Two centuries of monarch butterfly collections reveal contrasting effects of range expansion and migration loss on wing traits

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Migratory animals exhibit traits that allow them to exploit seasonally variable habitats. In environments where migration is no longer beneficial, such as oceanic islands, migration-association traits may be selected against or be under relaxed selection. Monarch butterflies are best known for their continent-scale migration but have repeatedly become established as nonmigrants in the tropical Americas and on Atlantic and Pacific Islands. These replicated nonmigratory populations provide natural laboratories for understanding the rate of evolution of migration-associated traits. We measured >6,000 museum specimens of monarch butterflies collected from 1856 to the present as well as contemporary wild-caught monarchs from around the world. We determined 1) how wing morphology varies across the monarch’s global range, 2) whether initial long-distance founders were particularly suited for migration, and 3) whether recently established nonmigrants show evidence for contemporary phenotypic evolution. We further reared >1,000 monarchs from six populations around the world under controlled conditions and measured migration-associated traits. Historical specimens show that 1) initial founders are well suited for long-distance movement and 2) loss of seasonal migration is associated with reductions in forewing size and elongation. Monarch butterflies reared in a common garden from four derived nonmigratory populations exhibit genetically based reductions in forewing size, consistent with a previous study. Our findings provide a compelling example of how migration-associated traits may be favored during the early stages of range expansion, and also the rate of reductions in those same traits upon loss of migration.

Significance

Monarch butterflies are famous for their seasonal migration in North America but have recently expanded around the globe. Using >6,000 monarchs collected over two centuries, we use the monarch’s recent global range expansion to test hypotheses about how dispersal traits evolve. Early monarch founders have large and elongated forewings, but postestablishment loss of migration repeatedly leads to smaller wings, a pattern detectable in both time series with historical specimens and experimentally reared monarchs. This research documents how migration-associated traits may be favored during range expansion but disfavored when species cease seasonal migration. Furthermore, it highlights the value of museum collections by combining historical specimens with experimental rearing to demonstrate contemporary evolution of migration-associated traits in natural monarch populations.

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During the early stages of species range expansions, dispersal rates along expansion fronts are expected to be elevated (19, 20). This phenomenon is well documented in the cane toad invasion of Australia, where toads along the range expansion edge have longer legs and disperse faster than individuals from the center of the expansion range (19, 21). Increased dispersal ability at range edges has also been demonstrated in experimental evolution studies (22–24). Genetic evidence supports serial stepwise dispersal in monarch populations in the Pacific and Atlantic (14, 25) (also see Fig. 1C), a pattern that is consistent with a natural range expansion (26). Thus, we expect that historical monarch specimens collected during the early phase of their global range expansion should have enhanced dispersal ability.

By contrast, once monarchs are established as nonmigratory populations, we expect for natural selection to drive a reduction in dispersal ability. This reduction could result from 1) directional selection operating on one or more potentially correlated traits to favor a new nonmigratory trait optimum and/or 2) a relaxation of directional or stabilizing selection previously associated with a migratory trait optimum (27). Previous research has shown that nonmigratory monarchs tend to have smaller and less elongated forewings (32–31), including for common-garden reared monarchs (28), and also that seasonal migration strongly selects for increased forewing size (32, 33). However, studies to date have not taken into account the recency of migration loss in the Pacific and Atlantic, nor the rate at which we might expect evolutionary responses to this loss of migration. Time series analyses taking advantage of historical specimen collections can provide insights into the nature and the pace of changes associated with the transition from migratory to nonmigratory status. We predict that in postestablishment Pacific and Atlantic populations, loss of migration should drive observable reductions in wing size and elongation, phenotypes that are seen in other longer established nonmigratory monarch populations in Central and South America and the Caribbean (28–31).

In this study, we use a combination of measurements from more than 6,000 museum specimens dating back to 1856 and a common-garden rearing experiment to test hypotheses about 1) dispersal traits in the early stages of range expansion and 2) postestablishment phenotypic trait evolution associated with loss of migration.

Range Expansion and Monarch Wing Morphology

Initial founders collected from the early phase of the Atlantic and Pacific expansion events generally had larger and more elongated forewings than the ancestral North American population (Fig. 2). Specimens from the United Kingdom, where monarchs may be blown from North America by storms but do not become established due to the absence of suitable host plants (34, 35), further support the idea of increased dispersal ability in founding individuals (Fig. 2). Likewise, the monarch’s seasonal range expansion during spring remigration in eastern North America also provides evidence for increased dispersal ability at the expanding range edge: early-arriving monarchs tend to have larger forewings (36), and monarchs that reach the northern range edge in eastern North America tend to be larger (SI Appendix, Fig. S1). These data are broadly consistent with models of dispersal evolution in island flora (37) as well as oceanic island bird assemblages (38), in which founding individuals exhibit highly dispersive traits. After establishment, selection then favors loss of dispersal ability.
North American populations (Fig. 5A). Puerto Rico had significantly smaller forewings than migratory monarch populations from Hawaii, Guam, Australia, and in common-garden rearing experiments, we found that nonmigratory Central American monarchs under comparable conditions (28). In contrast to Atlantic nonmigratory populations, wing size has increased in North and Central America, South America, and the Caribbean, where monarchs have likely been nonmigratory for much longer periods (14), did not show evidence for consistent directional evolution of their wing morphology (Fig. 4 and SI Appendix, Table S1) (39). By contrast, wing size has increased in migratory North American monarchs since 1870 (Fig. 4C and SI Appendix, Table S1) (35). Populations in Central America, South America, and the Caribbean, where monarchs have likely been nonmigratory for much longer periods (14), did not show evidence for consistent directional evolution of their wing morphology (Fig. 4D and E). In contrast to Pacific and Atlantic nonmigratory populations, wing size has increased in nonmigratory Central American monarchs through time (Fig. 4D and SI Appendix, Table S1). This increase in forewing size in Central America may reflect ongoing gene flow from North American migrants into Central America, a scenario that is consistent with an earlier study that reared migratory and nonmigratory populations (28) —31). When we analyzed these data to assess potential changes through time, we found significant decreases in both forewing size and forewing elongation through time for nonmigratory Pacific and Atlantic populations (Fig. 4 and SI Appendix, Table S1) (39). By contrast, wing size has increased in migratory North American monarchs since 1870 (Fig. 4C and SI Appendix, Table S1) (35). Populations in Central America, South America, and the Caribbean, where monarchs have likely been nonmigratory for much longer periods (14), did not show evidence for consistent directional evolution of their wing morphology (Fig. 4D and E). In contrast to Pacific and Atlantic nonmigratory populations, wing size has increased in nonmigratory Central American monarchs through time (Fig. 4D and SI Appendix, Table S1). This increase in forewing size in Central America may reflect ongoing gene flow from North American migrants into Central America, a scenario that is supported by the low levels of genetic differentiation between North and Central American monarchs (14, 40).

Morphological Evolution Inferred from Rearing Experiments

In common-garden rearing experiments, we found that nonmigratory monarch populations from Hawaii, Guam, Australia, and Puerto Rico had significantly smaller forewings than migratory North American populations (Fig. 5A). Thus, the forewing size trends we observed in our time series data likely reflect genetic differences in wing size rather than plasticity associated with environmental differences between collection locations, consistent with an earlier study that reared migratory and nonmigratory monarchs under comparable conditions (28). In contrast to size, forewing shape did not differ between North American and Pacific monarchs. Only the Puerto Rican population, which has likely been nonmigratory over a longer period (14), showed differences in forewing shape (SI Appendix, Fig. S2A). We found no wing morphological differences between eastern and western North American monarchs (Fig. 5A), consistent with studies suggesting that they form a single genetically undifferentiated population across the entire North American continent (14, 41) (also see Fig. 1C).

The only measured trait that consistently differed between migratory and nonmigratory monarchs reared under common conditions was forewing size. By contrast, traits that other studies have suggested are related to migratory status and flight ability, such as wing shape, body mass, wing loading, and mass allocation to thoracic tissue (42) did not consistently differ between migratory and nonmigratory populations (SI Appendix, Fig. S2), even though some of these traits are strongly correlated with forewing size (SI Appendix, Fig. S3). Only the longer established nonmigratory Puerto Rican population showed less elongated forewings and reduced body mass compared to other populations (SI Appendix, Fig. S2.A and B).

Morphological and genetic analyses that account for patterns of shared ancestry among populations (43, 44) did not indicate a strong signature of divergent natural selection in three Pacific island populations compared to ancestral North American populations (SI Appendix, Fig. S4). Both multivariate and univariate analyses (SI Appendix, Table S2) suggest that the observed levels of divergence among phenotypes we measured in North American and Pacific populations can be explained by genetic drift alone, which would be most consistent with relaxed selection on migration-associated traits postestablishment.

Interpretation and Conclusions

With long-term historical museum specimens and contemporary rearing experiments, we demonstrate that monarch butterflies have repeatedly expanded their range through dispersal of large-winged individuals, only to see a reduction in wing size associated with migration loss over the next ~1,000 generations. This result is striking because it involves a comprehensive timeline of two processes (range expansion, loss of seasonal migration) that are thought to have countervailing effects on the evolution of dispersal traits. While our finding of reduced forewing size in nonmigrants mirrors that of earlier studies comparing migratory and nonmigratory monarch populations (28–31), the size of our dataset and the analyses of morphological change through time allow for inferences about the mode of selection underlying these phenotypic differences.

Previous studies in monarchs found genomic evidence for selective sweeps associated with the loss of migration across a few hundred loci, including in recently established Pacific and Atlantic populations (14). These sweeps, which occurred across three independent losses of migration, are indicative of strong and parallel directional selection (14). None of the loci identified as being under strong selection in transitions to nonmigratory status have been implicated in wing size or wing shape evolution, though the haplotype showing the strongest signature of divergent selection is thought to be involved in wing muscle development and flight metabolism (14).

In practice, it is difficult to distinguish between 1) directional selection toward a nonmigratory phenotypic optimum versus 2) relaxed selection on traits maintained by migration (27). Unlike some other quintessential examples of relaxed selection, which involve discrete character changes with relatively simple genetic architectures—eye loss in cave fish (45), spine reductions in threespine sticklebacks (46), evolution of flightlessness in island birds (47)—monarch wing morphological traits are important for behaviors outside of migration, including foraging, predator avoidance, and mate pursuit. Furthermore, because we do not know...
which loci underlie wing morphological traits, we cannot directly
determine whether nonmigratory populations show an excess of loss
of function mutations (indicative of relaxed selection) versus re-
duced genetic diversity in the regions around these loci (indicative
of directional selection).

That said, our phenotypic data do provide two lines of evi-
dence to support relaxed selection associated with loss of mi-
gration. First, the pace of wing morphological evolution has been
quite gradual: for example, in common-garden reared monarchs
from Australia, average wing area (819 ± 15 mm²) was only 7.3%
smaller than average wing area from the ancestral migratory
North American population (884 ± 12 mm²). Given that mon-
archs have been established in Australia since at least 1871 (18
and Fig. 4C), this corresponds to a forewing size reduction of less
than 0.5 mm² per year, or less than 0.1 mm² per generation. This
result is also reflected in multivariate phenotypic analyses that
incorporate relatedness among populations, which suggest only a
modest signature of divergent selection between migratory North
American and nonmigratory Pacific populations (SI Appendix,
Fig. S4). Second, there is modest support for increased variation
in wing size in nonmigratory populations, consistent with a sce-
nario of relaxed purifying selection. However, this pattern of
increased variation does not hold across all nonmigratory popu-
lations and also is not present in common-garden reared
monarchs (SI Appendix, Fig. S5). It is also important to note that
neither of these two results precludes the possibility that large
and/or elongated forewings are actively selected against in non-
migratory populations; more research is needed to fully under-
stand how factors besides long-distance migration shape
monarch wing morphology.

Finally, this research highlights the importance of biological
collections in generating and testing evolutionary hypotheses. Researc
collections provided not only the specimens used for
measurement in this study, but also enabled the inference of the
monarch’s establishment history in the Atlantic and Pacific. As

Fig. 3. (A) The first principal component for forewing size and shape plotted together for 6,227 monarch records. Nonmigratory populations from the Pacific
and Atlantic have forewing morphology that is more consistent with the migratory North American population (large and elongated forewings; Upper Right
Quadrant) than with other nonmigratory populations from Central/South America and the Caribbean. The 95% data ellipses generated using the stat. ellipse
function are shown. (B) Example of female monarchs in standard pinning position. (Top) Migratory North American monarch, collected in San Francisco, CA.
(Bottom) Nonmigratory South American monarch, collected in Valle del Cauca, Colombia. Images are shown on the same scale and differ by nearly 40% in
their forewing area. Image credit: California Academy of Sciences.

Materials and Methods

History of Expansion out of North America. To describe establishment routes of
the monarch’s expansion into the Pacific (Fig. 1C), we generated restriction
site-associated DNA sequencing (RAD-seq) data from 281 monarchs from
North America and various Pacific Island groups using the methods de-
described in ref. 49 (SI Appendix, Table S3). Reads were mapped to v3 of the
monarch reference genome (50), and genotypes were called using SAMtools
(51). Next, genotypes were filtered and then used to build a neighbor-
joining tree using ANGSD (52) and the R package ape v5.0 (53). For
further details, see Supplementary Appendix. Establishment dates in the Pacific
and Atlantic were inferred from previously published literature (18) and
collection labels from museum collections. Establishment timing in the Ca-
ribbean, Central America, and South America is based on the supplementary
materials in ref. 14.

Museum and Wild-Caught Specimens. Data from museum specimens were
collected by photographing monarchs in standard pinning position (Fig. 3B)
with a scale bar using either a Nikon D7100 DSLR camera or imaging
equipment provided by museums. For a summary of museum specimens, see
SI Appendix, Table S4. Monarchs that were labeled as ex-ova, ex-larva, or ex-
pupa were not considered wild caught and were omitted from analyses, as
were specimens with major damage on both wings. Contemporary speci-
mens were either pinned and photographed or dissected and imaged using a
flatbed scanner (CanScan UDE 120) with a scale bar. All images were
measured using ImageJ v1.51 (54), with separate measurements taken for
right and left forewings when possible. To account for possible differences
in image generation, we included the collection identification (ID) as a
random intercept term in all statistical analyses. Date and locality of col-
collection were recorded for each specimen when available.

Common-Garden Experiment. For a full description of experimental rearing,
see ref. 55 and SI Appendix. Briefly, we collected gravid adult female
monarchs from six populations (SI Appendix, Table S5) or reared wild-caught
caterpillars and then crossed eclosed adults from these populations. We
generated eggs from 11 to 15 maternal families per population. Within 24 h

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of hatching, five neonate larvae were transferred onto a single live host plant from one of six milkweed species grown from seed. Wild plant seed was collected in regions of origin of each population, except for *Asclepias* *speciosa*, which was grown from commercially available seed. Plants were propagated in greenhouses at the University of California, Davis. Upon eclosion, adult F1 monarchs were given 6 to 8 h for their wings to dry and were then weighed to the nearest 0.1 mg. Monarchs were then frozen and later dissected and dried at 60 °C. We measured wing morphology using the procedure described above and also recorded the dry mass of abdominal and thoracic tissue separately. Phenotypes of interest for common-garden reared monarchs were forewing and hindwing morphological variables, eclosion mass (both wet and dry), wing loading (forewing area/wet mass), and dry mass of abdominal and thoracic tissue (SI Appendix, Fig. S2).

Data Analysis. As in Altizer and Davis (2010) (28), we combined measurements of forewing length, width, and area to generate principal components describing wing size, with size principal component 1 (PC1) explaining 96.4% of forewing size variation. Forewing aspect ratio (length/width) and forewing roundness (4π*area/perimeter²) were used to generate shape principal components, with shape PC1 explaining 86% of variation. Size PC1 and shape PC1 were then used as response variables in analyses of wing morphological variation. We included a fixed effect for region, with year as a continuous predictor and a region*year interaction. Butterfly sex was included as a fixed effect, latitude of collection and image type (scan vs. photo) were covariates, and collection ID and country/archipelago of collection were random effects. Continuous predictor variables (year, latitude) were centered and scaled. For both forewing size and shape analyses, the primary effect of interest was the interaction between region*year, as this effect captures the different evolutionary trajectories of the ancestrally migratory North American population, the recently derived nonmigratory Atlantic and Pacific populations, and the longer established nonmigratory Central American, South American, and Caribbean populations. Models were implemented in the lme4 package (56) in R v 3.6.3 (57) and were of the form:

response ~ region*year + sex + latitude + image type + (1|collection ID) + (1|archipelago)

For common-garden reared butterflies, we used the same size PC1 and shape PC1 measures and also conducted analyses of wet and dry body mass, wing loading (body mass/forewing area), and the mass of thoracic and abdominal tissue. In all analyses using common-garden reared monarchs, we included infection level (approximate log10 spore counts) with the protozoan parasite *Ophryocystis elektroscirrha* (OE) as a covariate in accordance with previous studies (58) (see results in SI Appendix, Table S7).

We used the program driftsel (44) to estimate the strength of divergent selection between migratory North American and nonmigratory Pacific populations. Briefly, we used RAFM (59) to estimate the ancestry-coancestry matrix using 1,000 randomly sampled single-nucleotide polymorphisms (SNPs) and then analyzed both univariate and multivariate signatures of selection for quantitative traits. To test for relaxed selection, we compared the coefficient of variation (CV) for wing morphological traits between...
Fig. 5. (A) Wing size of common-garden reared monarchs from six populations around the world. Abbreviations correspond to map at right. Numbers inside of boxplots correspond to total number of measured adults for each population. Measured offspring were the F1 progeny of wild-caught females from 11 to 15 maternal families per population. Letters above boxplots denote significant group-level differences in a linear mixed model after correcting for multiple comparisons. (B) Map of sampling locations. Eastern and western North American monarchs are migratory, and genomic data suggest that they form a single undifferentiated population (ref. 10 and Fig. 1C). Populations from Hawaii, Guam, and Australia are all nonmigratory and represent a serial stepwise contemporary range expansion across the Pacific over the last 170 y. The Puerto Rican population represents a less recent loss of migration. (C) Side-by-side comparison of a Puerto Rican (Left) and eastern North American (Right) monarch reared at the same time on the same individual host plant.

monarch populations for both wild-caught and common-garden reared monarchs. To test for among-group differences, we calculated 95% bootstrapped confidence intervals for CV, estimated using the package cvcov v.1.0.0 (60). Full details for all analyses are provided in the SI Appendix.

Data Availability. All raw data, metadata, and associated code used in analyses have been deposited with Dryad (https://doi.org/10.25338/B815TC) (61) and are also available through Github (https://github.com/micahfreedman/manuscripts). Images from museum specimens are copyright protected and cannot be made publicly accessible without permission but are available upon request.

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