zerene picta* from the Oregon Cascades with a female *S. c. cybele* from Michigan showed the dominance of the wide black median bars of *S. zerene* over the narrow median bars of *S. cybele* (Fig. 1, 13–15). On the ventral hindwing, the large median spots and narrow submarginal band of *S. zerene* were dominant over the small median spots and wide submarginal band of *S. cybele*. However, the pointed submarginal spots of *S. cybele* showed a dominant phenotypic expression over the rounded submarginal spots of *S. zerene*. When the primary hybrid was back-crossed to *S. cybele*, these characters showed recombination that indicated an independent segregation and inheritance of such traits. Thus, Figure 1 (12) shows a back-cross male with the small median spots and wide submarginal band of *S. cybele*, (16) shows a back-cross female with the large median spots and narrow submarginal band of *S. zerene*, and (20) shows a back-cross female with the large median spots of *S. zerene* combined with the wide submarginal band of *S. cybele*.

Figure 1 (9–11) shows a hybrid cross between *S. aphrodite* and *S. cybele* both from Michigan. The former species has little dark basal suffusion and thin veins in the male dorsal forewing, while the latter species has heavy dark basal suffusion and veins strongly thickened with androconial scales. On the ventral hindwing, *S. aphrodite* has large median spots combined with a narrow submarginal band, while *S. cybele* has small median spots and a wide submarginal band. The dorsal characters of the hybrid were intermediate between those of the parental species, indicating an incomplete dominance or polygenic inheritance of these traits. On the ventral hindwing of the hybrid, the larger median spots and narrow submarginal band of *S. aphrodite* were mostly dominant in expression over those of *S. cybele*. When the primary hybrid was back-crossed with *S. c. cybele*, these characters recombined independently in different individuals. This correlates with the dominance of similar *S. zerene* characters over those of *S. cybele* in the hybrid crosses previously discussed.

Figure 1 (17–19) shows a hybrid cross between *S. edwardsii* and *S. aphrodite ethne* (Hemming) both from Nebraska. The former species has a yellow-orange ground color on the dorsal wings, and a greenish brown disc and very large spots on the ventral hindwing. By contrast, the latter species has a reddish orange dorsal color, a red-brown disc, and smaller spots. In the hybrid, the reddish orange dorsal color of *S. aphrodite* and the very large spots of *S. edwardsii* showed a dominant expression. However, the expression of disc color was variable and intermediate, with some hybrid individuals having a pure dark brown disc and others showing a greenish brown disc (18). Similar results with mixed green and brown disc colors were seen in hybrid crosses

Fig. 3. Hybrid crosses of *Speyeria* species. Scale-bar equals 20 mm. (**1**) *S. c. cybele* ✗ *S. idalia* male dorsal, (**2**) *S. c. cybele* ✗ *S. idalia* male ventral, (**3**) *S. c. cybele* ✗ *S. idalia* female dorsal, (**4**) superhybrid (( *S. cybele pugetensis* ✗ *S. c. charlottii*) ✗ *S. nokomis nitocris*) ✗ *S. idalia* male ventral, (**5**) *S. idalia* ✗ *S. c. cybele* male dorsal, (**6**) *S. idalia* ✗ *S. c. cybele* male ventral, (**7**) *S. diana* ✗ *S. idalia* male dorsal, (**8**) *S. diana* ✗ *S. idalia* male ventral, (**9**) *S. diana* ✗ *S. n. nitocris* male dorsal, (**10**) *S. diana* ✗ *S. n. nitocris* male ventral, (**11**) *S. diana* ✗ *S. n. apacheana* male dorsal, (**12**) *S. diana* ✗ *S. n. apacheana* male ventral, (**13**) *S. c. cybele* ✗ *S. diana* male dorsal, (**14**) *S. c. cybele* ✗ *S. diana* male ventral, (**15**) *S. aphrodite* ✗ *S. diana* male dorsal, (**16**) *S. aphrodite* ✗ *S. diana* male ventral, (**17**) *S. a. atlantis* ✗ *S. diana* male dorsal, (**18**) *S. a. atlantis* ✗ *S. diana* male ventral, (**19**) *S. z. picta* ✗ *S. diana* male dorsal, (**20**) *S. z. picta* ✗ *S. diana* male ventral.

between *S. callippe* and *S. zerene*, and between *S. coronis* and *S. zerene*. This suggests an incomplete dominance in the expression of green and brown disc colors in *Speyeria*. Pure green and brown colors appear to represent homozygous expressions, while greenish brown color is probably a heterozygous expression. By contrast, the green disc color of *Mesoacidalia aglaja* was strongly dominant over the red-brown color of *S. nokomis nitocris* as previously discussed.

We should also mention the hybrid expression of sexual dimorphism within *S. cybele*. The eastern *S. c. cybele* and the southern Colorado *S. c. carpenterii* (Edwards) both lack sexual dimorphism in having orange males and females in dorsal colors. However, subspecies of the western *leto* complex including both *S. c. pugetensis* Chermock & Frechin from the Pacific Northwest and *S. c. charlottii* (Barnes) from northwestern Colorado show a very strong sexual dimorphism in which the females have a pale yellow dorsal ground color. A hybrid cross between *S. c. pugetensis* from Oregon and *S. c. cybele* from Michigan produced intermediate yellow-orange females. A back-cross of this hybrid to *S. c. cybele* produced a wide range of variation from pure orange to yellow-orange female phenotypes. These laboratory results are closely similar to the natural phenotypic variation in female coloration seen in populations of *S. cybele* from western Colorado and eastern Utah that are intermediate between *S. c. carpenterii* and *S. c. charlottii* in a broad clinal blend zone.

### Hybridization of *Speyeria nokomis*

*Speyeria nokomis* is slightly more divergent in wing pattern and large size compared to the various species in the central radiation of *Speyeria* discussed above. Figure 2 (3–12) shows the results of hybrid crosses made between *S. nokomis*, *S. cybele*, and *S. a. atlantis*. Crosses between *S. nokomis* and both *S. cybele* and *S. aphrodite* showed a Stage 2 level of genomic divergence. Minor Haldane effects were evident in that female hybrids were somewhat smaller than normal. Male hybrids were fertile. However, crosses between *S. nokomis* and both *S. atlantis* and *S. adiaste* showed much stronger Haldane effects where few or no female hybrids survived. Thus, a Stage 3 level of genomic divergence appears to exist between *S. nokomis* and members of the *callippe* species group.

A hybrid cross between *S. n. nitocris* (Edwards) from Arizona and *S. aphrodite* from Michigan produced similar results to those seen in the *aphrodite* × *cybele* cross shown in Figure 1 (9–11), except the dorsal dark basal suffusion of the hybrid male was greatly reduced and the forewing veins were thin without androconial scales as in *S. aphrodite*. On the ventral hindwing, the

larger median spots and narrow submarginal band of *S. aphrodite* were dominant over the small spots and wide submarginal band of *S. nokomis* just as in the *aphrodite* × *cybele* cross. A hybrid cross between *S. n. nitocris* and *S. a. atlantis* from Minnesota produced almost identical results as shown in Figure 2 (3–4), except the veins of the male dorsal forewing were thickened with androconial scales as in the *S. atlantis* parent.

In addition, *S. nokomis* exhibits a strong sexual dimorphism similar to that in the *leto* subspecies group of *S. cybele* in which the female dorsal ground color is yellow instead of orange. As with the hybrid cross between *S. c. cybele* and *S. c. pugetensis*, female hybrids of *S. nokomis* crossed with either *S. aphrodite* or *S. c. cybele* showed an intermediate yellow-orange ground color as shown in Figure 2 (7, 11).

Two subspecies of *S. nokomis* differ in the color of the disc on the ventral hindwing. In Arizona, males of *S. n. nitocris* have a dark reddish brown disc, while females have a reddish black disc. In Nevada and California, males of *S. n. apacheana* (Skinner) have a pure yellow disc and females have a green disc. The hybrid between these subspecies was intermediate with a light reddish brown disc in the male and a reddish green disc in the female. A hybrid cross between *S. n. nitocris* and *S. c. cybele* produced a dark reddish brown disc in both the male and female as shown in Figure 2 (6, 8), while a hybrid cross between *S. n. apacheana* and *S. c. cybele* produced a light reddish brown disc in the male and a dark greenish brown disc in the female as shown in Figure 2 (10, 12). The dorsal forewing veins of the hybrid males were thickened with androconial scales as in *S. cybele* as shown in Figure 2 (5, 9).

A hybrid cross between *S. adiaste* from the Coast Range of California and *S. n. nitocris* from Arizona combined a small butterfly with a pure yellow disc and unsilvered spots on the ventral hindwing and a large butterfly with a dark reddish brown disc and silver spots. The hybrid was intermediate in size and disc color between the two parental species, again showing the polygenic inheritance of size and disc color. As with the *aphrodite* and *atlantis* hybrid crosses described above, the large median spots and narrow submarginal band of *S. adiaste* were completely dominant over the small spots and wide band of *S. nokomis*. In addition, the unsilvered spots of the *S. adiaste* parent were completely dominant over the silver spots of the *S. nokomis* parent. As a result, the hybrid more closely resembled *S. adiaste* than *S. nokomis*. In populations of *Speyeria* that are polymorphic for both silver and unsilvered spots, it is not unusual to see intermediate phenotypes with partially silvered or unsilvered spots. These may represent heterozygotes with modifier genes

that allow partial expression or incomplete dominance of these traits, but this was not seen in the *adiaste* ✕ *nokomis* hybrid.

### Hybridization of *Speyeria idalia*

Multiple hybrid crosses were made between *S. idalia* from Nebraska and many other species of *Speyeria*. This species and *S. diana* are both highly divergent in wing color pattern from all other species in the genus, and show completely unique diagnostic characters not found in any other species. For *S. idalia*, these characters include (1.) a black dorsal hindwing with white median spots and white submarginal spots in the female, (2.) a black or reddish black disc on the ventral hindwing with solid black suffusion over a narrow submarginal band, and (3.) very large pointed or elongate median spots and pointed submarginal spots on the ventral hindwing.

In the various hybrid crosses with *S. idalia*, major Haldane effects occurred where hybrid females were severely stunted. They usually died in the pupal stage or were unable to expand wings upon eclosion, and were rarely able to develop into a deformed adult (Fig. 3-3). In addition, male hybrids appeared to be completely sterile, and we were never able to produce a hybrid back-cross with any other species. Thus, *S. idalia* exhibits a Stage 4 level of genomic divergence from other species.

Figure 2 shows the results of hybrid crosses made with *S. nokomis nitocris* from Arizona (13-14), *S. edwardsii* from Nebraska (15-16), *S. a. atlantis* from Minnesota (17-18), and *S. zerene picta* from Oregon (19-20). Likewise, Figure 3 (1-3) shows a hybrid cross with a male *S. c. cybele* from South Dakota. Figure 3 (5–6) shows the reciprocal cross using a male *S. idalia* and a female *S. c. cybele* to check for any sex-linked or sex-limited traits. None were found. A hybrid cross with *S. coronis* from Oregon produced similar results to those shown with *S. zerene*. Figure 3 (4) shows a super-hybrid cross where the primary *S. cybele pugetensis* ✕ *S. c. charlottii* hybrid was crossed with *S. nokomis nitocris*, and then this secondary hybrid was crossed with *S. idalia* to produce a tertiary hybrid.

In all of these various hybrids, the black dorsal hindwing and white spots of *S. idalia* were completely recessive in phenotypic expression to the normal dorsal orange colors of other *Speyeria* species. On the ventral hindwing, the sharp, pointed median and submarginal spots were strongly dominant over the rounded spots of such species as *S. zerene*, *S. coronis*, *S. cybele*, and *S. nokomis*. Likewise, the narrow submarginal band covered with black to reddish suffusion of *S. idalia* was completely dominant over the wide yellow submarginal band of *S. cybele* and *S. nokomis*, although a few of the

*cybele* hybrids showed a slight trace of the yellow band. However, the size of the median spots in hybrids was intermediate between the large spots of *S. idalia* and the small spots of *S. cybele* and *S. nokomis*. Also, the hybrid ground color of the disc on the ventral hindwing was usually a dark reddish brown as seen in the *zerene*, *atlantis*, and *cybele* crosses, but ranged between bright red-brown in the *nokomis* cross and pure black in the *edwardsii* cross.

Hybrids did show some unique phenotypic expressions depending on the parental species crossed with *S. idalia*. The cross with *S. nokomis* shown in Figure 2 (13–14) produced gigantic males with exceptional hybrid vigor, but they were often deformed and had difficulty with eclosion. This suggests a Stage 4 to Stage 5 level of genomic divergence between these two species. In the *zerene* ✕ *idalia* cross (19–20), the wide black median bars of the forewing in *S. zerene* were strongly dominant over the narrow bars of *S. idalia*, duplicating similar results in other hybrid crosses made with *S. zerene*. In the *edwardsii* ✕ *idalia* cross (15–16), the pale yellow-orange dorsal ground color of *S. edwardsii* was strangely dominant over the dark orange color of *S. idalia* on the forewing, which was the reverse of the *edwardsii* ✕ *aphrodite* cross shown in Figure 1 (17–19). However, the greenish brown color of the disc on the ventral hindwing of *S. edwardsii* was strongly suppressed by the black color of *S. idalia* in the hybrid.

### Hybridization of *Speyeria diana*

Multiple hybrid crosses were also made between *S. diana* from Virginia and many other species. Unique diagnostic characters of *S. diana* include (1.) very large wing size, (2.) intense solid black basal suffusion that extends to the median bars of the dorsal wings, (3.) extreme suppression of the spots on the ventral hindwing, and (4.) an extreme sexual dimorphism with orange-black males and blue-black females.

Major Haldane effects were evident in these hybrid crosses where females died as eggs in early embryonic development and never produced viable larvae. Hybrid males appeared to be completely sterile and no hybrid back-crosses were possible. Thus, *S. diana* exhibits a Stage 4 level of genomic divergence from most other species.

Figure 3 shows the results of hybrid crosses made with *S. idalia* from Nebraska (7-8), *S. nokomis nitocris* from Arizona (9-10), *S. n. apacheana* from Nevada (11–12), *S. cybele* from Michigan (13-14), *S. aphrodite* from Michigan (15-16), *S. atlantis atlantis* from New York (17-18), and *S. zerene picta* from Washington (19–20). A cross made with *S. coronis* from Oregon produced similar results to those shown with *S. zerene*.

The expression of dark black basal suffusion on the dorsal wings of hybrids was intermediate between that of *S. diana* and the various parental species. Thus, hybrids made with *S. n. nitocris*, *S. cybele*, and *S. atlantis* still showed very intense, dark suffusion. However, *S. n. apacheana* and *S. aphrodite* both have greatly reduced suffusion in the male, and their hybrids made with *S. diana* showed greatly reduced suffusion as well. Therefore, the intense solid black basal suffusion of *S. diana* appears to be the result of polygenic expression that has become fixated at one extreme end of a continuum.

On the ventral hindwing of hybrids, the suppression of the silver basal and submarginal spots was less extreme and intermediate compared with the parental *S. diana*. However, the suppression of the silver median spots in hybrids was nearly as great as in *S. diana* for virtually all crosses, even the cross made with *S. idalia*. This unique character of *S. diana* shows a strong dominant phenotypic expression over the normal median spots of other *Speyeria* species. In wing size, hybrids were usually intermediate between *S. diana* and the respective parental species, indicating a polygenic expression for size. We could not assess the inheritance of blue and orange colors in the hybrid females because they were completely non-viable.

The hybrid cross between *S. idalia* and *S. diana* exhibited major abnormalities in the males. Larvae showed a major defect in feeding behavior, and chewed randomly on leaf surfaces instead of feeding efficiently by chewing at the leaf margins. As a result, larvae grew very slowly and were quite stunted at pupation. Most individuals died as pupae or could not expand wings upon eclosion. Although several hundred larvae were reared, only a single individual was able to expand its wings as shown in Figure 3 (7–8). These two species display a Stage 5 level of genomic compatibility and divergence as a consequence, which is concordant with their extreme morphological divergence.

**Phenotypic Expression of Larval Color Patterns in Hybrid *Speyeria***

Most species of *Speyeria* have a larval color pattern that is mottled gray, black, brown, or yellow with two parallel mid-dorsal gray, yellow, or cream stripes. Such species include *S. mormonia*, *S. hollandi*, *S. sorocko*, *S. callippe*, *S. edwardsii*, *S. egleis*, *S. zerene*, *S. coronis*, *S. nokomis*, and *S. idalia*. Other species have pure black larvae that lack the pale body mottling and the pale mid-dorsal stripes. These include *S. hydaspe*, *S. cybele*, and *S. diana*. Still other species are polymorphic in larval color pattern with either pale striped morphs or pure black morphs including *S. atlantis*, *S. adiaste*, and *S. aphrodite*. These polymorphisms represent geographic

variations to a considerable extent in these species.

When we hybridized a pure black larval species such as *S. cybele* with a yellow-striped larval species such as *S. idalia*, an intermediate dusky striped and mottled phenotype was produced in the hybrid. Thus, hybrids display an incomplete dominance of larval color pattern expression in the heterozygous condition. Similar results were produced when *S. cybele* or black larval forms of *S. aphrodite* were crossed with various pale striped larval forms such as *S. nokomis*, *S. zerene*, *S. coronis*, and *S. edwardsii*. These intermediate dusky striped morphs are common in the wild within polymorphic populations of such species as *S. atlantis* and *S. aphrodite*.

### DISCUSSION

In general, there appears to be a close correlation between divergence in wing color pattern and genomic compatibility in the greater fritillary butterflies. Thus, the wing patterns of *Speyeria* and *Argynnis* are highly divergent, and there appears to be virtually no genetic compatibility between these genera. By contrast, *Speyeria* and *Mesoacidalia* both share the same basic wing color pattern, and still retain some genetic compatibility as evidenced by the production of viable hybrids. These results corroborate the results from the cladistic study of the fritillary butterflies by Simonsen et al. (2006). Likewise within *Speyeria*, all of the species within the central radiation of the genus are extremely conservative in sharing the same basic wing color pattern, and exhibit virtually no genetic incompatibility with fully normal, fertile hybrid males and females. These include the various species of the *callippe* group plus *S. aphrodite* and *S. cybele*.

In sharp contrast, both *S. idalia* and *S. diana* are extremely divergent in wing color pattern, both from each other and all of the other species as well. Our hybridization studies show that these two species of *Speyeria* have experienced a large amount of genetic divergence, and produce non-viable hybrid females and infertile hybrid males when crossed with all other species of the genus. Indeed, the divergence between *S. idalia* and *S. diana* is so great that even the hybrid males are only weakly viable. Two other species show minor genetic incompatibility with other species resulting in adverse Haldane effects in hybrid females, but hybrid males are fertile. These are *S. mormonia* and *S. nokomis*.

Although the pheromone mating system is probably at least 99% effective in promoting reproductive isolation among *Speyeria* species, hybridization accidents still occur at a low frequency in nature. For example, Scott (1981) observed a natural hybrid between *S. nokomis* and *S. cybele* in California. The

hybrid cross between *S. cybele* and *S. idalia* shown in Figure 3 (1–3) is not an artificial laboratory cross, but occurred naturally along the White River in Mellette Co. South Dakota. In this area, *S. cybele* occupies riparian forest habitats along the river, while *S. idalia* occupies the adjacent upland prairie. We collected the parental *S. idalia* female for breeding stock, but all of her progeny proved to be hybrids, showing that a male *S. cybele* had accidentally mated with the female in the wild.

Hybrid crosses that result in sterility as with *S. idalia* and *S. diana* are clearly an evolutionary dead end. However, we have found that most other interspecies hybrid crosses in *Speyeria* are fertile. Even if accidental hybridization in nature is a very rare event, it still provides a conduit for inter-species gene flow. As previously discussed, there are no constant diagnostic characters that consistently distinguish the various species of the *callippe* group due to overlapping character traits. In particular, *S. atlantis, S. hydaspe, S. callippe, S. egleis, S. zerene*, and *S. coronis* broadly overlap in characters across many geographic regions, which may result from low rates of hybridization taking place constantly through time. An earlier study of allozymes (Brittnacher et al. 1978) and several unpublished studies of the COI-COII sequence of mitochondrial DNA show similar overlapping genetic polymorphisms both within and between species of *Speyeria* at the molecular level. Proshek & Houghton (2012) have recently made similar observations with the COI sequence in the genus *Phyciodes* (Nymphalidae). Low rates of hybridization in nature would not be expected to affect the fundamental reproductive isolating mechanisms among the various species or to impact their fundamental identities as distinct biological species.

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