A Discovery of the Heritability of Female Mosaic Color-Morphism in the Eastern Tiger Swallowtail Butterfly *Pterourus (Papilio) glaucus* (C. Linnaeus 1758) (Lepidoptera: Papilionidae)

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Abstract - This paper is the second of two papers documenting continuing experimentation on the North American Swallowtail butterfly *Pterourus (Papilio) glaucus* (C. Linnaeus 1758). The first paper presented the results of coldshock experiments conducted on *P. glaucus* over multiple generations, and identified many previously unknown coldshock-induced aberrant wing patterns. During the course of these coldshock experiments, several mosaic color-morph females were randomly produced. This second paper details breeding experiments conducted on descendents of these ancestrally coldshocked lineage mosaic color-morph females over the course of six generations. In this experiment, it was discovered that this type of mosaicism is not only heritable, but that it can also occur in conjunction with the various types of coldshock aberrations that were produced in the first experiment in this series. In addition to the female mosaic color-morphs bred during the course of this experiment, a heritable mosaic orange aberration occurred in several of the male individuals. Although it is presumed that this orange mosaic aberration is unrelated to the female mosaic color-morph aberrations, as a possible consequence of inbreeding, the male orange mosaic aberration was produced in some female mosaic color-morph individuals resulting in "mosaic-within-a-mosaic" aberrations. A discussion and limited analysis of each of the observed mosaic aberrations is presented here, and an attempt is made to categorize some of the mosaic patterns obtained. Finally, a group of female individuals that exhibit both coldshock and mosaic color-morph aberrations is presented.

Keywords: Mosaic Color-Morph, Gynandromorph, Color Reversion, Color Replacement, Body Spot Mosaic, Streak Mosaic, Marbled Mosaic, Patch Mosaic, Brush Stroke Mosaic, Morph-Identity, Mosaic-Within-A-Mosaic Aberration

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INTRODUCTION

Pterourus (Papilio) glaucus (C. Linnaeus 1758) is the most widely distributed and well recognized of the North American Swallowtail butterflies. Either *P. glaucus* or one of its close relatives can be seen from Mexico to Canada and from coast to coast. As a result, it tends to be overlooked as the beautiful and remarkable butterfly that it truly is. The most unique attribute of *P. glaucus* is the evolution of its dimorphic female. The presumed ancestral yellow morph female is yellow like the male, and the more recently evolved melanic dark morph female is dark brown, sometimes approaching black. Some have even called the yellow morph of *P. glaucus* the "wild type" and the dark morph the "mutant type" (Koch, et. al. 1998). Both female morphs have the identical underlying black tiger stripes and border pattern, and the identical blue, orange, and cream-colored markings. Only the background color of the wings has the option of being either yellow or dark brown.

The two female morphs of *P. glaucus* provide a very flexible canvas upon which aberrant wing patterns can occur. Many unique and distinctive aberrations were produced during the course of our previous coldshock experiments. One such category of aberrant patterns resulted from the expansion or reduction of various sectors of the customary color template, which in some individuals yielded drastically different looking wing patterns. A second category of aberrations resulted from color substitutions, where alternate colors from the *P. glaucus* color palette replaced the genetically intended colors through processes that we have described as either Color Reversion or Color Replacement. A third category of aberrant wing patterns resulted from the coldshock-induced incorrect *blending* of the dark and yellow morphs in various ways that deviated from the customary genetically intended color pattern.

This paper presents the results of continuing experimentation on *P. glaucus*. Our first paper in this series investigated how *P. glaucus* reacts to coldshock, and documented the aforementioned wing pigmentation aberrations that resulted during the course of these experiments (Perlman, D. L. and Perlman, M. P. 2019a). This second paper is an outgrowth of the previous coldshock experiments, and commenced with the successful breeding of a randomly produced aberrant mosaic color-morph female from those experiments. Eventually this new mosaic color-morph breeding experiment resulted in the creation of a sustainable lineage of specimens that exhibited varying degrees of multigenerational hereditary mosaic color-morph aberrations.

This current paper includes numerous references to the first paper in this series. The first paper encompassed two consecutive identical coldshock experiments that were conducted on the descendents of two different populations of *P. glaucus*. For ease of readability, the first experiment in our first paper will be referred to as **PCE1** (Perlman Coldshock Experiment 1), conducted on the descendents of a dark morph female from far southern Illinois, U.S.A. The second experiment in our first paper will be referred to as **PCE2** (Perlman Coldshock Experiment 2), conducted on descendents of four dark morph females from northern Illinois, U.S.A. This current experiment and paper will be referred to as **PME1** (Perlman Mosaic Experiment 1), conducted on the descendents of a randomly produced mosaic color-morph female from the **PCE2** experiment.

MATERIALS AND METHODS

During the course of our predecessor PCE2 coldshock experiment, a total of nine prominently mosaic female specimens were produced (five of which are shown in Supplementary Materials Figs. 13 and 17). These mosaic females occurred even though none of the original mothers or intermediate parents showed the slightest outward indications of mosaicism. In addition to these prominently mosaic individuals, many more individuals were produced that had either tiny yellow body spots, or poorly saturated “smudgy” yellow patches on the wings. Despite conventional wisdom dictating that these were probably spontaneous random genetic anomalies, it seemed to us that this was more than a fortunate coincidence. We theorized that within the PCE2 gene pool, there existed a genetic tendency to create mosaics. It was decided to attempt breeding a mosaic specimen if the opportunity presented itself again. On August 8, 2013 a tenth prominent 1-Wing mosaic female eclosed, unfortunately also having a severe wing eclosion deformity (Fig. 1). This specimen became our original mother for this experiment that we now call PME1. (Subsequently, our coldshock experiments in PCE2 were discontinued so that we could devote all our efforts to this mosaic experiment. However, it is important to note that many of the coldshock-induced aberrations that were described in PCE2 had become heritable, and still randomly appeared throughout the course of PME1, sometimes in conjunction with mosaicism.)

Fig. 1 Original Mother of the PME1 Experiment
This is a photograph of the original mother of the PME1 experiment. It is an accidental byproduct of our predecessor PCE2 experiment. It is a 1-Wing mosaic displaying a Streak type mosaic pattern on the left hind wing. No other mosaic markings were present. Notice that the forewing also displays the type of yellow dusting that is typical of the minor coldshock yellowing that we produced in our PCE1 coldshock experiment. This female is a PCE2 F3 individual whose ancestors were coldshocked for three generations. This female was paired with a sibling male from the same coldshocked lineage. It is interesting to note that many of its F1 descendents in PME1 were radically more mosaic than this original mother, as can be seen in the photos included in the Supplementary Materials section of this paper.

The mother of our PME1 experiment was a PCE2 third generation female whose predecessors had all been coldshocked, with an overwintering diapause between the first and second generations. Its August 8th eclosion date allowed us just enough time to obtain chrysalides before winter. This female was hand-paired with a normal looking sibling male that had also sustained three generations of coldshock. The ova were obtained on fresh *Prunus serotina* clippings, and with relatively high productivity, we eventually obtained a total of approximately 100 chrysalides that comprised our new F1 generation. No further coldshocks were administered to this lineage. All were gradually chilled on the third day after chrysalis formation, to a relatively constant overwintering temperature of 35° to 38° F. (2° to 4° C.). The chrysalides were sprayed with water every two weeks throughout the winter, not allowing them to thaw, but also never allowing the refrigeration temperature to drop to freezing. The chrysalides were removed from diapause starting in early May of 2014.

In late May of 2014, our first group of F1 chrysalides from our mosaic mother started eclosing. The male offspring showed no mosaicism at all. However, as many as 40% of the female offspring were mosaic individuals, varying from tiny spots to major sized sections on multiple wings. Our presumption of mosaic heritability was proving to be correct. While the most severely affected individuals were retained for our collection, our intention was to continue breeding the more minimally affected females for as many future generations as possible. Breeding continued throughout 2014, 2015, 2016, and 2017, primarily by inbreeding mosaic females with sibling males.

In 2015 we experienced a significant decrease in productivity, and a simultaneous increase in eclosion deformities. In an effort to continue the project, a number of mosaic females were paired with wild-collected males. Fertility rates improved, although there appeared to be a noticeable decrease in the percentage of mosaics being produced in the wild-bred pairings. Having successfully restored some viability to our mosaic lineage, we returned to inbreeding in the subsequent generations. We ultimately concluded that not only is this type of mosaic color-morphism hereditary, but as intuitively expected, it is also a female-borne trait. In addition, although we have no conclusive statistics, it also appeared to us that pairings initiated with more severe mosaic females tended to produce an increase in the severity of mosaicism and an increase in the number of mosaic individuals produced, along a pathway that we call an Aberrant Trajectory. As of the date of this publication, thirteen successive generations of mosaic color-morph *P. glaucus* individuals have been bred, with an average frequency rate of 20% to 30% of the female individuals exhibiting minor to severe mosaic traits, possibly as high as 40% affected if we include those individuals exhibiting only minor body spots.

RESULTS

PME1 Experiment Statistics

This paper is based on six full generations of mosaic breeding experiments. Although the breeding of this lineage still continued, only these first six generations have been included in this report. The presentation which continues throughout the remainder of this paper is based on the following statistics:

1. Approximately 300 mosaic female individuals were produced during the course of PME1, which includes six complete generations of intentionally bred mosaic females.
2. Of the approximately 300 documented individuals referenced above, approximately 170 individuals are retained in our reference collection, and approximately 50 individuals were used as breeding stock and are photographed for reference. Most of the females that were used as breeding stock were minor 1-Wing mosaic individuals. The remaining approximately 80 individuals were either too minor to be of analytical interest, had extremely severe eclosion deformities so that the wings could not be examined fully, or died as pharate individuals.
3. The following analysis, and the classification categories, are based primarily on the approximately 170 individuals retained and photographed in our reference collection, and the approximately 50 individuals used as breeding stock (220 total individuals). While this collection of 220 specimens may be insufficient to formulate absolute “rules” that govern the formation of mosaic wing patterns, our intention is to draw conclusions that can be tested with future experimentation.

Mosaic Wing Surface Totals

Figure 2 presents the “mosaic wing surface” totals for PME1. This data is based on the accurate accounting of the 220 individuals referenced above. We have adopted some descriptive short cuts to make discussions about our mosaic individuals easier. To begin with, we have adopted the habit of referring to mosaic individuals by the number of *wing surfaces* that exhibit mosaicism. For example, a specimen that has mosaic markings on six wing surfaces is called in this paper a “6-Wing” mosaic.

Percentages of Mosaic Individuals By Generation

The generational percentages listed here are relatively close estimates, qualified and limited by the fact that numerous individuals did not eclose properly or died as pharate individuals. In addition, individuals that displayed only minor body spots were not included in these statistics. Inclusion of the Body Spot individuals would probably raise each of these percentages by as much as 10%.

Subsequent to breeding some of the F3 females with wild-collected males, we discontinued compiling these statistics for the generations F4 and later. We estimate that approximately 25% of the females produced displayed mosaicism. (This reflects a somewhat restored viability after the pairings with wild-collected males.)

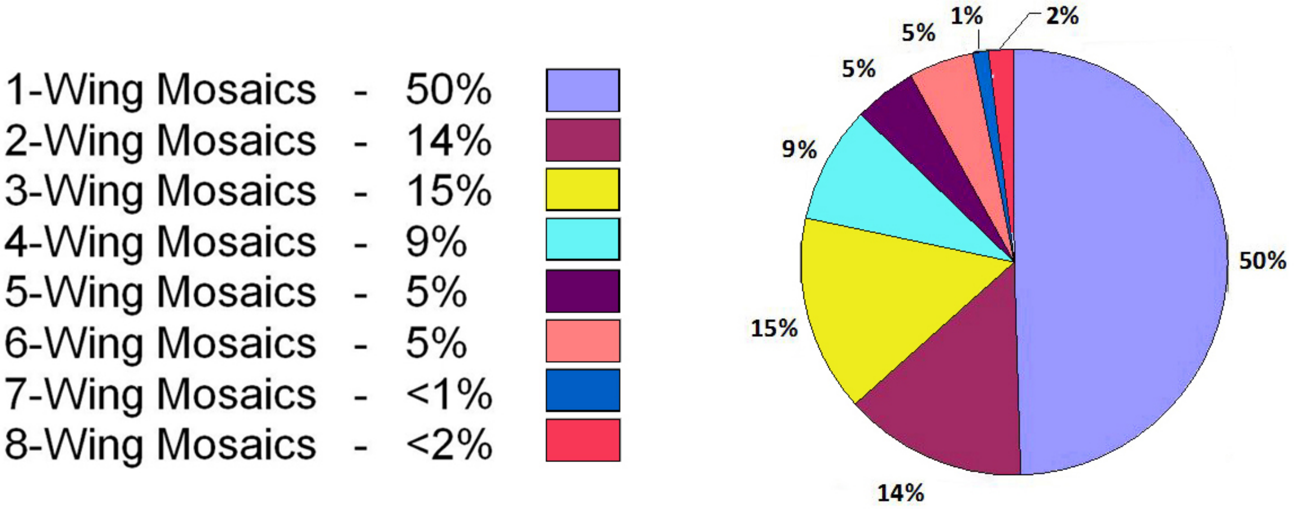

Fig. 2 Mosaic Wing Surface Totals Chart
The Mosaic Wing Surface Totals Chart above is a graphic representation of the breakdown of how the 220 mosaic individuals produced in PME1 were classified. As may be expected, the most severely affected individuals (based solely on the affected number of wing surfaces) were fewest in number. However, this ranking system does not take into account the severity of mosaicism on each wing surface. For example, a 1-Wing mosaic individual may have developed a mosaic area on its wing that far exceeds the total surface area covered by the smaller mosaic sections on a 3-Wing mosaic individual. The significance of this statistical information is yet to be determined, however, this chart is included as an aid towards understanding this phenomenon.

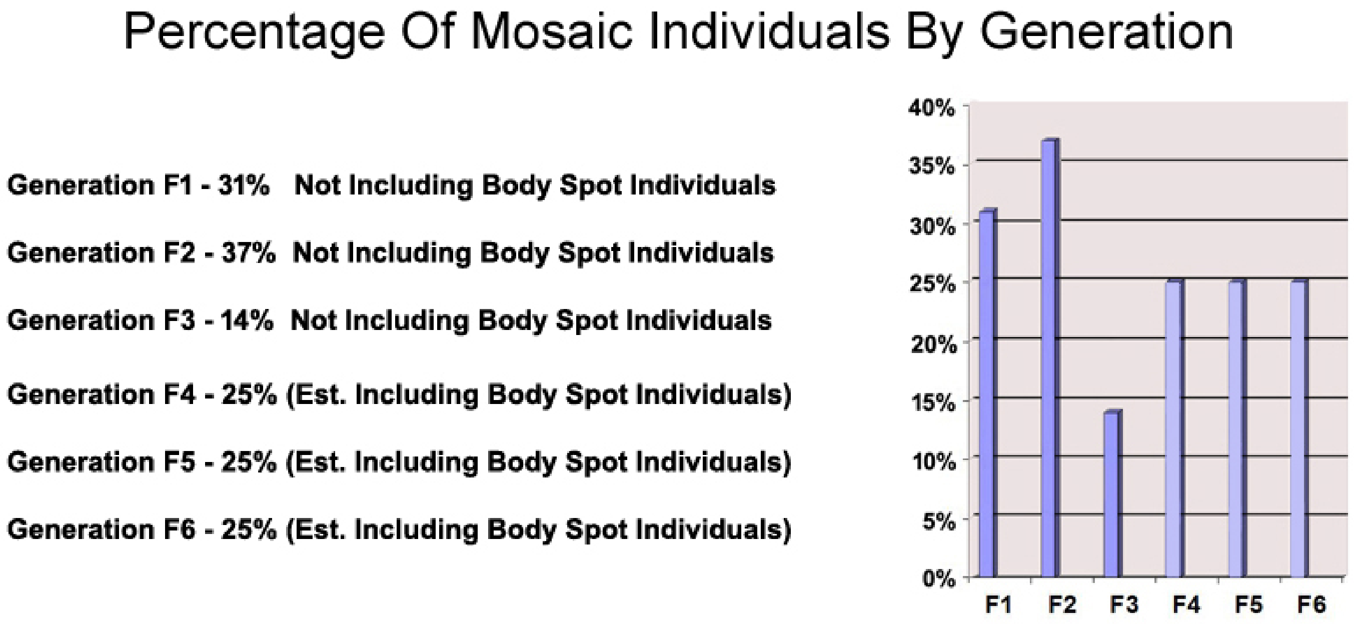

Fig. 3 Percentage Of Mosaic Individuals Chart
The Percentage Of Mosaic Individuals By Generation Chart above is a graphic representation of how many of the 220 mosaic individuals were produced in each generation. Body Spot mosaics were not included in this data. The F1-F3 columns represent very close estimates based on actual counts. Due to the introduction of wild collected males used as breeding stock for the F4 generation, we discontinued actual record keeping for F4-F6 generations. The F4-F6 columns represent our untabulated approximation of the percentages of mosaic wing individuals for these generations, and reflect a somewhat restored viability of the lineage as a result of the mothers of the F4 generation being paired with the wild males.

ANALYSIS

Mosaic Color-Morph Categories

Our collection of approximately 220 mosaic specimens (including breeding stock) is presumably the largest such collection of specimens with a known lineage in existence. As more and more mosaic individuals were produced, we began to notice that the shapes and distribution of the mosaic sections sometimes suggested similarities among the specimens. Based on the large number of specimens at hand, we believe it is possible to analyze mosaicism on a level not possible using a smaller selection of specimens. The discussion which follows is observationally based, and undertakes to organize the various mosaic aberrations into a coherent classification system with the intent being to lay the groundwork for future investigation.

A very broad range of mosaic variation was exhibited in the PME1 females. While every specimen is unique, and certainly all of the specimens share the basic “dark morph with yellow mosaic” characteristic, some interesting commonalities exist within particular groups of specimens.

As a matter of organization, we have divided our resulting collection of mosaic females into five basic categories, each with a descriptive name. These categories are: Body Spot, Streak, Marbled, Patch, and Brush Stroke (Fig. 4). These categories will be presented and discussed in the following pages of this paper. Each of these mosaic patterns can occur in conjunction with other mosaic patterns, either on the same wing or on different wings. By nature, these patterns are asymmetrical in their formation, are seldom mirrored on the opposite wings, and are typically not mirrored dorsal to ventral.

Visually it appears that mosaic sections have no borders, wing cell boundaries, or symmetry. Yet, we noted that there may be a few governing rules. For example, yellow mosaic sections do not override or modify the black pattern elements. Also, when a yellow mosaic section occurs, it apparently autonomously controls the pigmentation within its boundaries. For example, a yellow mosaic section on the dorsal hind wing controls the amount of blue dusting within its boundaries, which may be different than the amount of blue dusting displayed in the adjacent brown section of the wing. Finally, while the idea of trying to categorize seemingly random mosaic wing sections appears on its face to be impossible, we believe that enough similarities exist to support this concept. The following mosaic categories are based on our belief that similar looking mosaic wing sections result from similar developmental origins, and that eventually it will be possible to identify the specific causes for each of these categories.

We have speculated that there may be some inter-relationship between certain mosaic manifestations and their formation in certain zone locations on the wing surface. To this end, we have divided the wing surfaces into three zones from the costal to the anal margins. We have also illustrated these zones as part of some of the composite figures in an effort to aid in the discussion of certain characteristics.

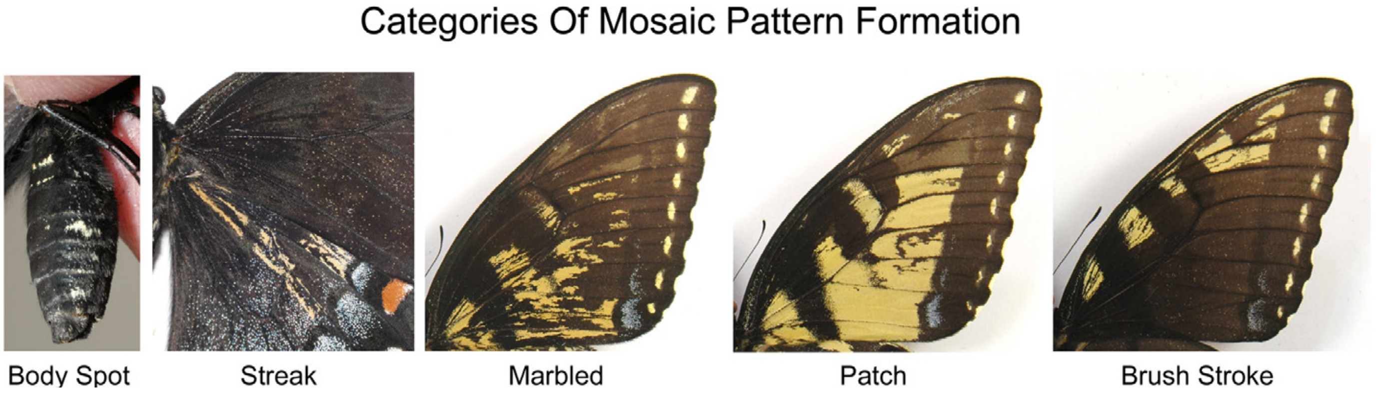

Fig. 4 Categories Of Mosaic Pattern Formation
This composite photo shows examples of the five mosaic categories that are discussed in this paper. From left: The Body Spot individual was an F1 female that was used as one of the mothers of the F2 generation. Notice that the spots are asymmetrical and distributed randomly on the abdomen. The Streak individual was an F4 female that was used as one of the mothers of the F5 generation. Notice that the streaks cross over the underlying black pattern stripe, but do not replace the black scales in within the stripe. The mosaic sections of the Marbled individual and the Patch individual occupy similar locations and sizes, with the difference being the degree of replacement by the yellow scales present. The brush Stroke individual exhibits almost complete replacement by the yellow scales, interrupted only by the underlying pattern stripes. Notice that the Brush Stroke occupies a costal zone of the forewing delineated by the Discal Cell midline and the M_1 vein. Finally, notice that in the Marbled and Patch individuals, the mosaic section does not encroach the area where the Brush Stroke occurs. This is not always the case, but many individuals exhibit this same tendency. Obviously the Discal Cell midline and the M_1 vein play an important role in the formation and delineation of mosaic sections on the forewing.

Body Spot Mosaic Pattern

The first category of mosaicism is what we refer to for obvious reasons as Body Spot. In most mosaic specimens, since the wing markings are the primary focus of attention, the body tends to be overlooked. Sometimes a mosaic wing pattern “carries over” onto the body, but many casual observers don’t realize that the body should be considered as part of the mosaic aberration. We became much more attuned to alterations in body pigmentation as a result of our PCE1 coldshock experiments, which usually resulted in an overall body yellowing in proportion to the severity of the coldshock wing aberrations. In PCE2, we noticed self-contained yellow mosaic body spots as early as the F1 generation. In retrospect, these yellow spots were the first indicators of the mosaicism that would eventually lead to our current PME1 experiment.

The body spots referred to in PME1 are most frequently abdominal in location with spots on the thorax occurring less frequently. They can appear as anything from very tiny spots consisting of a few scales, to more extended spots that sometimes extend across adjacent abdominal segments. In particular, we do not consider as Body Spots the general ventral yellowing of the last two abdominal segments, because these two segments are more prone to exhibit yellow scaling as one of the first indications of coldshock. Body Spots tend to be more numerous and larger when the wings are also mosaic, but we have come to consider their formation without mosaic wings to be an indicator of mosaic genetic tendencies (See Fig. 5).

Fig. 5 Body Spots As Indicators Of Genetic Tendency Towards Mosaicism
This composite photo shows examples of the Body Spot mosaic category. At left is a specimen that illustrates the type of zonal body yellowing sometimes seen in wild specimens and that is often typical of coldshocked specimens, but that is NOT included in the Body Spot category. The next six specimens illustrate increasing degrees of Body Spot mosaicism. Note that while the specimen at the far right exhibits numerous Body Spots, the yellowing on the last two abdominal segments is NOT considered to be Body Spot mosaicism because the yellowing of the last two abdominal segments is often seen in wild individuals and coldshocked individuals.

Streak Mosaic Pattern

The second category of mosaic patterns is the Streak mosaic pattern (Fig. 6). As the name implies, the Streak mosaic is usually a thin irregular line of yellow scales, frequently “clumpy” in thickness. Occasionally multiple streaks will exist on the same wing in generally parallel orientations. Streaks on the forewing are typically interrupted by the forewing stripes while maintaining their directionality, with streaks on the hind wing having fewer stripes interrupting their formation. Based on our examination of the Streak type mosaic individuals in PME1, we identified some important commonalities.

1. Streaks always develop in an outward direction from the body to the border, as if they are following an outward trajectory. We have not produced any Streak type individuals in which the Streaks exhibit any other directional orientation. All of the other Streak type specimens in our collection obtained from other sources also share this characteristic.
2. Streaks seem to be more commonly exhibited on the hind wing than the forewing. Admittedly this is a very tenuous conclusion. However, PME1 resulted in a ratio of approximately 2:1 hind wing vs. forewing Streak mosaics. Dorsal vs. ventral Streaks tended to be closer to a 1:1 ratio.
3. Almost all of the streaks developed in the central zones of the wings. Admittedly, this is also a tenuous conclusion. This was a realization that was not apparent until all of the specimens were photographed and sorted.

It is our speculation that these Streaks acquire their directional trajectory either as a consequence of the directional flow of signaling or pigment synthesis chemicals outward from the body during wing development, or as a consequence of “folds” in the developing wing which create a confinement for the yellow pigment synthesis chemicals. It is also possible that the relatively small surface area of the Streaks is an indication that these wing scale cells switch their “color-morph-identity” at the time of pigmentation rather than earlier in development, and subsequently propagate in an outward trajectory. Further experimentation is required for conclusive analysis.

Marbled Mosaic Pattern

The third type of mosaic pattern to be discussed is the Marbled mosaic pattern (Fig. 7). This pattern is more extensive than the Streak pattern, and can occasionally cover the entire wing. The most indicative characteristic is that although the surface area may be large, as the name implies, it is “marbled” in formation, usually with many alternating sections. In most instances it appears as if numerous locations on the wing changed color simultaneously, but did not merge into a contiguous section of the wing.

In contrast to the Streak type mosaic and the Brush Stroke type mosaic, the Marbled mosaic pattern does not appear to occur in particular zones on the wing, and often extends over the majority of the wing surface. However, like the Streak and Brush Stroke mosaic patterns, there does appear to be an outward trajectory to the Marbled mosaic. A careful examination of Figure 7 illustrates that in most of the examples, the yellow scales appear to extend outward from the body towards the wing margins, interrupted by the pattern stripes, but continuing outward after crossing the stripes.

It is our speculation that these Marbled mosaic areas acquire their directionality from a similar cause to that which occurs

Fig. 6 Streak Mosaic Pattern
This composite photo shows examples of the Streak type of mosaic aberration. Both single Streak and multiple Streak individuals are shown. All of these specimens are typical, and their Streaks share the common attribute of an “outward trajectory”. While Streak mosaic aberrations can occur in any location on the wing, the majority of Streak aberrations in PME1 occurred within the central wing zones depicted in white on the P. glaucus Wing Venation Diagram at right.

Fig. 7 Marbled Mosaic Pattern
This composite photo shows examples of the Marbled type of mosaic aberration. Rows 1 and 2 are examples of Dorsal Forewings and rows 3 and 4 are Ventral Forewings. Rows 5 and 6 show mostly Dorsal Hind Wings. Very few Ventral Hind Wings were affected by the Marbled pattern in PME1. It is unknown if this is statistically relevant. Some of the affected mosaic Dorsal Hind Wing areas are distinctly orange, however we have not attempted to speculate as to what caused the orange to occur. The orange appears to be a replacement color that is confined within particular sections of the marbling. It is important to note that many yellow morph female *P. glaucus* specimens have a distinct "mustard-yellow" color rather than bright yellow. It is possible that the orange exhibited in these mosaic sections is somehow related to this mustard color.

in the Streak mosaic. It is also probable that the switch in "color-morph-identity" that triggers the marbling occurs simultaneously in multiple locations on the wing rather than originating in a single location that then spreads erratically across the wing. Perhaps this also occurs nearer in time to the wing pigmentation process rather than during embryonic development.

Patch Mosaic Pattern

The fourth type of mosaic pattern to be discussed is the Patch mosaic pattern (Fig. 8). Like its closely related cousin the Marbled pattern, the Patch mosaic can be small or it can cover the entire wing. The difference is that the Patch mosaic is uniform in color and contiguous, with most or all of its enclosed scales being uniformly yellow. Because the Patch is uniform, it is not possible to detect any directionality in its formation. It is also impossible to speculate whether its "color-morph-identity" switched during the wing pigmentation process or during embryonic development, however, we favor the premise that its identity switch occurs later in development.

In PME1, the Patch mosaic frequently formed in conjunction with the Marbled mosaic, most often on the hind wings. Perhaps the most important observation is that the majority of the hind wing Patch mosaics exhibited a fairly obvious tendency to abruptly terminate approximately half way inside the Discal Cell. This boundary appears to exist near the midline of the Discal Cell, and apparently exerts its influence whether the patch is above or beneath the midline. This observation was not noted until the photographs were assembled, and likely would not be noticed without the large quantity of individuals available for examination. This mid-cell boundary was noted on both the dorsal and ventral surfaces.

Brush Stroke Mosaic Pattern

The fifth type of mosaic pattern to be discussed is the Brush Stroke mosaic pattern (Fig. 9). By definition, the Brush Stroke mosaic occurs only on the costal edge of the forewing. The name Brush Stroke is very descriptive of its appearance, a wide line with an outward trajectory interrupted only by the pattern stripes, but continuing from the body to the wing border. There is something extremely unique about this section of the forewing, which has as its boundary the midline of the Discal Cell and the M_1 vein. This Discal Cell midline boundary may have a corollary in the previous observation of the Patch boundary on the hind wing. The Brush Stroke mosaic can be fully formed in solid yellow, or Marbled, but it always occupies the same location.

When seeing the Brush Stroke for the first time, it probably gives the impression that it is a typical random formation with little statistical relevance. However, in PME1 it was the most commonly produced color-morph mosaic type. Figure 9 shows a large assortment of Brush Stroke aberrations from PME1. We also have in our collection, *P. glaucus* gynandromorphs with the Brush Stroke mosaic (Supplementary Materials Fig. 12), and numerous other species as well. We believe the Brush Stroke mosaic will prove to be an important discovery that will be relevant in future research.

Fig. 8 Patch Mosaic Pattern
This composite photo shows examples of the Patch type of mosaic aberration. In PME1, the Patch mosaic was more frequently produced on the hind wings. Row 2 shows several specimens that exhibit both the Patch and Marbled type mosaics on the same wing. Notice also that most of the hind wing Patch mosaics occupy half of the wing and are truncated near the midline of the Discal Cell. This observation may be important in future analysis of mosaic formation.

Fig. 9 Brush Stroke Mosaic Pattern
This composite photo shows examples of the Brush Stroke type of mosaic aberration. Some specimens are more intensely yellow and solid, while others are less developed. The top two rows are Dorsal and the bottom two rows are Ventral. Most of the Brush Stroke mosaics produced were not mirrored on the dorsal and ventral of the same wing, however both dorsal and ventral Brush Strokes were produced in relatively equal numbers. The Wing Venation diagram at right in row 1 illustrates that the Brush Stroke does not encroach the Discal Cell past its midline. Beyond the end of the Discal Cell, the Brush Stroke is bordered by the M_1 vein, and does not encroach into the central wing. In row 4 at right is a Marbled mosaic exhibiting no encroachment into the Brush Stroke area, as if it is being prevented from doing so by whatever influence enforces the boundary of the Brush Stroke mosaic category.

Observed Commonalities in Mosaic Traits

For analytical purposes, the four categories of wing mosaicism (not including Body Spots), while appearing random and asymmetrical, share the following commonalities:

1. Several of the mosaic types display a distinctly directional character in their formation. Specifically, there is an outward oriented trajectory, the exact cause of which is yet to be determined. To date, none of the mosaic specimens produced have displayed a different directional orientation.
2. None of the mosaic types cause variances in the black pattern elements.
3. All of the mosaic types can be partially mirrored left wing to right wing, or dorsal to ventral. However, most often no mirroring occurs.
4. Each of the mosaic types can form singly or in combination with any of the other types, either on the same wing or on different wings.
5. Some mosaic types tend to form most commonly in specific areas on the wing. In particular, the Discal Cell midlines of both the forewings and the hind wings tend to function as boundaries in the formation of certain mosaic types.

Orange Mosaic Aberrations in Male Specimens

Perhaps the most unexpected aberration type produced in PME1 was the formation of orange scales on the dorsal surfaces of male specimens, primarily for two reasons. First, this orange pigmentation does not exist on the dorsal surface of normal male specimens, except minimally in the Hind Wing Border Distal Band Spots. And second, we were exclusively experimenting on aberrant female lineages. The origin of these orange scales is unknown at this point, except to presume that our inbreeding of PME1 specimens caused genetic breakdowns in the males as well as the females. The orange mosaic males pictured in Figure 10 were produced in the F4 and F6 generations. None of the orange mosaic males were produced in the F5 generation. We attempted to breed one of the orange mosaic males, but were unsuccessful in producing viable ova.

The single unifying characteristic in these orange mosaic males was that the orange scales were only produced on the dorsal surface. All of the specimens exhibited the orange on the hind wings, but only half of specimens exhibited orange on the forewings as well. All specimens exhibited the orange in the Marbled Mosaic category. A total of 9 specimens were produced, the eight pictured below, and the ninth specimen which was used in a breeding attempt.

As can be seen in Figure 10, the orange scales are distinctly orange and not dark yellow. Frequently a mustardy orange-yellow color is produced in some summer brood yellow morph female specimens of *P. glaucus*. Usually these females are not as distinctly orange as our orange mosaic males. We presume that a Color Replacement has occurred in our males, rather than a mosaic of female orange scales on the male wing. We hope to determine the answer with future pigment chemistry analysis or genetic sex determination.

Fig. 10 Mosaic Orange Aberrations In Male Specimens (see additional in supp. mat.)
This series of photos illustrates the remarkable orange mosaic patches on the dorsal surfaces of eight individual male specimens (additional specimens are figured in supp. mat. Fig. 25). Although the full specimen photos do not as easily resolve the orange vs. yellow scales, the magnified views show this well differentiated marbling. Most of the orange sections display a distinct outward trajectory with some wing cells being entirely orange.

Orange "Mosaic Within a Mosaic" Aberrations in Female Specimens

Finally, the most amazing mosaic specimens we produced were two Orange Mosaic Females (Figs. 11, 25). These two specimens eclosed in the F5 generation. Presumably, the orange scales resulted from the same unknown causes that affected the F4 generation Orange Mosaic Males discussed above. These orange scales only appear in the yellow mosaic sections on the female wing, thus creating a "mosaic within a mosaic". This orange mutation is apparently carried within the gene package that causes the yellow mosaic sections to occur, however we are unable to explain the initial root cause of the formation of the orange pigment. Since the orange scales were first displayed in the F4 males, we initially presumed that this is a male aberration that has in all likelihood never been previously documented. At this point, we are now confronted with the uncertainty of whether this is a male aberration that has been transferred to the females, or whether it is a recessive female aberration that was transferred to the F4 males and did not show up in the females until the F5 generation. It must be emphasized again at this point that our specimens have not had the wing scales sex-identified, nor have the pigments been chemically analyzed. Both of these tests would vastly improve our chances of determining the origins of these orange mosaic specimens. To our knowledge, this "mosaic within a mosaic" aberration has never been seen previously in *P. glaucus,* or documented in any other specimens of any species.

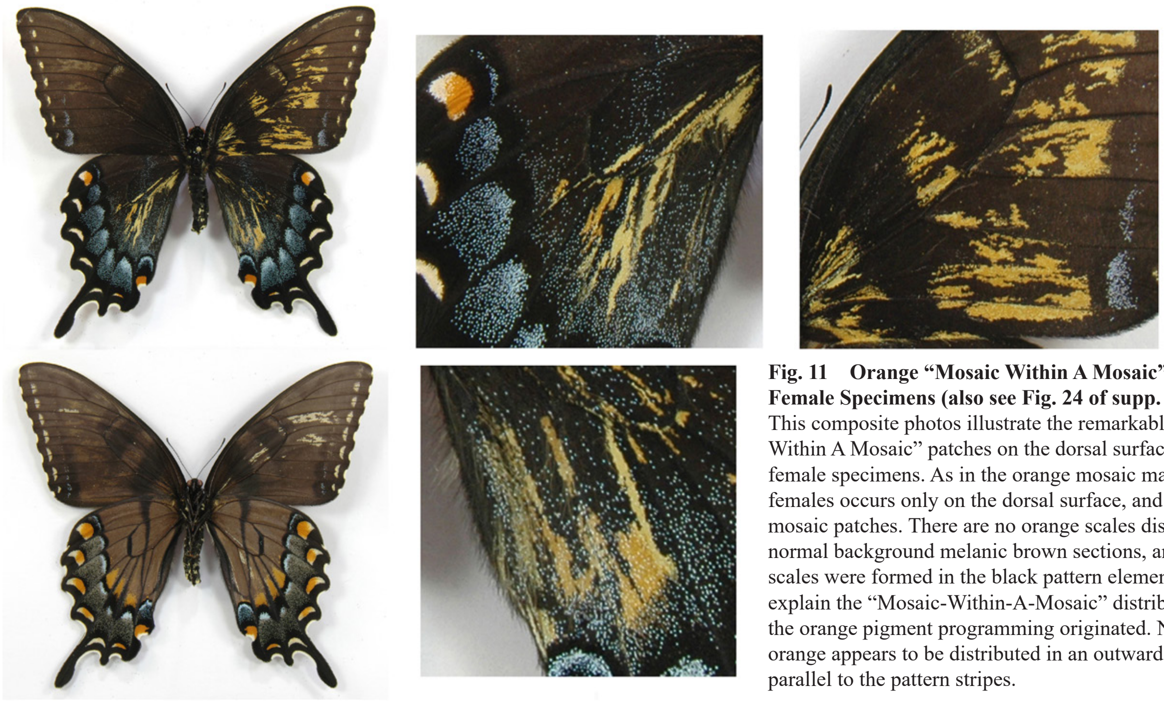

Fig. 11 Orange "Mosaic Within A Mosaic" Aberrations In Female Specimens (also see Fig. 24 of supp. mat.)
This composite photos illustrate the remarkable orange "Mosaic Within A Mosaic" patches on the dorsal surfaces of our two F5 female specimens. As in the orange mosaic males, the orange in these females occurs only on the dorsal surface, and only within the yellow mosaic patches. There are no orange scales distributed within the normal background melanic brown sections, and none of the orange scales were formed in the black pattern elements. We are unable to explain the "Mosaic-Within-A-Mosaic" distribution pattern, or where the orange pigment programming originated. Notice also that the orange appears to be distributed in an outward trajectory rather than parallel to the pattern stripes.

COMMENTARY

In this experiment we have explored the phenomenon of female mosaic color-morphism in *P. glaucus*. This is an iconic aberration that is frequently known to occur in this species. Numerous mosaic specimens have been photographed and presented in the literature (Scriber, et. al. 2009, Scriber and Evans, 1988). These mosaic specimens have historically been coveted by collectors worldwide. However, this aberration has not previously been extensively studied, and to our knowledge, *P. glaucus* mosaic color-morph specimens have never been deliberately bred. This lack of investigation is primarily due to the widespread belief that mosaic color-morphism is the result of one-of-a-kind mutations that result in specimens that are either infertile, or too rare and valuable as prized specimens to risk in futile breeding attempts. For context, in a previous series of breeding experiments over the course of five years, some 28,000 individuals of *P. glaucus* and hybrids with *P. glaucus* yielded a total of 37 mosiac color-morphs, presumably byproducts of the originally intended avenues of research (Scriber and Evans, 1988). By contrast, our PME1 experiment yielded close to 300 identifiable mosaic color-morph females in only six generations, all descended from a single female. Our experiments contradict the infertility assumption, and undoubtedly point the way to many new avenues of research, with implications in a wide range of disciplines.

A cursory glance at the photographs included in this paper immediately leads to the conclusion that there is "a lot going on" here. All of these different mosaic specimens originated from the same limited gene pool provided by the single mosaic female shown in Figure 1 paired with its sibling male. Yet despite the fact that we have shown that mosaic color-morphism is heritable, there appears to be no direct relationship between the ultimate mosaic pattern of the daughter and that of the mother. It has been demonstrated that a mother exhibiting any of our five categories of mosaicism can produce offspring also having any of our five categories of mosaicism. There is no predisposition for the daughter to resemble the mother other than that they both share the ability to develop mosaicism.

The chain of experiments that eventually resulted in this paper could not have occurred in a more compelling or advantageous sequence for us. Our first experiment, PCE1, demonstrated that the genetic color programming in the female *P. glaucus* can be overruled by coldshock to cause what we have called a Color Reversion from the more recently evolved brown to the ancestral yellow (Perlman and Perlman 2019a). Our second experiment, PCE2, demonstrated that the range of possible coldshock-induced color choices is not specifically limited to either brown or yellow (Perlman and Perlman 2019a). Rather, under the right circumstances most of the other colors in the *P. glaucus* color palette can be substituted for the yellow or brown programming in a process that we have characterized as Color Replacement. Both the Color Reversion aberrations produced in PCE1 and the Color Replacement aberrations produced in PCE2 can be viewed as "wing-wide" aberrations. In essence, the color substitutions can be distributed across the entire wing surface limited only by the black wing pattern elements.

Here in PME1, the mosaic sections of the wing can be viewed as "localized" aberrations, because the affected sections of the mosaic wing act autonomously. They implement the pigmentation instructions provided by their *incorrect* reprogramming. These mosaic wing sections share the characteristic of having well defined, though highly irregular and asymmetric boundaries. The cause of these mosaic wing sections is a programming error that prompts a switch in the genetic morph-identity of the wing scales within the mosaic boundaries. The biochemistry that allows the switch from brown to yellow pigment is well understood in *P. glaucus* (Koch, et. al 1998, Koch, et. al. 2000a, Koch, et. al. 2000b, Perlman and Perlman 2019a). These resulting mosaic sections stand in contrast to the scale-by-scale yellowing that occurs as the result of Color Reversion caused by coldshock. In other words, *on a scale by scale basis*, the same resultant pigment synthesis malfunction (yellow pigment being substituted for the intended melanic brown) can result in two distinct outward manifestations (yellow dusting or mosaicism) that have two distinct unrelated causes (genetic programming errors or environmentally caused programming errors).

The precise underlying triggering mechanism that resulted in mosaicism in our PME1 specimens is unclear, and possibly there can be multiple potential causes, including the ancestral coldshock that yielded the original parents of this experiment. Regardless of underlying triggering mechanism, our PME1 mosaic specimens are the result of genetic identity switches that occurred and further propagated during development and pigmentation. Genetic identity switches can occur at any point in time during a butterfly's development. Generally speaking, the later in development the switch occurs, the less of the individual will be affected. Due to the fact that PME1 did not produce any bilateral color-morphs or quadrilateral color-morphs, we believe that our mosaic color-morphs occurred later in development, possibly prepupal or just prior to the initiation of wing scale color identity selection and pigmentation. Based on the results of our experiments, we have come to consider that it is only the *mosaic ability* that originates as early as in the embryo, and that the final color and pattern choices occur as late as in the chrysalis, occasionally modified by environmental conditions during wing development. This may explain a host of observations such as directionality, adjacent areas exhibiting different types of mosaicism, and why no bilateral color-morphs were produced. More extensive investigation of this possibility is needed.

Mosaic female *P. glaucus* specimens frequently pose identification problems, as both mosaic gynandromorphs and mosaic color-morphs can be visually indistinguishable (Supplementary Materials Fig. 12). The resulting patterns exhibited in either of these mosaic aberrations can be identical, despite their differing underlying genetic causes. It seems plausible or at least possible that these similarities in mosaic patterns indicate analogous developmental pathways. This hypothesis would be extremely difficult to verify given the relative rarity of these aberrations and the unpredictability of their occurrence in the general population. However, it may be possible to explore this premise given a sustainable lineage such as that in PME1.

Taking this premise one step further, assuming that some mosaic gynandromorphs and some mosaic color-morphs owe their mosaicism to identity switches that occur later as the wing is developing, it may be logical to assume that the smaller the mosaic affected wing section, the later its identity switch occurred. Also, when a wing develops multiple tiny mosaic spots, it may be more logical to assume that the individual spots spontaneously developed in multiple locations on the wing surface, rather than that the spots all originated at a single cellular source and subsequently migrated as daughter cells to different locations on the wing. Perhaps it is even possible to count the number of yellow mosaic scales, mathematically calculate how many cell divisions occurred, and project backwards to determine precisely when the first mutation in the spot occurred. This premise would be particularly interesting to explore in the Streak type mosaic individual. Perhaps the visibly outward trajectory of the Streak indicates that the sequence of cellular propagation occurs with the oldest near the body and the youngest at its outer extent. Much more exploration of this premise is needed.

In PCE2, we made the observation that in some specimens, a lightening of the distal wing cells on the forewing tends to reveal a darker wing cell midline. These darker midlines are not normally visible in *P. glaucus*, however they are readily visible in other Papilionids such as in various members of the *P. memnon* (Linnaeus, 1758) group. These midlines obviously influence pigmentation saturation levels either biochemically or physically. Here in PME1, we observed that some of the mosaic wing sections are influenced by an invisible Discal Cell Midline. In many individuals, this Discal Cell Midline determines the boundary of the exhibited mosaic aberration. We cannot speculate on the significance of this observation. However, we expect this observation to show its relevance as further experimentation into wing developmental processes continues.

SUMMARY

Over the course of our PCE1, PCE2, and PME1 experiments, we have considered the possibility that the multitude of wing color aberrations that were produced may be partially attributable to the unique nature of the *P. glaucus* genome. It is possible that many of these aberrations occur precisely because of the shared chemical relationship between the yellow and melanic brown pigments, and therefore would not necessarily be exhibited in other species. This question may be resolved as more of the pigment chemistry in other species is deciphered. In addition, it is worth considering that ancestral coldshock may have been partially responsible for triggering the initial development of mosaic color-morphism by weakening the morph-stability of the affected individuals in the PME1 lineage. Based on the fact that numerous coldshock-induced aberrations occurred in conjunction with the mosaic aberrations observed in this experiment, the mosaicism may have its origins in the same coldshock-induced weakening of morph-stability that caused the various color and pattern aberrations. (See Supplementary Materials Figs. 18-23 for examples of these combination aberrations.) It is even possible that the original mosaic specimens would not have occurred without administering the coldshock. Further experimentation is needed to confirm this premise.

The following list is a summary of some of what we consider to be the most important discoveries and theories detailed in this paper. We intend them to primarily pertain to *P. glaucus* mosaic color-morphs, but with possible implications for other species as well:

1. Mosaic color-morphism is a heritable capability. Not only can multiple sibling sisters from the same brood exhibit this aberration in what has most often been assumed to be a “one-time” phenomenon, but this aberration can also be passed on and intensified in subsequent generations.
2. Mosaic color-morphism in *P. glaucus* is a female borne trait in that can be inherited by its descendents whether the fertilization occurs from an inbred sibling male, or a wild male.
3. Mosaic color-morphism was shown to affect a higher percentage of individuals within a brood, simultaneously affect more locations on the wing surfaces, and be more pronounced in intensity, when the mother was paired with a sibling multigenerational inbred male.
4. Mosaic color-morphism can occur in conjunction with most of the types of coldshock-induced aberrations, either on different wing surfaces or on the same wing surface.
5. For the first time here, a classification system has been proposed that has identified five common mosaic pattern types, consisting of Body Spot, Streak, Marbled, Patch, and Brush Stroke type mosaic patterns. Each of the five categories has coherent and definable commonalities. These same patterns can be identified in mosaic gynandromorphs of *P. glaucus*, and in mosaic specimens of other species as well.
6. Discal Cell Midlines function as definable boundaries in the formation of mosaic aberrations. In some of the mosaic categories presented herein, the Discal Cell Midlines may be the defining parameter.
7. Any of the five mosaic patterns can develop in combination with any of the other mosaic patterns. Despite the currently incomplete understanding of how these mosaic aberrations attain their final shapes, we have proposed the possibility that similar category patterns probably result from similar origins.
8. The five pattern classifications are affected in their formation by a variety of developmental factors. We

have suggested that some of the observed classifications of mosaicism are related to and controlled by specific locations on the wing surface, and that these patterns are related to a generalized outward pigmentation trajectory during wing development. The timing of the color-identity switch may also be involved along with many other unknown factors.

9. We have proposed that the mosaic patterns in our *P. glaucus* specimens may be caused by mutations in the embryo that create the *ability* to generate mosaic patterns, or mutations happening as late as in the chrysalis that *create* the actual mosaic patterns.
10. Based on the premise that the cellular morph-identity switch that causes mosaicism can occur up to the time of chrysalis formation, this model may suggest the conclusion that the smaller the mosaic wing section, the later in the wing development it is initiated.
11. The orange mosaic aberration which arose first in the males, and the following generation in the females, has not previously been documented as far as we have been able to determine. We are unable to ascertain its origins, or whether it is a genetically male or female aberration. We are also unclear as to whether it arose independently in both the males and the females, or was somehow transferred from one to the other.
12. Our research in PCE1, PCE2, and PME1 has enabled us to better understand the importance of pattern element stability, to further define the pigment color selection process, and to expand the known assortment of previously documented aberration types in *P. glaucus*. We believe our experimentation has augmented the possibility of deciphering the causes of these aberration types, and opened the doorway to further the understanding of wing developmental processes.

CONCLUSION

Our breeding experimentation with *P. glaucus* is ongoing. At the time of this publication, we will have completed the F13 generation of this mosaic lineage. Each succeeding generation has produced the now hereditary coldshock aberrations first seen in PCE1, and PCE2. In addition, new unique aberrations continue to arise in each generation, fueling our passionate interest in this project. As a consequence of concentrating on this mosaic lineage, many other interesting breeding opportunities have been bypassed or abandoned. We intend to experiment with some of these different aberrant types in the future. As our experimentation continues, we would be interested in communicating with other researchers who may be able to help us further expand our understanding of the nature and ramifications of our *P. glaucus* breeding projects.

The following avenues of research would significantly help to explain the aberrations that we have produced in PCE1, PCE2, and PME1:

1. A complete identification of the chemical composition of all of the pigments produced on the *P. glaucus* wing, and a complete sequence of steps required in the chemical synthesis of these pigments.
2. An analysis of the brown vs. the black melanic pigments to determine if they differ in chemical composition, or merely in saturation levels.
3. A genetic sex identification of the yellow mosaic scales to conclusively determine if any of our specimens are gynandromorphs or if all of them are mosaic color-morphs.
4. An identification of the specific genes responsible for pigment color selection and pattern stability.
5. An expanded investigation into how specific environmentally-induced distortions in the developmental timeline unlock the ability for wing scales to be pigmented with colors that contradict their originally intended genetic programming.
6. It is unknown if the unaffected female siblings in this experiment were "carriers" of the mosaicism, because none of them were bred as a control group.

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SUPPLEMENTARY MATERIALS

This Supplementary Materials section has been provided with the intention of expanding the audience for our research, and to provide additional space for specimen photos that are not contained in the main body of this paper. Background information specific to *P. glaucus* has been included for those readers who are not thoroughly familiar with this species. Also included are simplified explanations about wing pigmentation and mosaicism that relate specifically to *P. glaucus*. These are not intended to take the place of the more comprehensive explanations that are found elsewhere in the research literature. An expanded presentation of photographs of the PME1 specimens will be found in this section. These photographs are intended to show as many different combinations of mosaic aberrations as possible, and to show never before seen combination aberrations that mix mosaicism and hereditary coldshock aberrations in the same specimens. Our intention is that the reader will gain a fuller insight into the results obtained during our experiments, with our hope being that these photographs will lead to further experimentation by others. Finally, we suggest that readers who are not familiar with our *P. glaucus* coldshock paper (Perlman and Perlman 2019a) would benefit greatly from reading that paper also, because some of the information presented here is related to that previous paper.

Explanation of How Yellow vs. Brown Pigmentation Occurs

Like every species, *P. glaucus* can develop numerous different types of wing pattern aberrations. One such very distinctive category of aberrations consists of those specimens that combine the dark and yellow morphs in the same individual. Unlike many other dimorphic or polymorphic species, *P. glaucus* has a well documented ability to produce unique combination aberrations that blend the dark morph and yellow morph in striking ways. These blended individuals can be produced either as intermediate morphs that have a somewhat evenly-dusted “smoky” appearance, or as mosaic morphs in which the melanic brown and the yellow pigmented scales are distributed in uniformly mono-colored sections that are randomly located on the wing surface.

The “smoky” intermediate individuals and the mosaic individuals are two very different looking types of aberrations, but in reality, they are more closely related than their appearances would imply. These blended morphs occur in *P. glaucus* primarily as a consequence of shared ancestry and shared chemistry, and lie somewhere between genetic programming and genetic flexibility. In order to understand how these two types of aberrations can occur, it is first important to understand how the yellow and brown background colors of normal yellow and dark morph females are created.

Fortunately, previous research on *P. glaucus* wing scale pigmentation has provided us with an underlying understanding of how the color choice between yellow and brown is accomplished. Two basic discoveries encapsulate this process. The first of these discoveries is that in *P. glaucus,* the same precursor chemicals can be used to produce either yellow or brown pigments. This yellow or brown potential depends upon the availability of the catalyst enzyme BAS (N-β-alanyldopamine synthase) (Koch, P. B., et. al., 2000a and 2000b). Yellow is produced when BAS is abundant, and brown is produced when BAS is deficient. It is important to note that this shared pigment chemistry between the two morphs of *P. glaucus* is extremely unique, and does not exist in most other polymorphic species.

The second of these discoveries is that there exists an orderly wing pigmentation sequence. Accordingly, wing scales get pigmented specific colors during their specific windows of opportunity. This sequence dictates that the synthesis of yellow pigments precedes the synthesis of brown pigments (Koch, P. B., et. al. 1998). It appears that this pigmentation sequence is universal for all Lepidoptera species, regardless of the species and regardless of the specific pigment chemicals being synthesized. Therefore, simply summarized, in *P. glaucus,* yellow scales mature and get pigmented earlier, at a time when BAS is available, and brown scales mature and get pigmented later, when BAS is not available.

So how do the shared chemistry and the universal pigmentation sequence determine which female color morph will be produced? BAS production is *genetically controlled*, and is released or suppressed during the formation of the wings. Its concentration levels are genetically synchronized with wing scale development rates so that the correct color for either morph will be properly implemented. Thus, under normal circumstances the yellow morph individuals are produced only in the earlier yellow window of opportunity, and the brown dark morph individuals are produced only in the later melanic brown window of opportunity.

Evolution has genetically stabilized this pigmentation process in the two morphs of *P. glaucus* to the point that in the wild, yellow mothers can genetically produce only yellow daughters and brown mothers can genetically produce only brown daughters. This process is heritable and cannot be altered under normal circumstances. As a result, blended individuals of either the “smoky” intermediate type or the mosaic type can only occur as the result of incorrect implementation of the pigmentation sequence programming.

*Why do these two types of yellow aberrations occur in dark morph female*s? Generally speaking, the smoky intermediate individuals are ***not*** genetic mutations. They occur as the result of extreme environmental conditions, like coldshock, that disrupt the pigmentation process temporally and/or chemically *on a scale by scale basis*. Disruptions like coldshock cause the genetically intended brown scales of the dark morph female to accept an incorrect fate determination, thus maturing early and producing yellow instead. This disruption results in a “salt and pepper” scale distribution across the wing surface.

Fig. 12 Comparison Of *P. glaucus* Female Mosaic Color-Morphs To Mosaic Gynandromorphs
This composite photo shows examples of the two types of mosaic aberrations that are possible in *P. glaucus*. At left is an 8-Wing female mosaic color-morph, dorsal and ventral. In center is an 8-Wing mosaic gynandromorph, dorsal and ventral. Notice that aside from the positioning of the mosaic sections, these two specimens have many similarities, thus creating some confusion in identifying which type of mosaic aberration has been produced. In the right panel are examples of similar individual wing comparisons, with the female mosaic color-morph at the left and the mosaic gynandromorph at the right. Referencing this composite photo in conjunction with the explanations in this section of the paper should help clarify any potential confusion. In the female mosaic color-morph specimen at the left, the yellow sections are composed of yellow morph *female* wing scales. In the mosaic gynandromorph specimen in the center, the yellow patches are composed of yellow *male* wing scales. The bodies of both specimens are mosaic, with the female mosaic color-morph having a female body, and the gynandromorph having a male body. Generally speaking, the yellow scales of the normal male wing are more vibrant in color than the yellow scales of the normal yellow morph female wing. In addition, the male hind wing black borders do not contain the extensive blue dusting present in both morphs of the female. Therefore, the easiest decisive identification of a mosaic individual can be accomplished by examination the abdomen tip, or when the mosaic yellow section is on the dorsal hind wing and displays adjacent extensive blue in the black border of the affected wing cells. More difficult to observe, are subtle differences in the intensity of the pigmentation. Some of these subtle differences can be seen in the mosaic gynandromorph specimen in center. Notice that the left forewing apex of the gynandromorph displays differences in the darkness of the border, the darker sections being the more vibrant color of the male. Also, notice the ventral forewing in the same location. In the two cells that are male, the yellow is brighter, and the brown is darker than the remainder of the wing which is the female section.

In the right panel, a variety of female mosaic color-morph individuals are shown at left, compared to the individual wing sections highlighted from the mosaic gynandromorph specimen above. The top pair shows a female mosaic color-morph wing exhibiting blue in the forewing border, compared to the male gynandromorph wing having no blue. The second pair shows isolated ventral forewing views of the two specimens in the left panel above, with the male mosaic section displaying more intense yellow and brown. The third pair shows a dorsal hind wing of a female mosaic color-morph with all the blue, compared to the gynandromorph wing where the blue is absent from the male mosaic section and present in the female section. The fourth pair shows two ventral wings with almost identical mosaic sections. It cannot be determined by observation alone which is the female mosaic color-morph and which is the mosaic gynandromorph. This is perhaps the best example of why it is easy to confuse the two types of mosaic individuals in *P. glaucus*.

These smoky intermediate aberrations were investigated and discussed in our previous PCE1 experiment (Perlman, D. L. and Perlman M. P., 2019a).

By contrast, mosaic individuals ***are*** caused by genetic mutations. Incorrect genetic instructions are given to selected sections of the wings that allow both the yellow and the brown pigmentation processes to proceed normally, but only in these well-defined patches. In a dark morph mosaic individual, the yellow patches which should have been brown, are essentially being prematurely pigmented out of sequence according to the incorrect genetic instructions they have been given.

It is these dark morph *P. glaucus* females with yellow mosaic patches that are the subject of this paper. PME1 is the first experiment of its kind that has successfully bred mosaic individuals in quantity. It has provided the opportunity to examine and compare mosaic individuals in many configurations, and has afforded us a window into the mechanism whereby mosaic individuals are produced.

Gynandromorph vs. Female Mosaic Color-Morph

There are two distinct ways in which a dark morph female *P. glaucus* can form yellow mosaic aberrations. The first is gynandromorphism, which is a mosaic of both sexes, and the second is same-sex female mosaic-morphism, which is a mosaic of its two genetically stable female morphs. Both are genetic malfunctions. Since these two types of mosaicism are especially confusing in *P. glaucus*, the following explanation will be helpful. (See Supplementary Materials Fig. 12 for a pictorial comparison.)

A gynandromorph is a butterfly that possesses both sexes within the same individual. The sexes are not blended, but are retained in the affected individual as distinct sections. In butterflies this can be very dramatic, especially when the two sexes are different in color or shape. Gynandromorphism is genetic in origin and usually occurs when developing cells divide improperly, yielding daughter cells that switch their sexual identity. In the most extreme case, the first embryonic cell divides improperly yielding one male and one female daughter cell. If these cells continue propagating without further incident, the resulting adult will be a *bilateral* gynandromorph, half male and half female, split right to left down the center of the body. If the initial improper cell division occurs later in development, less of the resulting adult will be the opposite sex. This second type is called a *mosaic* gynandromorph. Mosaic gynandromorphs can be mostly male or mostly female. Sometimes mosaic sections develop at multiple locations on the wings, which indicates that improper cell divisions occurred in multiple locations. This explanation is grossly oversimplified, but it should suffice for the discussion which continues.

As one would imagine, gynandromorphs are exceedingly rare, although they can occur in any species of butterfly. By contrast, same-sex mosaic-morphs are blended versions of the same sex. Same-sex mosaic-morphs are theoretically much more rare. *Why?* Because in order to produce a same-sex mosaic-morph, a species must have multiple morphs of the same sex. Since most species of butterflies do not have same-sex polymorphism, there are fewer species that possess the ability to produce a same-sex mosaic-morph. Same-sex mosaic-morphs can also be either bilateral or mosaic. Like a bilateral gynandromorph, a bilateral same-sex mosaic-morph occurs when the first embryonic cell divides improperly, with each of the two daughter cells getting programming for a different morph. And like mosaic gynandromorphs, same-sex mosaic-morphs occur later in development, and can also occur when multiple locations on the wing spontaneously switch their programming to an alternate programming mode.

Interestingly, in mosaic-morphs there are relatively few genes that control which colors and patterns will be produced on a wing surface. Since these genes do not control sexual identity, it is potentially a simpler matter for these genes to switch their original programming to another of their possible programming modes and still yield a viable adult. Having said that, mosaic-morphs are not common at all. This is partially because in many polymorphic species, the morphs are geographically isolated and stabilized, making it less likely that the controlling genes will switch their programming. One of the very unique characteristics of *P. glaucus* is that both of its female morphs exist sympatrically in relatively equal numbers. The males seem to show no selection preference for either morph. In addition, due to the fact that both of the female morphs are sympatric, they both share the same local male gene pool, and it cannot be determined by examining the male which morph of the female was its mother.

P. glaucus mosaics often pose a problem in determining whether the mosaic is a gynandromorph or a female mosaic color-morph. This is because when examining a dark morph female with a yellow mosaic section, it is impossible to determine visually if the mosaic section consists of yellow male scales (a gynandromorph), or yellow female scales (a mosaic-morph). The only two possible rapid confirmations of gynandromorphism in *P. glaucus* would be if the abdomen tip displays mixed genitalia, or if the mosaic section extends into the hind wing border where the pattern differs between the sexes (See Supplementary Materials Fig. 12.) If instead, the yellow patch is on the forewing or on the central hind wing, confirmation may require genetic testing.

Although we have not conducted sexual identification genetic testing of the yellow mosaic sections, we have concluded that the mosaic individuals produced in PME1 are of the female mosaic color-morph type. Of the approximately 300 mosaic individuals produced in PME1, none of them have male genitalia, and none of them have mixed hind wing border patterns. Therefore, the presentation in this paper is based on the assumption that PME1 has produced *P. glaucus* female mosaic color-morphs.

Mosaic Examples Produced In PME1

The following composite photos present a large sampling of the mosaic color-morph aberrations produced in PME1. This fairly comprehensive assortment is provided in an effort to not only illustrate as many mosaic combinations as possible, but also to demonstrate the large range of variation that can result from what is probably a minimal number of genetic causes. It is hoped that this section will serve as a baseline for future comparison. The composite photos are presented in a relatively generational and chronological order, and were chosen for their variety and intensity.

Combinations Of Mosaicism With Residual Inherited Coldshock Aberrations

PCE2 produced numerous types of coldshock aberrations. These were presented in our first paper (Perlman, D. L. and Perlman M. P., 2019a). Of particular interest, the majority of these aberrations became heritable to some degree, and continued to arise in non-coldshocked generations. Since this current PME1 experiment originated using PCE2 coldshocked lineage, it is not surprising that some of these coldshock aberrations continued to arise in PME1. What is most surprising, is that many of these coldshock effects were able to form in conjunction with mosaic effects – yielding two totally different types of aberrations in the same specimens! The resulting specimens are spectacular. Rather than reiterate our analysis of coldshock effects, we have chosen to show these incredible specimens with limited comments, confined to the Figure photograph descriptions, with the recommendation that fuller descriptions can be accessed by referring to our first paper.

Fig. 13 PCE2 Females - Byproducts Of Coldshock Experiment
This composite photo shows four of the more interesting mosaic specimens which were the "accidental" spontaneous occurrences produced in our predecessor PCE2 experiment. These are sibling sisters of the mother of the PME1 experiment (shown in Fig. 1). These specimens were all coldshocked, although none of them display prominent coldshock effects. Specimen 3 above displays a mirrored location mosaicism with a Marbled type dorsal and a Patch type ventral.

Fig. 14 PME1 F1 Generation (Panel 1 above and for panels 2-4 see next page)
This composite photo shows 16 mosaic F1 individuals, daughters of the pairing of the original mosaic mother and an inbred sibling. None of these specimens were coldshocked, although the three preceding generations were coldshocked. All of these specimens were overwintered in diapause. The sixteen specimens in Fig. 14 plus some minor mosaic individuals and some Body Spot individuals eclosed from the approximately 100 chrysalides in this pairing. Several of the more minor mosaic females were used as breeding stock for the F2 generation. Given that approximately half of this brood were males, we determined that 31% of the females were mosaic with an additional approximately 10% exhibiting only Body Spots. The original mother was the 1-Wing minor mosaic shown in Fig. 1. Notice that the heritability of the minor original mosaic aberration was significantly intensified in many of its descendents.

Fig. 14, panels 2-4)

Fig. 15, panels 1-3)

Fig. 15 PME1 F2 Generation - Specimens Produced From Inbred F1 Females And Males (Panels 4-5; for panels 1-3 see previous page)
This composite photo shows 20 mosaic individuals that were all descended from inbred pairings of F1 individuals.These specimens were not coldshocked or overwintered. They were the 2nd brood "summer" individuals. Notice that there is still a wide variation in the amount of blue on the dorsal hind wings. Also, there are some specimens exhibiting lightness of the ventral surfaces, which is a typical coldshock characteristic. The 2nd specimen in the 2nd frame is unusual in that only the hind wings are mosaic. Most multiple wing mosaics display a combination of forewing and hind wing mosaicism in the same individual. This F2 generation produced the highest percentage of mosaic individuals in the PME1 experiment, at 37%.

Fig. 16 F3 And F4 Generation - F3 Generation Was Inbred, F4 Generation Used Wild Male Fathers
This composite photo shows 8 mosaic individuals that were all descended from inbred original lineage females paired using both inbred males and field-collected wild males. The F3 generation experienced drastically diminished viability, with most of the inbred pairings being either infertile, or refusing to be hand-paired. The F4 generation, which was the result of pairings with wild males, was viable, and these chrysalides were subsequently overwintered to continue this experiment the following spring. However, statistical record keeping for PME1 was officially terminated with this F4 generation due to the initiation of comingled generations and bulk rearing techniques. The F4 individuals exhibited both a drastically diminished incidence and severity of mosaicism, possibly due to the introduction of their wild male parents. Subsequently, several pairings in succeeding generations were successful with inbred males, and this breeding continued. The F5 and subsequent generations were also somewhat diminished in the incidence and severity of mosaic effects, and also exhibited a high percentage of Insufficient Scale Quantity aberrations and death as pharate individuals.

Fig. 17 Most Extreme 8-Wing And 6-Wing Mosaic Individuals
This composite photo shows 4 mosaic individuals that were the most extremely mosaic in the PME1 experiment. From left, a spontaneously occurring 6-Wing mosaic specimen from PCE2 prior to our deliberate breeding of mosaics; an 8-Wing F1 mosaic, the most extreme of all and a daughter of the original mosaic mother shown in Fig. 1; an 8-Wing mosaic from the F2 generation; and an 8-Wing mosaic from the F3 generation.

Fig. 18 Mosaic Aberrations With Coldshock-Induced Heritable Traits And Wing Deformities
This composite photo shows 4 individuals that exhibit prominent mosaic aberrations in conjunction with wing eclosion deformities, and wing characteristics that are typical of the coldshock-induced aberrations that were produced in PCE2. Specimen 1 exhibits minor lightening on the ventral. Specimen 2 exhibits blue trailing up the forewings. Specimen 3 exhibits minor yellow forewing discal spots and minor “White Triangles” aberrations. Specimen 4 exhibits lightened ventral hind wings.

Fig. 19 Mosaic Aberrations With Coldshock-Induced Heritable Insufficient Scale Quantity (ISQ) Aberrations
This composite photo shows 4 individuals that exhibit Forewing ISQ aberrations in conjunction with minor mosaic aberrations. Specimen 1 displays minor mosaic patches on the right ventral hind wing, and also displays a distinct lightening of the forewing at the M_3 vein. Specimens 2 and 3 display mosaic patches on the ventral. Specimen 4 displays its mosaic pattern on the left dorsal forewing in the same area affected by the ISQ aberration.

Fig. 20 Mosaic Aberrations With Coldshock-Induced Heritable Extreme Insufficient Scale Quantity (ISQ) Aberrations
This composite photo shows 4 specimens with extreme ISQ aberrations in conjunction with mosaic aberrations. Specimen 1 is a 3-Wing mosaic. Specimen 2 is a 2-Wing mosaic with a partially developed Banded aberration as described in PCE2. Specimen 3 is a 5-Wing mosaic. Specimen 4 is a 3-Wing mosaic.

Fig. 21 Mosaic Aberrations With Coldshock-Induced Heritable Lightened Ventral Aberrations
This composite photo shows 4 specimens that display lightened ventral aberrations in conjunction with mosaic aberrations. Specimens 1 and 2 are examples of what we described as the *pelli* aberration in PCE2. Specimen 3 has an overall light ventral with evident border intrusion caused by Pattern Drift on the forewing ventral margin. Specimen 4 is a 3-Wing mosaic that exhibits an extremely lightened ventral, and both surfaces exhibiting yellow Forewing Discal Cell Spots and Pistol Grip markings as described in PCE1 and PCE2.

Fig. 22 Mosaic Aberrations With Multiple Coldshock-Induced Heritable Aberrations
This composite photo shows 4 specimens that display more extreme mosaicism in conjunction with a variety of heritable coldshock aberrations. Specimen 1 is a 3-Wing mosaic with a lightened ventral, Pistol Grips, and bluer than normal hind wing margin. Specimen 2 is a 6-Wing mosaic with a developing Banded aberration and a speckled appearance due to a minor ISQ aberration. Specimen 3 is a 7-Wing mosaic with a bluish-white lightened ventral, and extensive blue trailing up the dorsal forewing margin. Specimen 4 is a 4-Wing mosaic with a White Triangles ventral aberration, blue trailing up the dorsal forewing margins, and prominent blue Pistol Grips.

Fig. 23 Mosaic Aberrations With Multiple Extreme Coldshock-Induced Heritable Aberrations
This composite photo shows 4 specimens that display mosaicism in conjunction with the most extreme coldshock aberrations that we produced. Specimen 1 is a 3-Wing mosaic with an extremely yellowed Color Reversion type left dorsal forewing. Specimen 2 is a yellow Banded aberration with its mosaic patch in the yellow band. Specimen 3 is a 2-Wing mosaic in conjunction with a Banded aberration and extreme ISQ. Specimen 4 is a 1-Wing mosaic that has within its mosaic patch an extremely blurred hind wing stripe due to extreme Pattern Breakdown.

Fig. 24 Orange “Mosaic Within A Mosaic” Aberrations In Female Specimens (also see Fig. 11)
This composite photos illustrate the remarkable orange “Mosaic Within A Mosaic” patches on the dorsal surfaces of our two F5 female specimens. As in the orange mosaic males, the orange in these females occurs only on the dorsal surface, and only within the yellow mosaic patches. There are no orange scales distributed within the normal background melanic brown sections, and none of the orange scales were formed in the black pattern elements. We are unable to explain the “Mosaic-Within-A-Mosaic” distribution pattern, or where the orange pigment programming originated. Notice also that the orange appears to be distributed in an outward trajectory rather than parallel to the pattern stripes.

Fig. 25 Mosaic Orange Aberrations In Male Specimens (see additional in Fig. 10)
This series of photos illustrates the remarkable orange mosaic patches on the dorsal surfaces of eight individual male specimens. Although the full specimen photos do not as easily resolve the orange vs. yellow scales, the magnified views show this well differentiated marbling. Most of the orange sections display a distinct outward trajectory with some wing cells being entirely orange.