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Research Article

Wing Color of Monarch Butterflies (*Danaus plexippus*) in Eastern North America across Life Stages: Migrants Are "Redder" than Breeding and Overwintering Stages

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Monarch butterflies are famous among insects for their unique migration in eastern North America to overwinter sites in Mexico and their bright orange wing color, which has an aposematic function. While capturing migrating monarchs in northeast Georgia, USA, I noticed that many appeared to have unusually deep orange wings. I initiated the current study to compare wing hues (obtained using image analysis of scanned wings) of migrants (captured in 2005 and 2008) to samples of breeding and overwintering monarchs. Consistent with initial observations, migrants had significantly lower orange hues (reflecting deeper, redder orange colors) than breeding and overwintering monarchs. There was also a difference in hue between sexes and a relationship with wing size, such that larger monarchs had deeper, redder hues. The reasons for the color difference of migrants are not apparent, but one possibility is that the longer-lived migrant generation has denser scalation to allow for scale loss over their lifespan. Alternatively, this effect could be confined to the subpopulation of monarchs in the Southeastern United States, which may not be well represented at the Mexican overwintering sites. In any case, this discovery highlights the many questions emerging on the significance of wing color variation in this species.

1. Introduction

The monarch butterfly (*Danaus plexippus*) is one of the world's most well-known insects, being easily identifiable by its bright orange and black wing colors (Figure 1). It is the posterchild for insect conservation, and its image even graces the front cover of this journal. One of the reasons for this attention is certainly the amazing annual migration of the population in eastern North America, which starts in breeding grounds in Canada and the northern United States, and ends some 3000 km away at a select few overwintering sites in the mountains of Central Mexico [1, 2]. There, they form massive clusters of millions of butterflies, and wait until spring, when they remigrate northward to repopulate the breeding grounds [3, 4]. This unique life cycle is completed in many generations. Breeding monarchs normally live for one month, undergoing several generations per summer. The final generation of the late summer is the one that undertakes the migration and overwintering phase, so that these individuals live up to 9 months [5]. Over many decades, a considerable body of literature has been developed around this unique insect, although recent discoveries are revealing how many questions about it yet exist. For example, the functional significance of the orange coloration of the monarch's wings has long been known to be a warning to predators of its toxicity from the cardenolides the larvae sequester from the host plants, which are members of the genus *Asclepias* [6]. However, recent work using modern image analysis techniques has demonstrated there is considerable variation in the degree or shade of orange color among monarchs [7]. This discovery together with the use of today's image analysis computer programs that can easily quantify color variation on images of butterfly wings, opens up a new body of questions regarding the significance of this variation.

The current study was initiated because of certain casual observations regarding monarch butterfly wing color. Specifically, I noticed that monarchs captured during the fall migration sometimes appeared (with the naked eye) to have

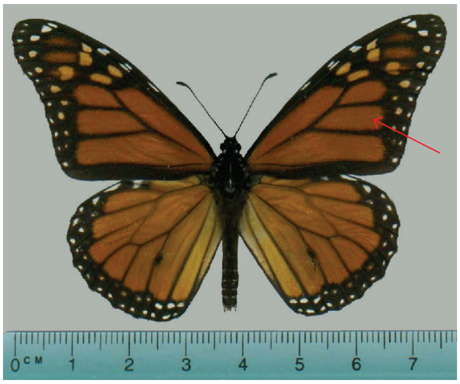

Figure 1: Monarch butterfly, *D. plexippus*. This individual, a male, was captured in September 2005 by AKD. Arrow indicates the cell used for measuring color in this study.

more reddish-colored wings than those reared in lab experiments [7, 8] and than those that had been captured during the summer months. I subsequently developed the current project aimed at determining if this difference was real, by using an image analysis approach to objectively quantify the orange color of monarch wings, similar to that performed recently [7]. With these data, I statistically compared wing colors of monarchs captured during migration to a sample of breeding monarchs and to a collection of overwintering monarchs, and I report the results of this exploration here.

2. Methods

2.1. Butterfly Sources. Three sets of monarch butterfly specimens were examined in this study. The first group ($n = 39$) was collected during the summer of 1997 in Minnesota and Wisconsin (hereafter called "breeding" group), between July 11 and August 30, although 85% of these were collected before August 15. These specimens had been stored at -20°C (individually in glassine envelopes) since capture. The second set were 75 individuals collected at two of the Mexican overwintering sites in February 2008 (hereafter called "overwintering" group). These were netted from random clusters and brought to the lab (with all applicable permits), where they were also stored at -20°C. The third group consisted of monarchs captured during the fall migration in 2005 and 2008 ($n = 29$ and 31; hereafter called "migrant" group) and were killed via freezing immediately after capture and stored at -20°C. All migrants were captured while nectaring on blooming vegetation near Athens, Ga, USA (33.9°N–83.4°W), during the periods of October 14–22, 2005 and September 11–29, 2008.

2.2. Scanning and Processing Wings. All frozen stored specimens were thawed and their forewings were removed for scanning. Scanning procedure generally followed procedures established in prior work [7, 8]. Wings were placed face down on a standard flatbed scanner connected to a laptop computer, and scanned at 300 dpi. The exposure settings on the scanner were set so that the original wing color was maintained (i.e., so that the scanner software did not manipulate the images). All forewings were scanned in this manner (i.e., with this scanner and with these settings). When scanned, the sex of each monarch was also recorded.

When all wings were scanned, each forewing image was imported into the image analysis software, FoveaPro (Reindeer Graphics, Inc., NC, USA) (http://www.reindeergraphics.com/), which works in the Photoshop environment. This program is ideally suited to measure color, and has been used by the author in prior studies [7–11]. Wing color measurement generally followed procedures established in prior work that focused on this species [7] with slight modification. Briefly, the central wing cell of the right forewing (Figure 1) was selected using the "magic wand tool." I considered this selected area to be representative of the orange color of the entire wing. Further, choosing this location to measure provided a convenient border for the selected area. However, since female monarchs have more black scales over their wing veins, which makes their veins appear thicker than males, this means that in general, the selected area was smaller in females than in males. Next, a color-measure routine was initiated which returned the average hue, saturation, and brightness values for all pixels selected (usually between 5 and 10 thousand pixels). In this case, only the hue values were retained, which is in contrast to prior work, where the saturation score was the focus [7]. The hue was more appropriate to examine in this study since the magnitude of the color variation was thought to be large based on initial observations of migrant monarchs (i.e., ranging from pale yellow to orange to dull red). Hue is measured in degrees (i.e., 0–360), with 0 representing perfect red, and in this study the scores tended to be between 25 and 40 (see results). Finally, a secondary routine was run which returned the area of the entire forewing in mm^2, based on prior calibration of the software using a scanned ruler.

2.3. Data Analysis. Since two sets of migrants were obtained in this study, I initially examined these data for possible differences using a two-sample t-test, where the wing hue was the dependent variable and the year was the independent. This test revealed no significant variation (df $= 58$, $t = -0.811$, $P = .421$), therefore these data were pooled for subsequent analyses. I then used general linear modeling procedures to examine the possible variation in monarch wing color (hue) among life stages, that is, breeding, migrating, and overwintering. Thus, the analysis included wing hue as the response variable, "group" as a categorical independent, as well as gender. Finally, butterfly size (indexed by forewing area) was included as a continuous covariate. All two-way interaction terms were initially included but removed if found nonsignificant. All analyses were conducted using Statistica 6.1 software [12].

3. Results

Across all monarchs examined in this study ($n = 174$), there was considerable variation in wing hue scores, which ranged from 25.3 to 40.1 degrees (Figure 2). Lower hue scores

Table 1: Summary of general linear model examining factors influencing wing hue of monarch butterflies in this study. Groups were "breeding," "overwintering," and "migrating." Nonsignificant interaction terms (i.e., where $P > .05$) were removed.

Variable	df	MS	F	P-value
Sex	1	475.93	187.47	<.0001
Group	2	391.56	154.23	<.0001
Wing area	1	12.35	4.87	.0288
Sex*group	2	10.99	4.33	.0147
Error	167	2.54	—	—
Total	173	—	—	—

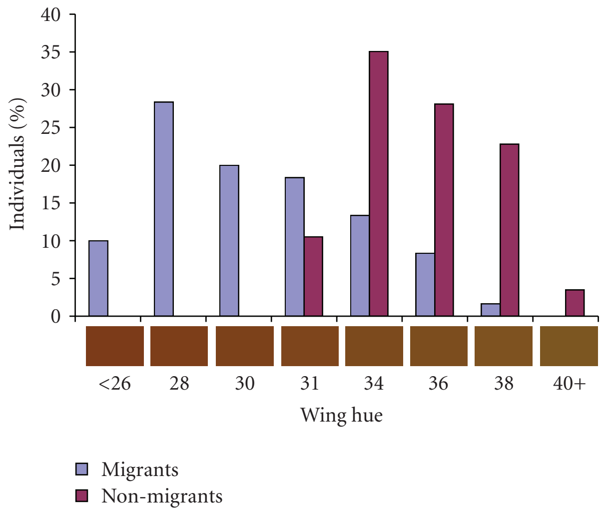

Figure 2: Frequency distribution of wing color (hue score, measured in degrees) observed among all migrating ($n = 60$) and nonmigrating ($n = 114$) monarchs. Colored scale included which shows the hue scores of each category.

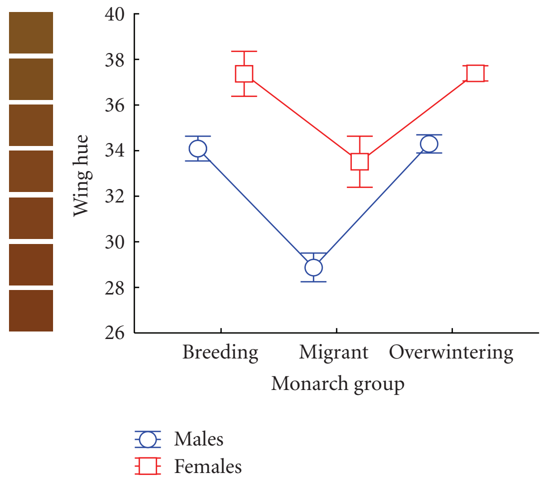

Figure 3: Average wing hue score (in degrees) across all groups for male ($n = 107$) and female ($n = 67$) monarchs in this study. Error bars represent 95% CI.

reflect deeper orange colors, almost nearing red, while the higher scores indicate generally paler, more yellowed wings. In the statistical analysis of wing hue, there was a significant effect of group ($P < .0001$; Table 1) in that migrants had significantly lower hues than all other life stages (Tukey's pos-hoc test; Figure 3). The mean hue of migrants was 30.5 and the hues of breeding and overwintering monarchs were 35.2 and 35.6, respectively. To visually depict this large difference between migrants and other groups, Figure 4 shows selected forewings from each group. Note the color of the orange wing cells in each group. Furthermore, the distribution of migrant monarchs is graphed separately from other nonmigrant groups in Figure 2 to highlight the large difference in wing color of migrants.

In addition to the differences among stages, I also discovered that males differ in wing hue from females ($P < .0001$; Table 1). In all three life stages, males had significantly lower hue scores than females (Figure 3). In other words, the orange color on male monarch wings tends to be deeper, or more reddish, while female monarchs have a paler orange color. However, the magnitude of this gender difference differed among groups, as evidenced by the significant interaction term of sex*group in the final model ($P = .0147$; Table 1). This interaction effect can also be seen in Figure 3, where the overall difference between male and female hue scores was the greatest in the migrant group (4.6 degrees difference), versus 3.3 degrees for breeding and 3.1 degrees for overwintering monarchs.

Finally, there was a surprising effect of wing size on hue score ($P = .0288$; Table 1). This effect was negative, such that large wings tended to have lower hue scores. Put another way, larger monarchs tended to be more red-colored. The interaction terms of wing area*sex and wing area*group were not significant, meaning that this relationship held for both sexes and across all life stages.

4. Discussion

The results of this exploratory study corroborate the initial observations made of the wings of migrating monarchs. That is, migrating monarch butterflies appear to have significantly deeper orange wings than they do during the summer. Further, I observed this phenomenon in two separate migrations (2005 and 2008), and in both cases the migrants' wing colors were similarly deep orange or nearly red (and were statistically similar). Thus, this phenomenon is not random but appears real. This discovery then represents the first morphological trait shown to be different among the final summer migratory generation and the mid-summer breeding generations. The reason for this difference is not clear, although one could speculate that the deeper orange color may help to absorb solar energy, which would enable flight at lower temperatures. Alternatively, the deep orange could reflect some physiological shift by larvae of the final generation (i.e., allocating resources from other processes to pigment production).

Besides the differences in wing color found between migrating and breeding monarchs, a more surprising result

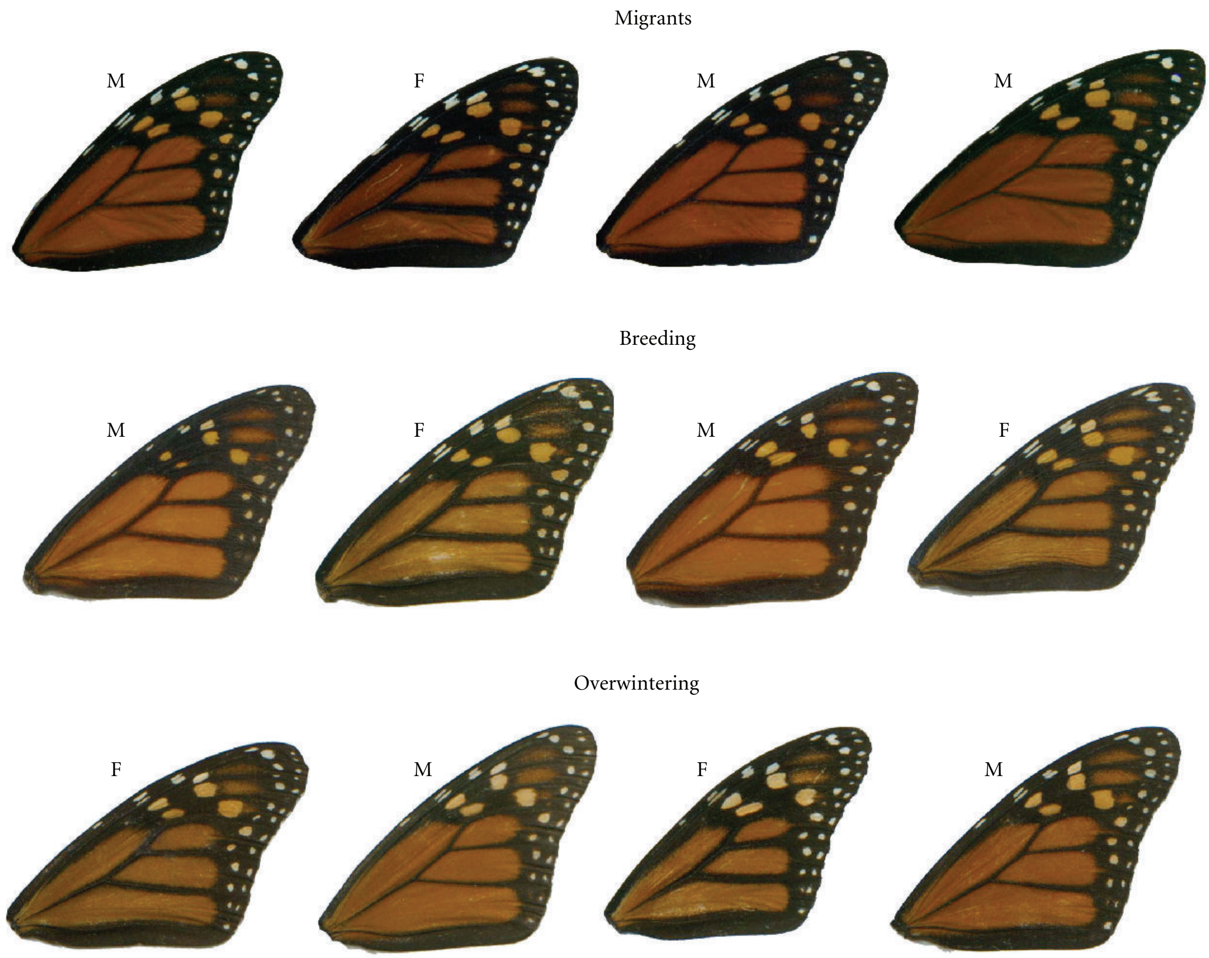

Figure 4: Forewings of selected migrant, breeding, and overwintering monarch butterflies. Note the difference in orange color between migrants and all others. All wings were scanned with the same scanner using the same settings. Males (M) and females (F) are indicated.

of this study was that the wing color of *migrating* monarchs did not resemble that of *overwintering* monarchs. The overwintering monarchs all appeared to have the same pale-orange color of breeding monarchs. How then, is this possible if they are all cohorts of the same generation? Does the migrant wing color fade over time while at the overwintering locales? While there is little research on this subject, the answer to this question would probably be yes. This generation is certainly the longest lived by far, and it may make sense that the migratory generation produces heavily pigmented wings to account for the eventual scale loss over time.

A second explanation may be that this phenomenon (of deeper-orange migrants) is unique to the population of monarchs in the southeastern United States, and that monarchs from this area are not well represented at the Mexican overwintering site. In support of this idea, recent work examining migration routes of monarchs indicated that monarchs migrating along the eastern seaboard have a reduced chance of reaching the overwintering sites than those in the interior United States [13]. Furthermore, migrating monarchs captured and tagged in the Florida Panhandle have only a 1 in 4000 chance of being recovered at Mexico overwintering sites (cited in [13]). In contrast, the normal Mexican recovery rate for tagged monarchs from the eastern population as a whole is 1 in 250 [14]. Thus, monarchs migrating through Georgia may not be well represented at the Mexican overwintering site. Clearly, future work should examine wing colors of migrating monarchs from other regions to determine how widespread this phenomenon is. Only then, will we know if this phenomenon is unique to the southeast, or if all migrants display this trait. If the latter is true, then the discrepancy between migrant and overwintering wing color would become even more perplexing.

There were two other surprising findings in this study. First, I found that males and females differ statistically in wing hue, with males tending to have a deeper orange color than females. This species was already known for another sexually dimorphic trait, in that females have a greater degree of black pigmentation [8], see Figure 4, but it would seem that the sexes differ in other features as well. Secondly, there was an unexpected relationship found (although wing size was included as a covariate in the analysis to account for this possibility) between wing size and hue, such that larger individuals tended to have a deeper orange to dull-red color. Like most butterflies, large body size in monarchs is generally considered an indicator of fitness [15, 16], so deeper orange wing color may be a sign of more robust individuals. However, a greater understanding is needed of the causal mechanisms behind wing color formation before this idea can be fleshed out further.

Clearly, there are a number of findings from this work that open up a large number of additional questions on the significance of monarch butterfly wing color, and which will take much more study to address. However, since even these simple comparisons of wings between breeding, migrating, and overwintering monarchs here proved fruitful, the possibility for further exciting discoveries in this research avenue is high. This is especially true since the discoveries made here only serve to emphasize the large gaps that remain in our knowledge of the biology of this unique and charismatic insect.

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