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Got milkweed? Genetic assimilation as potential source for the evolution of nonmigratory monarch butterfly wing shape

Kyra J. A. Nixon | Harald F. Parzer

Department of Biological Sciences, Fairleigh Dickinson University, Madison, New Jersey, USA

Correspondence
Harald F. Parzer, Department of Biology, Fairleigh Dickinson University, Madison, NJ, USA.
Email: hparzer@fdu.edu

Abstract

Monarch butterflies (*Danaus plexippus*) are well studied for their annual long-distance migration from as far north as Canada to their overwintering grounds in Central Mexico. At the end of the cold season, monarchs start to repopulate North America through short-distance migration over the course of multiple generations. Interestingly, some populations in various tropical and subtropical islands do not migrate and exhibit heritable differences in wing shape and size, most likely an adaptation to island life. Less is known about forewing differences between long- and short-distance migrants in relation to island populations. Given their different migratory behaviors, we hypothesized that these differences would be reflected in wing morphology. To test this, we analyzed forewing shape and size of three different groups: nonmigratory, lesser migratory (migrate short-distances), and migratory (migrate long-distances) individuals. Significant differences in shape appear in all groups using geometric morphometrics. As variation found between migratory and lesser migrants has been shown to be caused by phenotypic plasticity, and lesser migrants develop intermediate forewing shapes between migratory and nonmigratory individuals, we suggest that genetic assimilation might be an important mechanism to explain the heritable variation found between migratory and nonmigratory populations. Additionally, our research confirms previous studies which show that forewing size is significantly smaller in nonmigratory populations when compared to both migratory phenotypes. Finally, we found sexual dimorphism in forewing shape in all three groups, but for size in nonmigratory populations only. This might have been caused by reduced constraints on forewing size in nonmigratory populations.

KEYWORDS
forewing evolution, genetic assimilation, geometric morphometrics, migration, monarch butterfly

1 | INTRODUCTION

Migration, the synchronized directional movement of a population, is a seasonal behavior found in many animal species (Liedvogel et al., 2011). It is thought that migrating animals can reduce resource competition, avoid predation, or avoid unsuitable environmental conditions due to seasonal changes, as is the case for monarch butterflies, *Danaus plexippus* (Alerstam et al., 2003). Monarchs are native across North America, however, populations of this mostly tropical genus are unable to survive the freezing winter temperatures (Miller et al., 2012). Thus, populations that occur outside of tropical ranges overwinter as adults in warmer locations each year, most famously in the fir forests in Mexico's Central Highlands (Urquhart & Urquhart, 1978). While several hundred other butterfly species are known to migrate for similar reasons (Chowdhury et al., 2021), *D. plexippus* migration is unique among all butterfly species due to its enormous distance, as single monarch individuals travel up to 3000 miles from Canada to central Mexico to their overwintering grounds each year (Li et al., 2016; Solensky, 2004). To navigate this journey, monarchs possess a sun compass, in which they use daylight cues to aid in their directions (e.g., Guerra et al., 2014; Reppert & de Roode, 2018), as well as a magnetic compass which allows them to orient themselves in the proper direction, even in the absence of guiding daylight (Guerra et al., 2014; Reppert & de Roode, 2018). Interestingly, the overwintering individuals do not complete their return journey to the north within the same generation as they do when migrating southward. Instead, after completing their overwintering in Mexico, monarchs migrate to the Gulf Coast to lay their eggs, after which they usually die (Miller et al., 2012). Then, each developing generation moves progressively further north, laying eggs along the way such that the newly hatched generation continues the trek on their own (Miller et al., 2012), presumably using similar navigational mechanisms as southward migrating monarchs (Guerra et al., 2014). The northern limit of their range is therefore only reached after third to fourth generations of monarchs within the same year (Miller et al., 2012). As both migratory types are part of the same population and only differ in the seasonal environment in which they develop, differences in migratory behavior between north- and south-migrating individuals are achieved by phenotypic plasticity and not by genetic differences.

However, not all monarchs migrate (Pierce et al., 2014; Reppert & de Roode, 2018). For example, individuals found in southern regions such as Florida, as well as (self-)introduced populations on various Caribbean, Atlantic, and Polynesian Islands and larger regions like Australia, tend to stay year-round at these locations (Freedman et al., 2020; Pierce et al., 2014). This range of migratory behaviors (from migratory to nonmigratory populations) has led to noted variation in forewing size among migratory types, which can be detected even with simple morphometric tools (Altizer & Davis, 2010; Li et al., 2016). Here, it was shown that both southward-bound long-distance migrants (hereafter, migratory monarchs) and multigenerational northward-bound migrants that travel shorter distances (hereafter, lesser migratory monarchs) possess larger and more elongated wings when compared to nonmigratory monarchs (Freedman et al., 2020; Li et al., 2016), suggesting selection based on different flight requirements of these populations. While the exact genetic underpinnings of wing shape differences between nonmigratory and migratory monarchs are not known, the Collagen IVs alpha-1 gene, which plays a role in muscle development and efficiency, is cited as a possible target gene (Zhan et al., 2014). However, it is unclear if this gene would affect the above-mentioned forewing shape variation and/or if other additional mutations contribute to the observed differences. Regardless of the exact underlying genetics, these differences have been shown to be heritable, with forewing shape varying between genetic lines (Altizer & Davis, 2010; Li et al., 2016).

While it seems clear that there are adaptive wing shapes between migratory and nonmigratory populations (Altizer & Davis, 2010), possible variation between migratory and lesser migratory monarchs is of special interest, but poorly understood. Unlike migratory and nonmigratory populations, both migratory and lesser migratory behavioral phenotypes develop from the same genetic background; thus, any differences in migratory behavior and associated phenotypes are induced by the environment alone, therefore not heritable, but phenotypically plastic (e.g., Goehring & Oberhauser, 2002; Moczek, 2010; West-Eberhard, 2003). If wing shape and size are phenotypically plastic traits, as previously suggested with dietary change experiments (Berns, 2014; Soule et al., 2020), then the induced phenotypic variation could serve as an important source for heritable variation via genetic assimilation (Moczek, 2010; Via & Lande, 1985; Waddington, 1953, 1956), which is also known as genetic accommodation (West-Eberhard, 2003).

Genetic assimilation is a process in which an environmentally induced phenotype, like monarch butterfly wings, becomes part of the genotype (Waddington, 1953, 1956). For example, when a mutation reduces sensitivity to the environment of a phenotypically plastic trait, such as the differences in forewing shape between migratory and lesser-migratory monarchs, it might allow for

rapid divergence and subsequent modification between populations and species (e.g., Moczek, 2007; Pfennig et al., 2010; Suzuki & Nijhout, 2008). A wide range of organisms, including Lepidopterans, show that genetic assimilation might play an important role in adaptive evolution (e.g., Badyaev et al., 2017; Bock et al., 2018; Jones & Robinson, 2018; Kelly, 2019; Kulkarni et al., 2017; Levis et al., 2017; Robinson, 2013; Suzuki & Nijhout, 2006) and has been suggested as a source for butterfly wing shape evolution (Rossato et al., 2018). Thus, genetic assimilation, using the pre-existing phenotypic plasticity observed in monarch butterfly wings, could at least partly explain both the shape and size forewing adaptations described for island populations (Freedman et al., 2020). It has been pointed out that testing the presence of genetic assimilation in nature is difficult (Hall, 2001; Matsuda, 1982), as one must show that plasticity is already available *before* the evolution of genetically fixed traits. We suggest that monarch butterflies, with their highly plastic phenotypes found in migratory populations and their limited plasticity observed in island populations, provide an excellent opportunity to investigate the importance of genetic assimilation in phenotypic evolution. We tested this hypothesis by comparing the shape of three monarch migratory types: nonmigratory (island), migratory (south-bound), and lesser (north-bound) migratory individuals. Due to the importance of forewings in butterfly flight behavior (e.g., LeRoy et al., 2019), which is believed to evolve independently from the highly variable hindwings (Owens et al., 2020), we focused our study on forewing shape, using geometric morphometrics. In addition, we also tested for shape and size variation in forewings between the sexes for all three groups to test if migration behavior might constrain the evolution of sexual dimorphism in butterfly wing shape.

2 | MATERIALS AND METHODS

2.1 | Specimen collection and migratory status assignment

A total of 87 monarch individuals were utilized from the collection at the *American Museum of Natural History* (*AMNH*) (Table 1) and assigned to the following migratory status: "migratory" (southbound individuals returning to the overwintering grounds to Mexico), "lesser migratory" (individuals which were part of the multigenerational northwards migration), and "nonmigratory" (individuals found on Caribbean Islands, which are known to not migrate (Urquhart, 1960). Migratory (M) and lesser migratory (LM) status were assigned by the collection date and site. For this, we compared the collection date with published peak migration abundance midpoint dates for

TABLE 1 Species, sex, and sample size used.

Migration status	Sex	Location	N
Nonmigratory	Female	Caribbean (DR, PR, HT)	11
	Male	As females	19
Migratory	Female	East Coast and Mid-West (CT, IN, NJ, NY, VT)	18
	Male	East Coast and Mid-West (CT, MA, NJ, NY)	12
Lesser migratory	Female	East Coast and Mid-West (IN, NJ, NY, OT, VT)	15
	Male	East Coast and Mid-West (CT, NJ, PA, VT)	13

Note: Nonmigratory individuals were collected from three Caribbean Islands (Dominican Republic, Haiti, Puerto Rico), migratory and lesser migratory individuals were collected from the East Coast and the Mid-West, United States or Ontario, Canada. Abbreviations for US state names are given (nonmigratory females: DR = 3, PR = 7, HT = 1; nonmigratory males: DR = 6, PR = 11, HT = 2; migratory females: CT = 1, IN = 5, NJ = 6, NY = 4, VT = 2; migratory males: CT = 1, MA = 4, NJ = 3, NY = 4; lesser migratory females: IN = 3, NJ = 5, NY = 3, OT = 1, VT = 3; lesser migrator males: CT = 3, NJ = 4, PA = 2, VT = 4).

each latitude (Monarchwatch, 2021; Taylor et al., 2019). If an individual was found within a range of ± 10 days of the peak abundance date for its latitude, we assigned it as M (Supporting Information S1: Table 1). However, if it fell outside of this range, we assigned it as LM (Supporting Information S1: Table 1). No individual assigned as LM overlapped with any documented migration peaks; in fact, all LM individuals were caught at least 44 days before their respective mid-migration peak point. No specimen collected showed any signs of wear and, therefore, must have been killed fairly recently after their eclosure (Watt et al., 1977).

2.2 | Geometric morphometric analysis

To determine the shape and size of each individual's forewing, a landmark-based approach was utilized through a geometric morphometric analysis (Adams et al., 2004; Bookstein, 1989). Each specimen was placed on a horizontally positioned Styrofoam board and photographed using a Nikon 5100 (55 mm objective) that was attached to a camera stand positioned at a fixed distance from the specimen. The pictures were all taken by the same person (H. F. P.).

The photos were then converted through tpsUtil32 into a thin plate spline format (tps) (Rohlf, 2015). A series of seven landmarks were placed along each forewing (K. J. A. N.), outlining the forewing veins of the distal tips which were readily visible among all specimens using tpsDig2.32 (Rohlf, 2015) (Figure 1).

FIGURE 1 Image of a female monarch butterfly (*Danaus plexippus*), showing the position of landmarks (lm = 7) placed on the right forewing. Landmarks were placed on the distal tip of most forewing veins. [Color figure can be viewed at wileyonlinelibrary.com]

Next, a Procrustes least-square superimposition of the landmarks was performed (Rohlf & Slice, 1990) to remove any differences in translation or rotation between the samples (Zelditch et al., 2004) using MorphoJ (Klingenberg, 2011). Additionally, MorphoJ (Klingenberg, 2011) was used to obtain the principal components from the transformed landmarks to identify shape variables, along with the centroid size (which we used as a measurement for forewing size, Zelditch et al., 2004). Differences between principal component scores were evaluated using multivariate analysis of variances, and canonical variate analysis (CVA) was used to test for differences between migration status in Minitab18 (Majeske, 2008).

3 | RESULTS

3.1 | Shape

Supporting previous studies, we found a significant difference in the canonical variates (CV) of wing shape between migratory and nonmigratory monarch butterflies (Table 2, Figure 2b). However, we were unable to find a significant difference in the wing shape of lesser migratory and nonmigratory populations, or lesser

migratory and migratory phenotypes, suggesting that the lesser migratory wing shape is an intermediate phenotype between the two (Table 2, Figure 2a,b). When using a Procrustes analysis, we found a significant difference between all possible group comparisons in the Mahalanobis distances (Table 2, Figure 2a), as well as a significant difference between migratory and nonmigratory populations in the Procrustes distances (Table 2, Figure 2a).

3.2 | Size

A comparison of forewing sizes using an analysis of variance on centroid sizes revealed significant differences between all groups (Table 3). This difference in forewing size appears to be driven by smaller wings found in the nonmigratory populations, as we could not detect a significant difference between lesser migratory and migratory phenotypes (Table 3, Figure 3).

3.3 | Sex differences

We also detected significant differences in forewing shape between sexes in all three groups (Table 3,

TABLE 2 Shape divergence of forewings between and within nonmigratory, migratory, and lesser migratory monarch butterflies using principal component analysis.

Shape comparison	Wilk's	*F* Value	Mahalanobis distance	Procrustes distance
All types	0.90080	2.172 ns		
Lesser migratory versus nonmigratory	0.96530	0.935 ns	1.8005***	0.088 ns
Migratory versus nonmigratory	0.86297	4.526**	2.1643***	0.0163***
Lesser migratory versus migratory	0.93906	1.687 ns	1.1310*	0.011 ns
Male versus female	0.89708	4.704**	1.4847***	0.0154***

Note: Significance of the statistical analysis is designated on the scale of "ns," indicating a nonsignificantnonsignificant *p* value of *p* > .05, while the significant *p* values are marked "*" for *p* ≤ .05, "**" for *p* ≤ .01, and "***" for *p* ≤ .001.
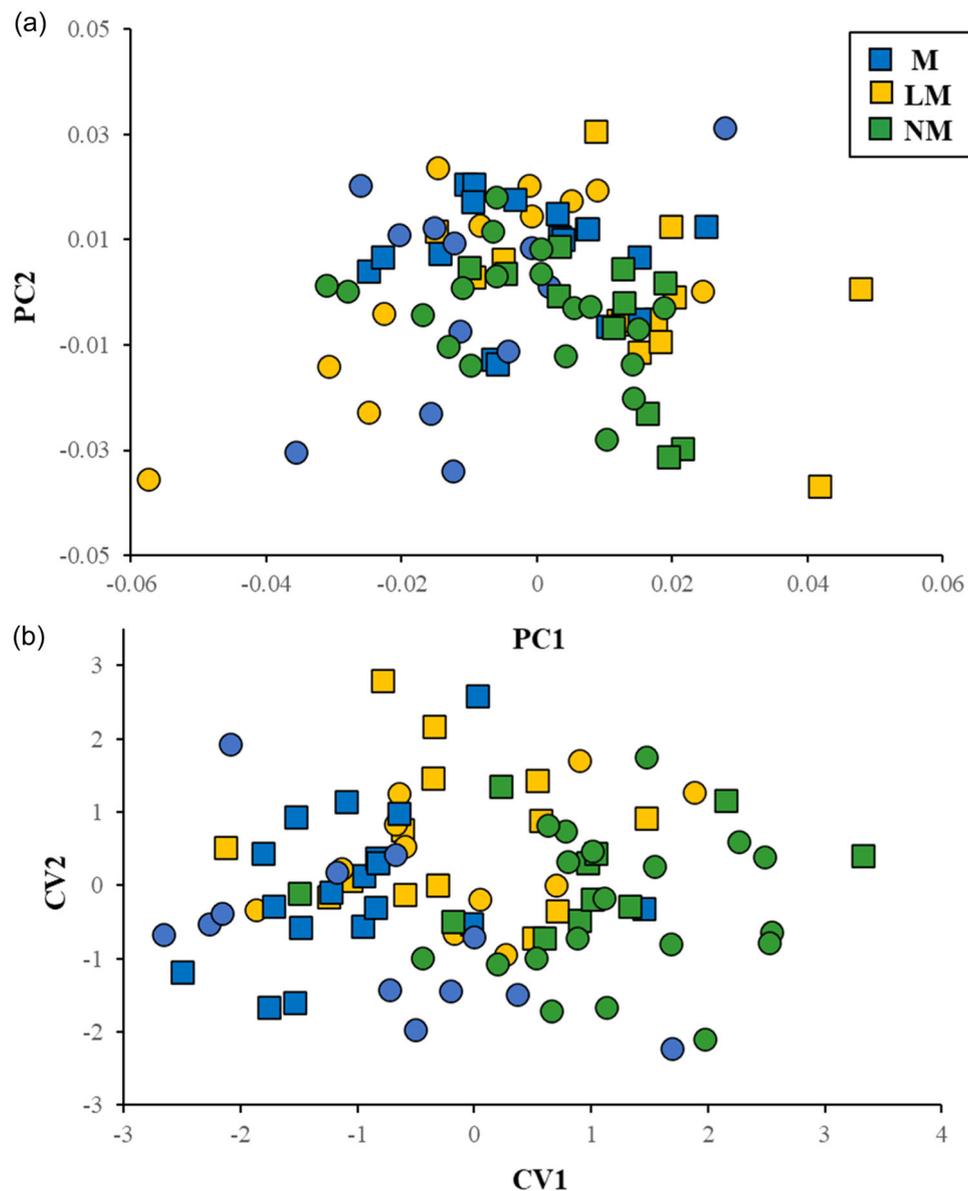
FIGURE 2 Shape variation of the forewings for three migratory behavioral phenotypes. (a) Principal components. PC 1 explains 31.9% and PC2 explains 24% of the total variation. (b) Canonical variates. Each symbol represents an individual. LM (ochre square and circles), lesser migratory individuals (female, *n* = 15; male, *n* = 13); M (blue squares and circles), migratory individuals (female, *n* = 18; male, *n* = 12); NM (green squares and circles), nonmigratory individuals (female, *n* = 19; male, *n* = 11). [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Size divergence of forewings between and within nonmigratory, migratory, and lesser migratory monarch butterflies using ANOVA.

Size comparison	p Value	df	Test statistic
All types	>.0001**	2	$F = 17.44557$
Lesser migratory versus nonmigratory	>.0001***	53	$t = 3.961596$
Migratory versus nonmigratory	>.0001***	58	$t = 5.401828$
Lesser migratory versus migratory	.1703	53	$t = -1.39013$
Lesser migratory: female versus male	.3866	23	$t = -0.88264$
Migratory: female versus male	.5029	28	$t = -0.67883$
Nonmigratory: female versus male	.0219**	28	$t = -2.42756$

Note: Centroid size was used as an estimate for forewing sizes. Significance of the statistical analysis is designated on the scale of "ns," indicating a nonsignificant p value of $p > .05$, while the significant p values are marked "*" for $p \leq .05$, "**" for $p \leq .01$, and "***" for $p \leq .001$.

Abbreviation: ANOVA, analysis of variance.

Figure 2). In addition, we found that males develop larger wings when compared to females in non-migratory populations but could not find any significant differences for size in any of the migratory groups (Table 3). While nonmigratory males have larger forewings than females, both males and females have smaller forewings when compared to individuals of the same sex of the other migration phenotypes (Table 3).

4 | DISCUSSION

Previous studies indicate that butterfly wing morphology is influenced by a wide variety of biotic and abiotic factors, including migratory status, latitude and longitude of populations, as well as the genetic background of butterflies (e.g., Dennis & Shreeve, 1989; LeRoy et al., 2019). In this study, we focused on the monarch butterfly species *D. plexippus*, well-known long-distance migrants from North America, overwintering in Mexico, and elsewhere (Reppert & de Roode, 2018). Here, it has been shown that nonmigratory populations, as found on various Caribbean islands, have heritable differences in wing shape when compared to conspecific migratory populations found on the mainland (Altizer & Davis, 2010; Li et al., 2016). It has been suggested that these shape differences reflect adaptations to flight

associated with different migratory behaviors (Altizer & Davis, 2010; Li et al., 2016).

Unlike the migration to their overwintering grounds, migratory populations repopulate the north, not in one, but over the course of several generations (Flockhart et al., 2013; Miller et al., 2012). Thus, migratory populations develop into two distinct behavioral phenotypes: long- and short-distance migrants (Reppert & de Roode, 2018). Given their different flight behaviors, we predicted that these two phenotypes would have different wing shapes. However, as these two types develop through phenotypic plasticity, any observed differences in wing shape would be based on environmental factors. Our results support this hypothesis: we found not only strong differentiation between nonmigratory and migratory wing shapes as previously demonstrated (Altizer & Davis, 2010; Dockx, 2007; Freedman et al., 2020; Li et al., 2016), but also detected significant wing shape differences among migratory types as well. Interestingly, differences in wing size were mostly driven by the smaller wings of the measured nonmigratory populations. Finally, we found sexual dimorphism in wing shape between all groups, however, size dimorphism in only the nonmigratory populations.

4.1 | Shape differences

Confirming the results of previous studies (Altizer & Davis, 2010; Dockx, 2007; Freedman et al., 2020; Li et al., 2016), our geometric morphometric analysis revealed significant shape differences between migratory and nonmigratory monarch populations. An earlier common garden experiment suggested that these differences are line-specific and, therefore, heritable (Altizer & Davis, 2010). Like other long-distance fliers (LeRoy et al., 2019), migratory monarchs have been found to have more elongated forewings, when compared to the nonmigratory conspecifics (Altizer & Davis, 2010; Dockx, 2007). Such high wing aspect ratio is thought to be mostly found among butterflies with gliding flight behavior (DeVries et al., 2010; LeRoy et al., 2019), such as monarchs (Gibo & Pallett, 1979), which allows them to fly great distances with reduced metabolic costs without losing much flight height (LeRoy et al., 2019). Interestingly, our study also revealed significant shape differences in the forewings of migratory and lesser-migratory phenotypes which are known to be induced by various environmental factors, such as declining food quality and changes in temperature and light availability (Goehring & Oberhauser, 2002), and thus can be considered an example of developmental plasticity (West-Eberhard, 2003). To be more specific, it has been
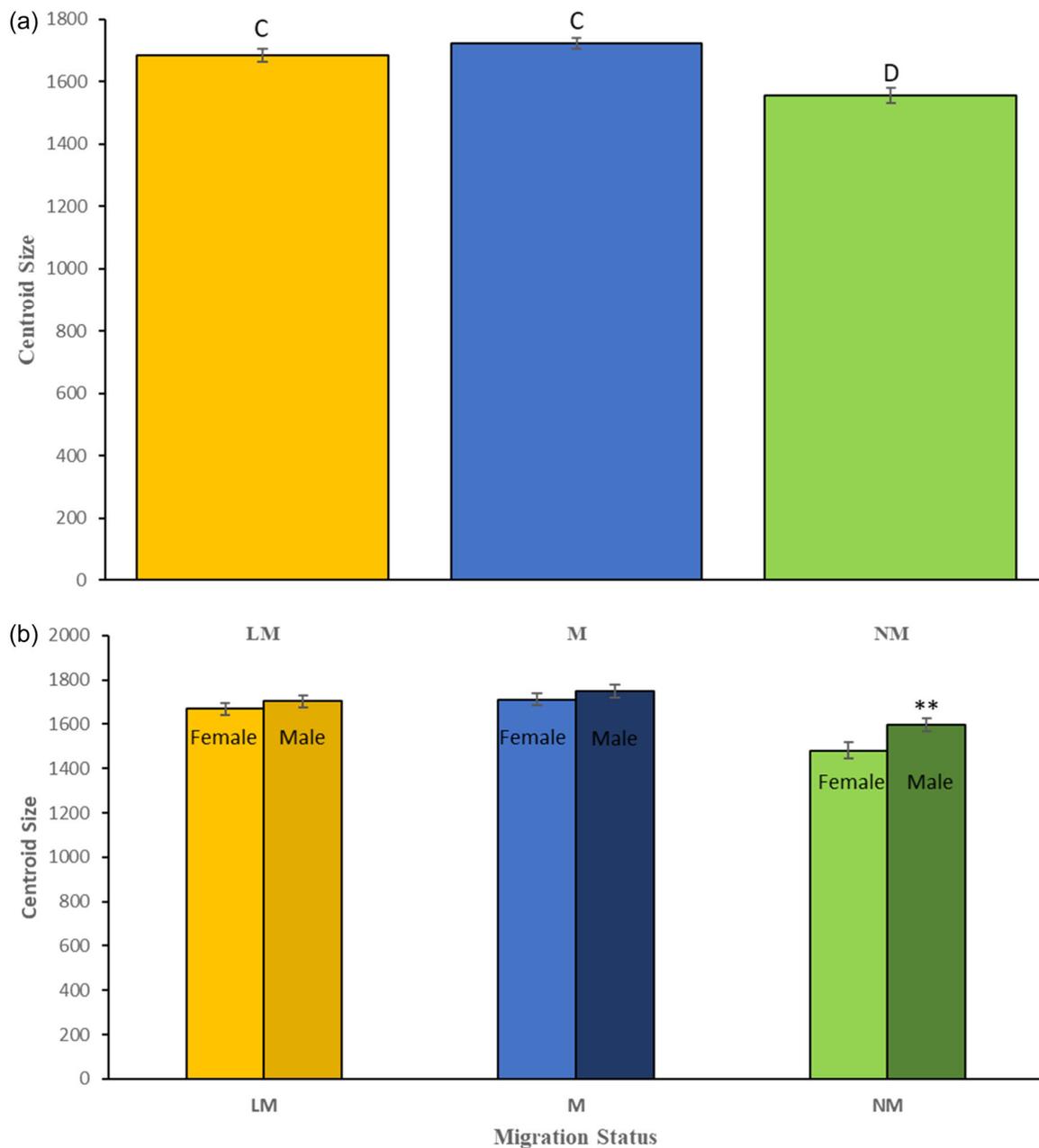
FIGURE 3 Mean size variation of the forewings for three migratory behavioral phenotypes. Error bars indicate standard errors. Centroid size was used as an estimate for forewing size. (a) mean of all individuals for each migratory status. Shared letter on top of the bar indicates non-significance between the groups. (b) mean of all individuals of each migratory status, separated by sex. Significant p-values are marked "*" for $p \leq .05$, "**" for $p \leq .01$. LM (ochre bar), lesser migratory individuals; M (blue bar), migratory individuals; NM (green bar), nonmigratory individuals. [Color figure can be viewed at wileyonlinelibrary.com]

shown that changes in cardenolide concentrations in milkweed are an important mediator of wing shape development (Berns, 2014; Soule et al., 2020). For example, monarch caterpillars which were fed on plants with higher cardenolide concentrations (*Asclepias curassavica*) develop forewings similar to what has been found in island populations (Soule et al., 2020). On the other hand, when raised on plant species with lower cardenolide concentrations (*Asclepias incarnata*), wing

shapes share similarities with migratory individuals (Soule et al., 2020). Interestingly, cardenolide concentrations do not only vary across milkweed species, but are also seasonally affected within a species (Nelson et al., 1981). Here, plants at the end of the growing season tend to have reduced cardenolide concentrations when compared to plants at the beginning of the growing season (Nelson et al., 1981). Thus, lesser migratory individuals, which develop early in the season should be

exposed to higher cardenolide concentrations during larval development. However, migratory individuals, which develop late in the season, are expected to be exposed to lower cardenolide concentrations. We suggest that the difference in wing shape found in the migratory and lesser-migratory are mediated by seasonal changes in plant toxin concentrations. This is supported by the finding that nonmigratory individuals, which are mostly feeding on high cardenolide milkweed species (e.g., *A. curassavica* [Faldyn et al., 2018; Soule et al., 2020]) exhibit wing shape more similar to the lesser migratory phenotype than the migratory phenotype.

As differences between migratory and lesser migratory individuals are solely environmentally induced, we hypothesize that these two developmental pathways are mediated at least partially by the available cardenolide concentrations. This might have allowed individuals to rapidly adapt to nonmigratory phenotypes by canalizing the already available wing shapes of lesser-migratory phenotypes when entering the Caribbean islands at least 2000–3000 years ago (Freedman et al., 2020; Zhan et al., 2014) and starting to feed on the tropical, high-cardenolide milkweed. We suggest that this pathway became canalized through subsequent modifying mutations (Waddington, 1942), allowing them to express the phenotype even when not exposed to tropical milkweed (Altizer & Davis, 2010). Thus, individuals which entered the tropical islands might have been preadapted to change their wing shape by being exposed to a high-cardenolide food source, and a genetic modifier allowed these developmental adjustments to become heritable (Moczek et al., 2011; Waddington, 1942, 1953, 1956; West-Eberhard, 2003). However, further studies would need to investigate if the observed changes between migratory and lesser migratory individuals are indeed adaptive and/or evolved through other evolutionary mechanisms.

4.2 | Size differences

Our study confirms previous findings (Altizer & Davis, 2010; Dockx, 2007; Freedman et al., 2020; Li et al., 2016), which show that nonmigratory populations have significantly smaller wings when compared to migratory populations. While the elongation of the forewing has been thought to be an important phenotypic adaptation to long-distance migratory behavior (LeRoy et al., 2019), Li et al. (2016) suggest that size itself might be a more important predictor of migratory status. It is thought that the ancestor of all monarchs was most likely migratory with larger forewings (Freedman et al., 2020) and subsequently underwent a reduction of

wing size when invading islands and becoming non-migratory (Freedman et al., 2020). Smaller and shorter wings, as found on these islands, are thought to allow for slower, more controlled flight behavior (LeRoy et al., 2019) as well as an overall reduction in flight, thus limiting individuals drifting off into the ocean. Smaller wings also likely provide the additional benefit of saving resources, which could be utilized for other traits during development, especially in holometabolous insects (e.g., Emlen, 2001; Parzer & Moczek, 2008).

Since selection on reduced wing size has not been found in populations that established themselves already a few thousand years ago in the Caribbean and Central/South America (Freedman et al., 2020; Zhan et al., 2014), these populations might have reached their adaptive peaks fairly rapidly. However, populations that only recently arrived on the Pacific and Atlantic Islands might still be responding to selection for reduced wing size (Freedman et al., 2020). Interestingly, despite the different wing sizes found in migratory and nonmigratory populations, evidence of stabilizing selection on wing size has not been found in either of them (Dockx, 2007).

We suggest that similar to wing shape, genetic assimilation might be an important component for the reduction of wing size in monarchs. Like wing shape, wing size is strongly affected by food quality: monarchs raised on milkweed with higher cardenolide concentrations tend to have smaller wings, whereas those raised that were exposed to lower concentrations develop larger wings (Soule et al., 2020). Thus, as outlined above, monarchs, which arrived on tropical islands with year-round high-concentrate cardenolide milkweed were possibly preadapted to develop smaller wings. Subsequent mutations might have stabilized and further reduced size via genetic assimilation, as we described for wing shape above. Therefore, the astonishing range expansion of the monarch butterfly might be at least partially explained by phenotypic plasticity of their wings.

4.3 | Sexual dimorphism in wing shape and size

We detected sexual dimorphism in forewing shape in all three groups, supporting previous studies, which found sex differences in forewing shape in lab-reared non-migratory (Altizer & Davis, 2010; Berns, 2014) and migratory (Altizer & Davis, 2010) individuals, and in some wild-caught migratory and nonmigratory populations (Li et al., 2016). In addition, we found that males of nonmigratory populations have larger wings when compared to nonmigratory females. Interestingly, we were unable to find this pattern in the two migratory groups.

Other studies found that males have larger wings in some of the migratory populations measured (Altizer & Davis, 2010; Berns, 2014; but see Li et al., 2016), however, these results were not consistent across all studies. It is possible, as most of these studies focused on lab-reared individuals, that the observed sexual size dimorphism found in migratory populations is a consequence of the lab-rearing environment, and thus, while certainly interesting and suggesting heritability for forewing size, might not reflect patterns observed in nature. If our results are confirmed, size dimorphism in this species might be considered a novel trait in nonmigratory populations, which are thought to have evolved from migratory populations (Freedman et al., 2020). The lack of sexual dimorphism in migratory individuals could be caused by functional constraints, which have been shown to limit evolutionary outcomes (Parzer et al., 2018). Due to the differences in flight demand between migratory and nonmigratory populations, natural selection has been suggested to act on the various aspects of flight efficiency in migratory populations, including wing shape and size (Altizer & Davis, 2010; Zhan et al., 2014). As long-distance migrants are thought to require a minimum wing size to minimize the energetic costs for their long journey to the south (LeRoy et al., 2019), we assume that natural selection overrides sexual selection in migratory monarch populations. However, if that is true, nonmigratory populations, which are, with their overall reduced wing size, possibly released from such functional constraints, allowing sexual selection to act upon the wing size of the two sexes. Regardless, in monarch butterflies, courtship consists of two phases: an aerial phase and a ground phase. During the aerial phase, the male positions himself to be in view of females in the area (Pliske, 1975) and actively pursue females when they enter his territory by chasing after them. If successful, the males then attempts to subdue her down by clutching her body with his legs (Pliske, 1975). During this time, females appear to try to escape, thus requiring the male wings to not only carry himself, but also the female (Pliske, 1975). During this phase, the male also tries to use his hair pencil to initiate copulation. Mating happens once the couple has landed on the ground (ground phase) (Pliske, 1975). Thus, while natural selection might favor smaller wings in non-migratory populations (Freedman et al., 2020), as we observed in females, the necessary aerial displays by males might have forced males to maintain larger wing size (albeit still smaller than is observed in migratory populations) (Pliske, 1975). Indeed, it has been shown that forewing size affects mating success of males in a lab setting (Davis et al., 2007). Here, males with larger wings tend to have increased mating success, when compared to males with smaller wings (Davis et al., 2007). However, as it appears that larger wings are counterintuitively not associated with flight endurance (Davis et al., 2012), female choice might still be an important component in the observed sexual dimorphism.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Harald F. Parzer http://orcid.org/0000-0002-1413-2755

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SUPPORTING INFORMATION

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