

Research Article

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Yiwen Li*, Amanda A Pierce, Jacobus C. de Roode

Variation in Forewing Size Linked to Migratory Status in Monarch Butterflies

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Abstract: Long-distance migration can be seen throughout the animal kingdom and can have large impacts on population dynamics and species distributions. The act of migration itself also affects the evolution of a species, as evolutionary forces select for certain characteristics in animals conducting long-distance migration. Monarch butterflies are best known for their annual migration from Canada and the northern United States to central Mexico, but some populations of monarchs have lost the ability to migrate. Previous research found that migratory monarchs had larger, more elongated wings than their non-migratory counterparts and it was hypothesized that these traits were beneficial for migration. However, Bergmann's rule - which predicts larger body sizes with increasing latitude - could also explain this pattern as migratory populations are found at higher latitudes. To understand the role of wing dimensions in migration, we examined forewing size and shape of migratory and non-migratory monarchs from seven worldwide populations varying in latitude. Results showed that larger forewing size was indeed correlated with migratory status rather than latitude. However, migratory monarchs did not have more elongated forewing shape than non-migratory monarchs across the globe. Our study indicates that size may play a larger role than shape in long-distance migratory capability.

Keywords: long-distance migration, *Danaus plexippus*, wing morphology, Bergmann's rule

1 Introduction

Seasonal animal migrations occur in response to changing habitats, competition, and predation [1-7]. Migratory behavior involves synchronized and directional movement [8], and can have large effects on species interactions and habitat ecology [9-11]. Migrating species have evolved to adapt to a changing environment [5] and to survive the migration and thus, their body characteristics are under strong selective pressure. The benefits associated with increased accessibility of geographical and ecological resources are compounded with increased energy demand and mortality risk during migration. This results in morphological and behavioral adaptations, which themselves may be costly to maintain [5]. For flying animals, traveling such distances requires a number of migratory traits such as efficient metabolism, flight directionality, and flight endurance [8,12,13]. Indeed, several morphological traits, such as body shape or size, have been found to differ between migratory and non-migratory species. For example, a study examining phenotypic variations of Libellulid dragonflies revealed several flight associated traits differentiating migratory and non-migratory species, including wing size [14]. Accordingly, further investigation into the traits that are of importance to migration can help us better understand the evolution of migration.

Each autumn, millions of monarch butterflies, *Danaus plexippus*, migrate from Canada and the northern parts of the United States to overwinter in central Mexico [15]. In addition, monarchs occur west of the Rocky Mountains, from where they migrate to the California coast [16]. Eastern monarchs may fly over 4000 km to reach their overwintering sites whereas western monarchs generally fly less than 500 km. While many animals acquire migratory behaviors through learning [4], this is not the case for monarch butterflies. A migration cycle is complete after a monarch butterfly travels to Mexico and 3-4 generations of its offspring make the return trip back north [17]. Therefore, because multiple generations separate each migration event, the migratory behavior in monarchs is innate.

*Corresponding author Yiwen Li, Emory University, USA, E-mail: yiwen.li@emory.edu

Amanda A Pierce, Emory University, Department of Biology; University of North Carolina at Chapel Hill, Department of Biology

Jacobus C. de Roode, Emory University, Department of Biology

Although the annual migration of monarch butterflies is a renowned phenomenon, monarchs have colonized many parts of the world where they have formed non-migratory populations [18,19]. These populations not only differ in migratory status, but are also genetically differentiated and have been shown to differ in the Collagen IV alpha-1 gene, which is involved in muscle development and efficiency [18-20]. Previous work on wing morphology by Altizer & Davis (2010) indicated that migratory monarchs from eastern and western North America have larger wings than non-migratory South Florida and Hawaii monarchs and larger, more elongated wings than the non-migratory populations from Puerto Rico and Costa Rica. Both wild-caught and lab-reared monarchs supported these findings, which implies that monarch wing morphology is at least partly genetically based. However, there is a second competing hypothesis as to why the examined non-migratory monarchs had smaller wing sizes than their migratory counterparts, as studies have shown environmental factors can also impact these traits. A study on morphological adaptations of the common nightingale revealed that, rather than being associated with migration distance, elongated wings were positively associated with longitude and phenology at breeding destinations [21]. This suggests morphological changes thought to be related to long-distance migration may be driven by environmental factors at destination regions instead. Another study examined phenotypic consequences of a rapid range expansion in the damselfly, *Erythromma viridulum*, and revealed a positive correlation between latitudinal variation and body size in newly formed populations; this suggests that Bergmann's rule was maintained through dispersal [22]. Bergmann's rule states that in species that are broadly distributed, populations of larger body size tend to be found in colder environments, or increasing latitudes, while species of smaller body size are found in warmer regions, typically closer to the equator [23,24]. Because monarch butterflies have also experienced a recent range expansion to colonize a wide range of latitudes [18,19], it is important to explore the impact of latitudinal variation on monarch body traits in addition to their migratory status [18]. In the study by Altizer & Davis (2010), the butterfly populations with smallest wing sizes also happened to be those closest to the equator. In ectotherms the relationship between morphology and latitude is not solely a result of phenotypic plasticity to temperature, but can be the result of genetic differentiation among populations [25,26]. Thus, the phenotypic and genetic variation in wing morphology found by Altizer & Davis could be at least partly driven by latitudinal variation.

Therefore, the goal of this project was to assess whether forewing size and forewing shape differ based on the migratory status of monarch butterflies or whether they vary by latitude instead. If the latter, we would expect monarch populations at lower latitudes to have smaller wings and monarchs at higher latitudes to have larger wings. However, if migration is instead driving this pattern, we would expect wing size and shape variation to be determined by migratory status. We included monarch butterflies previously caught during 2007-2012 that differ in migratory status, and included non-migratory populations from varying latitudes. We examined the forewing size and shape differentiation among migratory monarch butterflies from eastern and western North America, and non-migratory monarchs from South Florida, Hawaii, Spain, Morocco, and Aruba.

2 Methods

2.1 Geographic sampling locations

We used monarchs from eastern North America, western North America, South Florida, Hawaii, Spain, Morocco, and Aruba (Figure 1). Collection years and latitude of sampling site are listed in Table 1. All monarchs were net-captured as adults by scientists and migratory monarchs were captured during the migration season. Latitudes for eastern and western United States monarchs are not listed because these samples were collected at migratory stopover and overwintering locations, not where monarchs developed and resided during the breeding season. The western overwintering location of Pismo Beach, CA has been shown to receive the most migrants coming from the northern region of the intermountain west [27]. The eastern site in St. Marks, Florida is a stopover location for monarchs traveling along the eastern seaboard [28]. Sample numbers and mean forewing measurements are listed in Table 2.

2.2 Wing measurements

We focused on forewings because the previous study by Altizer & Davis (2010) indicated that monarch forewings cover the majority of their hindwings during soaring flying, and thus forewing morphology is more likely to contribute towards the exposed wing surface area. Forewings were scanned and the Fovea Pro plugin (Reindeer Graphics, Inc., Asheville, NC) for Adobe Photoshop was used to measure forewing area, length, breadth and perimeter

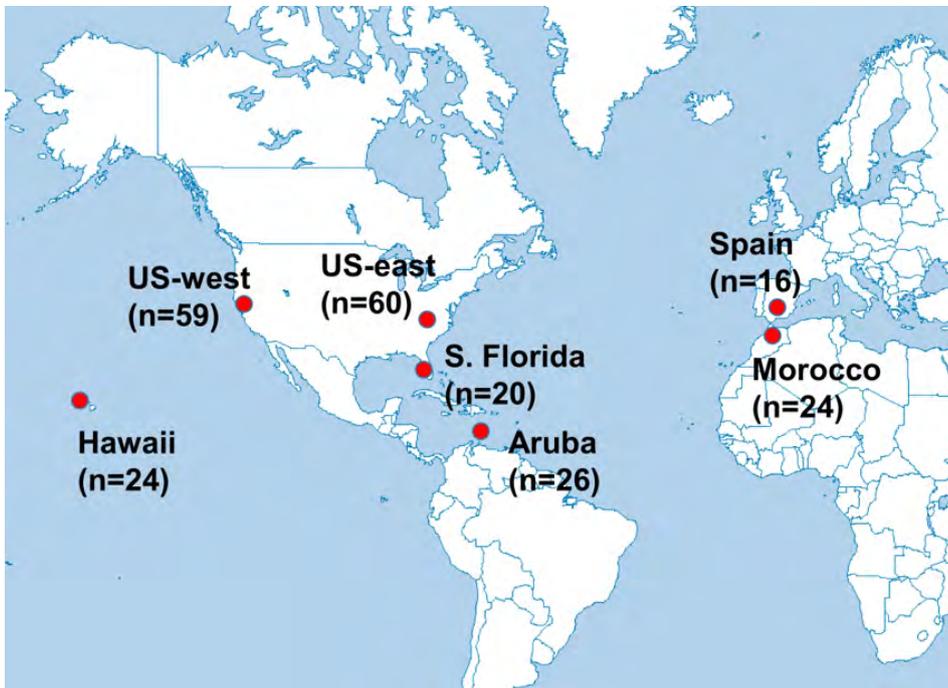


Figure 1. Sampling locations of seven migratory and nonmigratory monarch butterfly populations. Sample sizes are within parentheses.

Table 1. Sampling data information: location, year and latitude.

Sampling location	Sampling city/region	Migratory Status	Year collected	Latitude
western US	Pismo Beach, CA	Migratory	Feb. 2008	
eastern US	St. Marks, FL	Migratory	Oct. 2007	
South Florida	Miami	Non-migratory	April 2011	25.7753
Aruba	Noord	Non-migratory	Jan. 2012	12.5667
Hawaii	Hawaii; Maui; Kauai	Non-migratory	Nov. 2009	21.3114
Spain	Cordoba	Non-migratory	Sept. 2012	37.8833
Morocco	Belyounech	Non-migratory	Oct. 2012	35.1714

(Figure 2). Forewing area in millimeter² was measured of the total forewing surface region, length in millimeters was measured from the distal tip to the thorax attachment point of the forewing, breadth in millimeters was perpendicular to the length, and perimeter was measured in millimeters for the entire forewing. From this we calculated aspect ratio, by dividing length by breadth of the forewing, and roundness, by using the equation $4 \cdot \pi \cdot \text{area} / (\text{perimeter})^2$, as described in Altizer & Davis (2010). Using Principal Component Analysis (PCA), forewing area, length, and width were reduced into one variable to measure forewing size, while forewing aspect ratio and roundness were reduced to a second variable to measure forewing shape. ANOVA using population as the fixed effect variable and Tukey's Test were then performed to examine the forewing size and shape differences among the migratory and non-migratory populations. We also tested for the effect of

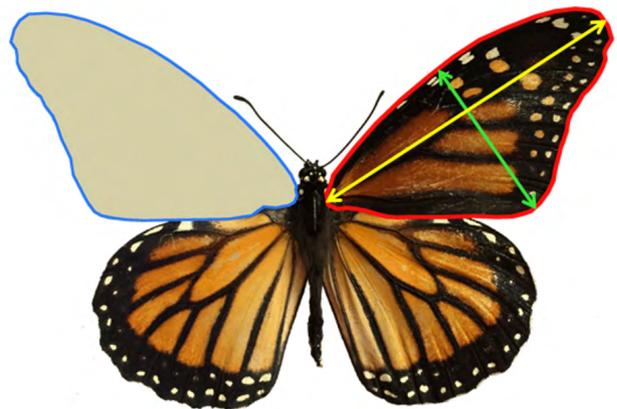


Figure 2. Monarch butterfly photograph, indicating forewing measurements: area (grey shading with blue border), length (yellow arrow), breadth (green arrow), and perimeter (red border).

Table 2. Mean forewing dimensions. Means are shown separately for males and females (\pm SE).

Sex	Population	N	Length (mm)	Breadth (mm)	Aspect Ratio	Area (mm ²)	Roundness
Male	eastern US	40	51.946 \pm 0.257	26.358 \pm 0.162	1.972 \pm 0.008	865.220 \pm 8.828	0.663 \pm 0.002
	western US	36	51.003 \pm 0.255	25.868 \pm 0.141	1.972 \pm 0.007	837.908 \pm 8.129	0.664 \pm 0.002
	S. Florida	14	48.618 \pm 0.659	24.868 \pm 0.308	1.955 \pm 0.014	775.912 \pm 18.911	0.661 \pm 0.004
	Aruba	17	44.414 \pm 1.132	23.520 \pm 0.582	1.912 \pm 0.012	682.712 \pm 32.030	0.686 \pm 0.003
	Hawaii	10	47.574 \pm 0.880	24.333 \pm 0.428	1.955 \pm 0.015	737.184 \pm 23.345	0.661 \pm 0.006
	Spain	8	48.474 \pm 0.426	24.827 \pm 0.311	1.954 \pm 0.019	776.952 \pm 16.027	0.680 \pm 0.004
	Morocco	14	48.242 \pm 0.986	23.977 \pm 0.586	2.016 \pm 0.085	744.276 \pm 30.472	0.662 \pm 0.005
Female	eastern US	20	50.633 \pm 0.484	25.735 \pm 0.283	1.969 \pm 0.008	826.185 \pm 16.662	0.665 \pm 0.003
	western US	23	51.174 \pm 0.390	26.070 \pm 0.188	1.963 \pm 0.009	844.022 \pm 11.086	0.665 \pm 0.003
	S. Florida	6	48.477 \pm 1.713	24.964 \pm 1.001	1.941 \pm 0.015	788.981 \pm 54.473	0.675 \pm 0.004
	Aruba	9	44.414 \pm 0.600	23.208 \pm 0.323	1.914 \pm 0.011	665.206 \pm 17.078	0.681 \pm 0.003
	Hawaii	14	48.043 \pm 0.602	24.536 \pm 0.329	1.959 \pm 0.041	760.131 \pm 17.541	0.672 \pm 0.003
	Spain	8	47.229 \pm 1.221	24.067 \pm 0.562	1.962 \pm 0.019	737.622 \pm 33.177	0.676 \pm 0.007
	Morocco	10	48.287 \pm 0.925	24.374 \pm 0.481	1.982 \pm 0.011	756.003 \pm 27.497	0.667 \pm 0.002

migratory vs. non-migratory type using model comparison and a linear mixed effects model with migratory status as a fixed effect and population as a random effect using the lme4 package [29]. Finally, to test whether forewing size was correlated with latitude, we performed a correlation analysis for non-migratory monarch populations using the rcorr function in the Hmisc package [30]. All statistical analyses were performed in R 3.1.2 [31]. Each analysis was performed separately for males and females and corrected for multiple comparisons using a Bonferroni adjustment ($p=0.025$).

3 Results

3.1 Wing morphology

Migratory monarchs from eastern and western North America had significantly larger wings, but their wings were not consistently more elongated than those of non-migratory monarchs (Figure 3). ANOVA and Tukey's test confirmed the largest difference in forewing size was between North American migratory populations (US-east and US-west) and non-migratory Aruba monarchs for both males (US-east vs. Aruba, $p<0.01$; US-west vs. Aruba, $p<0.01$) and females (US-east vs. Aruba, $p<0.01$; US-west vs. Aruba, $p<0.01$). Pairwise comparisons among populations for male and female wing size are shown in Table 3. Overall, migratory status was found to affect wing size in males ($\chi^2(1)=8.67$, $p=0.003$) and females ($\chi^2(1)=8.88$, $p=0.003$).

ANOVA and Tukey's test also showed significant differences in forewing shape between North American migratory populations and Aruba non-migratory monarchs for both males (US-east vs Aruba, $p<0.01$; US-west vs Aruba, $p<0.01$) and females (US-east vs Aruba, $p<0.01$; US-west vs Aruba, $p<0.01$). In all of these cases, migratory monarchs had more elongated wings than the non-migratory Aruba monarchs. Additionally, with respect to forewing shape, male Aruba monarchs had significantly less elongated forewings than males in Morocco and S. Florida ($p<0.015$) while female Aruba monarchs had significantly less elongated forewings than females in Morocco ($p<0.01$). While migratory populations were found to have more elongated wings than the non-migratory Aruba population, we did not find an overall effect of migratory status on wing shape in either males ($\chi^2(1)=0.70$, $p=0.40$) or females ($\chi^2(1)=2.39$, $p=0.12$).

3.2 Latitude

In the non-migratory populations, we also tested whether the variation in forewing size was due to latitudinal differences rather than migratory status. We performed a correlation analysis examining male forewing size and population latitude and did not find a significant relationship ($r=0.77$, $p=0.13$). Similarly, we found no significant relationship between latitude and female forewing size ($r=0.47$, $p=0.43$). We also did not find a significant relationship between latitude and forewing

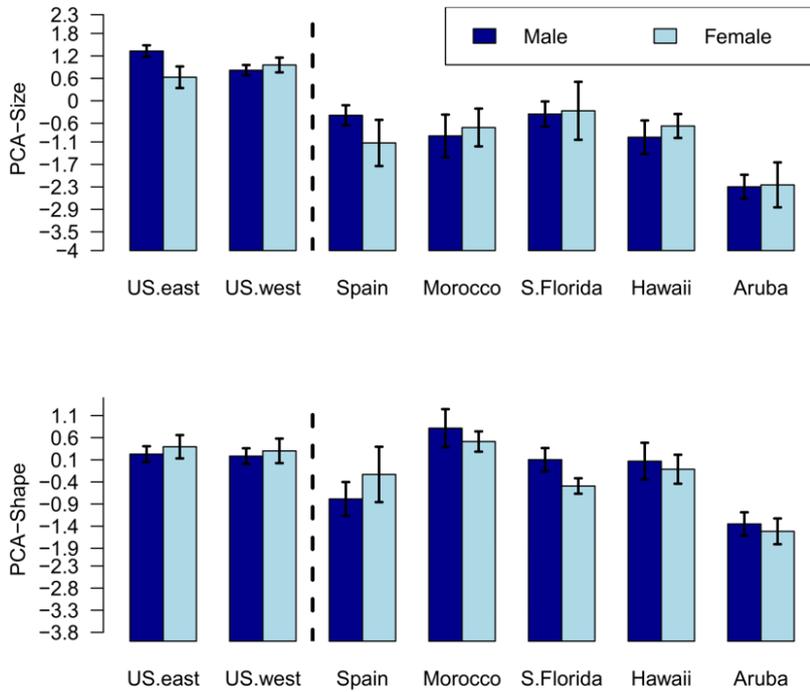


Figure 3. Average male and female forewing size and shape from migratory monarchs (eastern and western North America) and non-migratory monarchs (South Florida, Hawaii, Spain, Morocco, and Aruba). The y-axes represent the average (± 1 s.e.) forewing size (greater PCA-size means larger wings) and forewing shape (greater PCA-shape means more elongated wings) among different monarch populations, as based on principal component analysis. The non-migratory populations are ordered along the x-axis by decreasing latitude. Migratory monarchs (US-east and US-west) had significantly larger forewing size than non-migratory populations (South Florida, Hawaii, Spain, Morocco, and Aruba). In contrast, migratory monarchs did not show a strong pattern of forewing elongation comparing to the non-migratory populations. Aruba monarchs had significantly less elongated forewings than many populations.

Table 3. Forewing Size Comparison. Males and females are compared in separated tables. Sizes are considered to be equal if they are not significantly different from one another using an $\alpha=0.025$ cut-off value.

Male	Population	East	West	Morocco	Spain	S. Florida	Hawaii	Aruba
	East		E=W	E>M	E>S	E>S.F	E>H	E>A
	West	W=E		W>M	W=S	W=S.F	W>H	W>A
	Morocco	M<E	M<W		M=S	M=S.F	M=H	M=A
	Spain	S<E	S=W	S=M		S=S.F	S=H	S>A
	S. Florida	S.F<E	S.F=W	S.F=M	S.F=S		S.F=H	S.F>A
	Hawaii	H<E	H<W	H=M	H=S	H=S.F		H=A
	Aruba	A<E	A<W	A=M	A<S	A<S.F	A=H	

Female	Population	East	West	Morocco	Spain	S. Florida	Hawaii	Aruba
	East		E=W	E=M	E=S	E=S.F	E=H	E>A
	West	W=E		W=M	W>S	W=S.F	W>H	W>A
	Morocco	M=E	M=W		M=S	M=S.F	M=H	M=A
	Spain	S=E	S<W	S=M		S=S.F	S=H	S=A
	S. Florida	S.F=E	S.F=W	S.F=M	S.F=S		S.F=H	S.F=A
	Hawaii	H=E	H<W	H=M	H=S	H=S.F		H=A
	Aruba	A<E	A<W	A=M	A=S	A=S.F	A=H	

shape in non-migratory males ($r=0.47$, $p=0.42$) or females ($r=0.78$, $p=0.12$).

4 Discussion

Our study quantified phenotypic traits that are important in facilitating long-distance migration. To perform long distance migration, migratory monarchs evolved morphological and physiological adaptations to develop more efficient metabolism, perform flight directionality, and enhanced flight endurance [19,32-34]. Analyzing traits linked to migratory status of monarch butterflies provides us with insights into how evolutionary selective pressures favor certain characteristics for long distance animal migration. These morphological changes of migratory traits found in monarchs are also evident and applicable to other species, which experience similar selective pressure during long-distance migration. In birds, for instance, migratory species were found to display greater wing spans to facilitate efficient flight compared to non-migratory bird populations [35]; likewise, in dragonflies wing shape significantly varied with migratory status [36].

We determined if differences in wing size and shape were correlated with migratory status or were instead correlated with latitude and thus more consistent with Bergmann's rule. Bergmann's rule states that larger body sizes tend to be found at increasing latitudes, while smaller body sizes tend to be located in warmer regions and closer to the equator [23,24]. Our results found no relationship between monarch butterfly forewing size and population latitude and instead found that forewings were significantly larger in migratory versus non-migratory monarchs. This is similar to the Altizer & Davis (2010) study on monarch wing morphology which showed that eastern and western North American migratory populations have larger wings compared to non-migratory populations in South Florida, Hawaii, Puerto Rico and Costa Rica. It would therefore seem that it is indeed migratory status, rather than latitudinal differences, that best explains the differences in forewing size.

Aerodynamic theory suggests that greater wing size is crucial for long-distance migration [37] and a previous study revealed that birds with larger wing size fly faster than birds with smaller wing sizes given the same amount of energy input [21]. This phenomenon of energy conservation with greater wing size is therefore beneficial for long-distance migratory species, as the traveling conditions are less predictable and food sources along the route could be scarce. For migratory monarchs, more efficient flying with less energy being consumed

per distance flown could result in fewer stopovers and would provide a quicker and more likely arrival at the final destination. In contrast, non-migratory monarch populations in South Florida, Hawaii, Spain, Morocco, and Aruba are not under strong selective pressure for long-distance travel, and our results confirmed that they developed smaller forewings. Shorter forewings allow for slow and more controlled flight [38], which may be beneficial for non-migratory, resident monarchs.

Our results also indicate that forewing shape does not significantly differentiate eastern and western North America migratory monarchs from the non-migratory monarch populations. Butterflies in general utilize a combination of efficient gliding and active flapping [39]. Theoretically, species with elongated forewings and a larger aspect ratio employ extensive gliding which lowers their energy consumption whereas species using active flapping flight display more compacted forewing shape which allows for increased maneuverability [40]. However, migrating monarchs are frequently exposed to wind drift and are known to perform intermittent gliding and soaring flight among active flight [41,42]. As suggested, more elongated forewings may be beneficial for lower energy consumption and gliding behavior, but may also hinder active flight, which is also an important part of migration. This trade-off may explain the non-significant forewing shape differences among migratory and non-migratory monarch populations.

While the Altizer and Davis (2010) study found that eastern monarchs, which travel the longest migratory distances, had more elongated forewings than most non-migratory populations, when looking at wild caught eastern and western migratory monarchs they primarily found a significant difference in wing shape when compared with Costa Rica and Puerto Rico populations. This is similar to our findings in that we did not see a consistent difference in forewing shape based on migration status across all examined populations. While forewing shape may be beneficial to long-distance migration, as is the case in many other migrating species, it appears that forewing size is under greater selection in monarch butterflies. A recent study examining natal origin of western migrating monarchs found a positive relationship between distance to the overwintering location and forewing size [27]. However, the same study did not find a relationship between more elongated wings and greater migratory distance. This is in accord with our study, which suggests that forewing size, rather than shape, is an important contributor to the migratory ability of monarch butterflies. Conversely, it may be the case that changes in wing size rather than wing shape evolve faster in the

non-migratory populations, leading to greater differences in size among migratory and non-migratory monarchs. In this scenario, elongated wings may still be beneficial for long-distance migration, but could explain why more rounded wings are not consistently seen in non-migratory populations. Indeed, a study examining migratory eastern North American monarchs found that earlier migrants tend to have more elongated forewings compared to later migrants, which suggests that elongated wings may provide a benefit in long-distance monarch migration [43].

We found that monarchs in Aruba have significantly rounder wings compared to many other sampling locations, including other non-migratory locations like Hawaii and Morocco. Again, Altizer and Davis (2010) found the forewings of monarchs in Costa Rica and Puerto Rico to be less elongated than both migratory groups and another study found that monarchs in Cuba have less elongated wings as well [44]. While these locations are all non-migratory, they also all share a general locality of being in or sharing coastline with the Caribbean Sea. Since other non-migratory populations from Hawaii, S. Florida and Morocco did not exhibit significantly rounded wings, it is possible that rounded wings may instead be correlated with some aspect of the Caribbean, rather than with migration status.

Overall, our results suggest that forewing size is a major morphological trait related to monarch migration. As such, identifying the genes underlying wing size, for example through Quantitative Trait Analysis on the F2 offspring of migratory and non-migratory monarchs, would be a fruitful avenue towards characterizing the genetic basis of migration.

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