



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

Micah G. Freedman^{a,b,1,2}, Hugh Dingle^c, Sharon Y. Strauss^{a,b}, and Santiago R. Ramírez^{a,b}

^aCenter for Population Biology, University of California, Davis, CA 95616; ^bDepartment of Evolution and Ecology, University of California, Davis, CA 95616; and ^cDepartment of Entomology and Nematology, University of California, Davis, CA 95616

Edited by Marlene Zuk, University of Minnesota, St. Paul, MN, and approved September 17, 2020 (received for review January 22, 2020)

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 in North America 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 raised 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.

relaxed selection | migration | monarch butterfly | morphology | range expansion

Long-distance migration has evolved across the tree of life as a way for organisms to exploit seasonally variable environments (1). Migratory species are characterized by suites of traits that enable long-distance movement, including morphological (2), physiological (3), and navigational adaptations (4). Loss of migration is also a common phenomenon, both over macroevolutionary (5, 6) and contemporary timescales (7, 8). When lineages shift from migratory to nonmigratory status, traits that were previously important for long-distance movement may undergo relaxed selection or may be actively selected against as populations move toward new nonmigratory trait optima. Signatures of selection associated with migration loss should be especially pronounced in situations when traits important for migration are physiologically costly to maintain.

The functional importance of migration-associated traits is often inferred using phylogenetic comparative approaches that contrast migratory and nonmigratory species (9, 10) or divergent populations of species that differ in aspects of their migratory behavior (11–13). While broadly informative, approaches involving comparisons that span macroevolutionary timescales may integrate over millions of years of both adaptive and nonadaptive evolutionary processes. Intraspecific studies using taxa that have

repeatedly transitioned between migratory and nonmigratory status over recent time scales can be especially helpful for understanding how natural selection shapes migration-associated genes and traits (14).

One species that shows repeated contemporary shifts in its migratory status is the monarch butterfly (*Danaus plexippus* (L.)). The monarch is best known from its ancestral range in North America, where it carries out a multigeneration annual migration that can involve individual butterflies flying more than 3,000 km in their lifetime (15). However, over the past 180 y, monarchs have expanded their range outside of North America and can now be found in locations throughout the Pacific and Atlantic (14, 16, 17) (Fig. 1A). Monarchs are also established in Central and South America, as well as the Caribbean (16–18); these southern populations likely became established tens of thousands of years ago and are phenotypically (16) and genetically distinct (14) (SI Appendix). In almost all locations where monarchs have become established, they form year-round breeding, nonmigratory populations, owing to the year-round availability of their milkweed host plants in these locations. Each major expansion event out of North America corresponds to an independent loss of migration, with evidence for convergent genomic evolution associated with this transition (14). As a large, colorful

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.

Author contributions: M.G.F., H.D., S.Y.S., and S.R.R. designed research; M.G.F. and H.D. performed research; M.G.F. analyzed data; and M.G.F., H.D., S.Y.S., and S.R.R. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

Published under the PNAS license.

¹Present address: Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637.

²To whom correspondence may be addressed. Email: mfreedman@ucdavis.edu.

This article contains supporting information online at <https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2001283117/-DCSupplemental>.

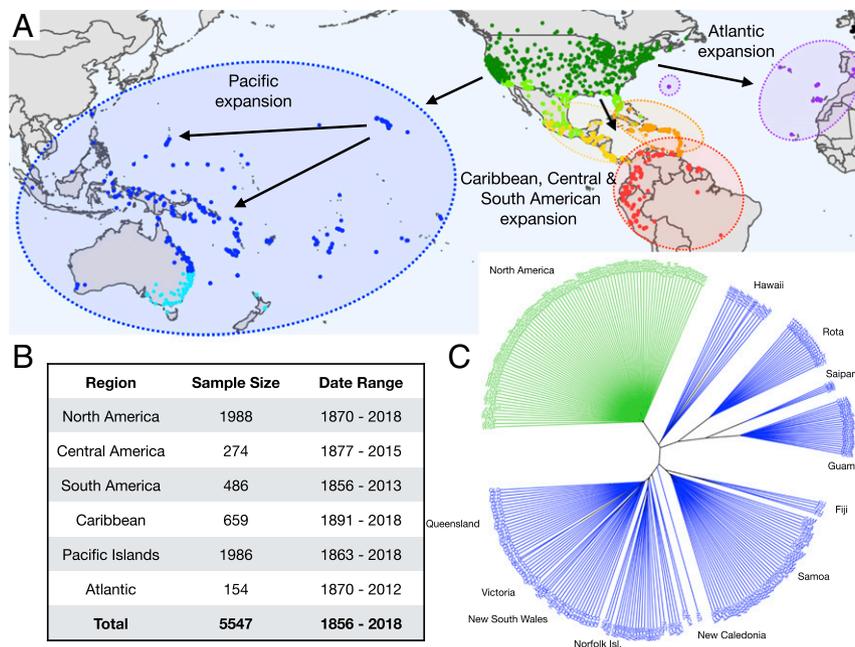


Fig. 1. (A) Map showing sampling locations for monarchs measured in this study. The three out-of-North America expansion events (Pacific locations shown in blue, Atlantic in purple, and Central/South American/Caribbean in yellow, red, orange, respectively) are shown with arrows and correspond to those identified by ref. 10. The earliest historical records from the Pacific expansion event are from 1841 (Hawaii); earliest records for the Atlantic expansion event are from 1847 (Bermuda). The timing of the expansion into Central and South America and the Caribbean is uncertain but likely occurred tens of thousands of years ago. Records in light green and light blue correspond to monarchs whose migratory status is ambiguous based on their location and time of collection. (B) Number of recorded monarchs and date range of collection for each region. Note that sample sizes do not include North American monarchs with ambiguous migratory status ($n = 493$) or potentially migratory monarchs from Australia and New Zealand ($n = 411$). (C) Neighbor-joining tree showing relationships among eastern and western North American and Pacific Island populations. For the Pacific expansion event, monarchs first became established in Hawaii and then separately expanded westward into the Mariana Islands (Guam, Saipan, Rota) and southwestward toward Australia.

butterfly species, the monarch is often collected by amateur and professional entomologists, thus providing excellent records of arrival times and phenotypes in novel ranges.

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 (28–31), 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.

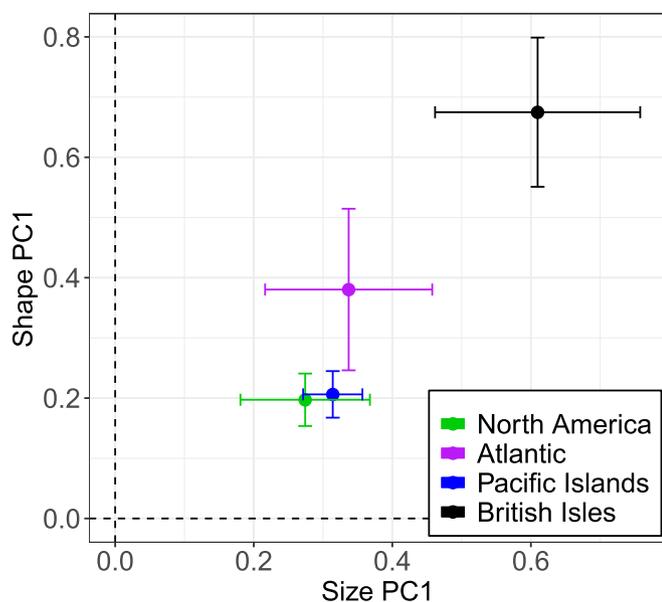


Fig. 2. Wing morphology of monarchs collected during the first 50 y postestablishment from various locations in the Atlantic ($n = 31$) and Pacific ($n = 452$). Higher values for Size PC1 correspond to larger forewings, while higher values for Shape PC1 correspond to more elongated forewings. North America includes only individuals collected prior to 1920 ($n = 192$). Monarchs from the British Isles ($n = 18$) are off-course North American migrants (ref. 35) that never became established as breeding populations; as such, these individuals should be representative of true founders. Points represent mean ± 1 mean standard error (MSE). Group means are only significantly different between North American and British samples.

Time Series Using Museum Collections Reveal Contemporary Evolution

Museum specimens of monarchs from around the world show substantial variation in their forewing morphology (Fig. 3), corroborating earlier research comparing migratory and nonmigratory monarch 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 $\sim 1,000$ 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

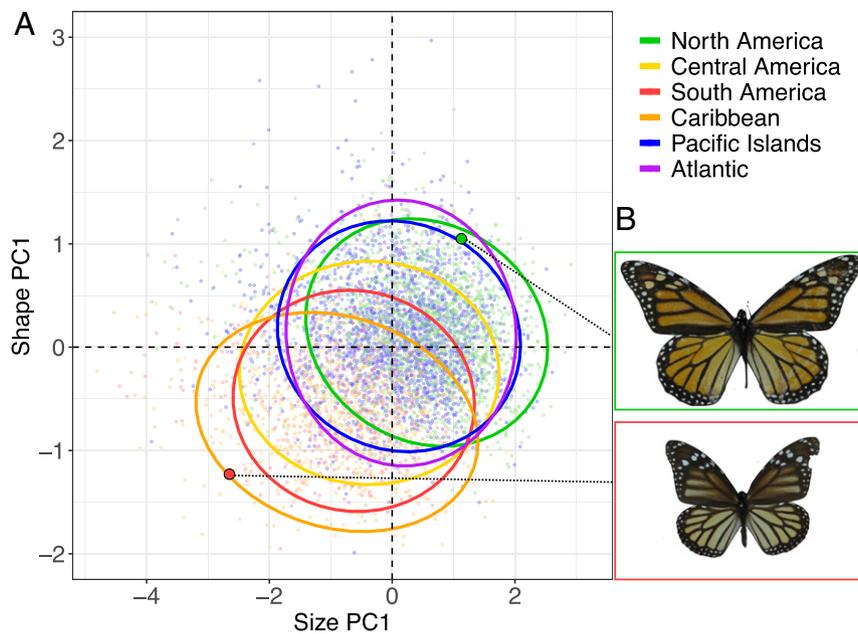


Fig. 3. (A) The first principal component for forewing size and shape plotted together for 6,227 monarch records. Nonmigratory populations from the Pacific and Atlantic have forewing morphology that is more consistent with the migratory North American population (large and elongated forewings; *Upper 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.

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 reduced genetic diversity in the regions around these loci (indicative of directional selection).

That said, our phenotypic data do provide two lines of evidence to support relaxed selection associated with loss of migration. First, the pace of wing morphological evolution has been quite gradual: for example, in common-garden reared monarchs from Australia, average wing area ($819 \pm 15 \text{ mm}^2$) was only 7.3% smaller than average wing area from the ancestral migratory North American population ($884 \pm 12 \text{ mm}^2$). Given that monarchs 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 mm^2 per year, or less than 0.1 mm^2 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 scenario of relaxed purifying selection. However, this pattern of increased variation does not hold across all nonmigratory populations 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 nonmigratory populations; more research is needed to fully understand 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. Research 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

species' range expansions associated with climate change and human introductions became more common (48), the value of biological collections will only increase.

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 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 Caribbean, 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 specimens were either pinned and photographed or dissected and imaged using a flatbed scanner (CanoScan LiDE 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 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

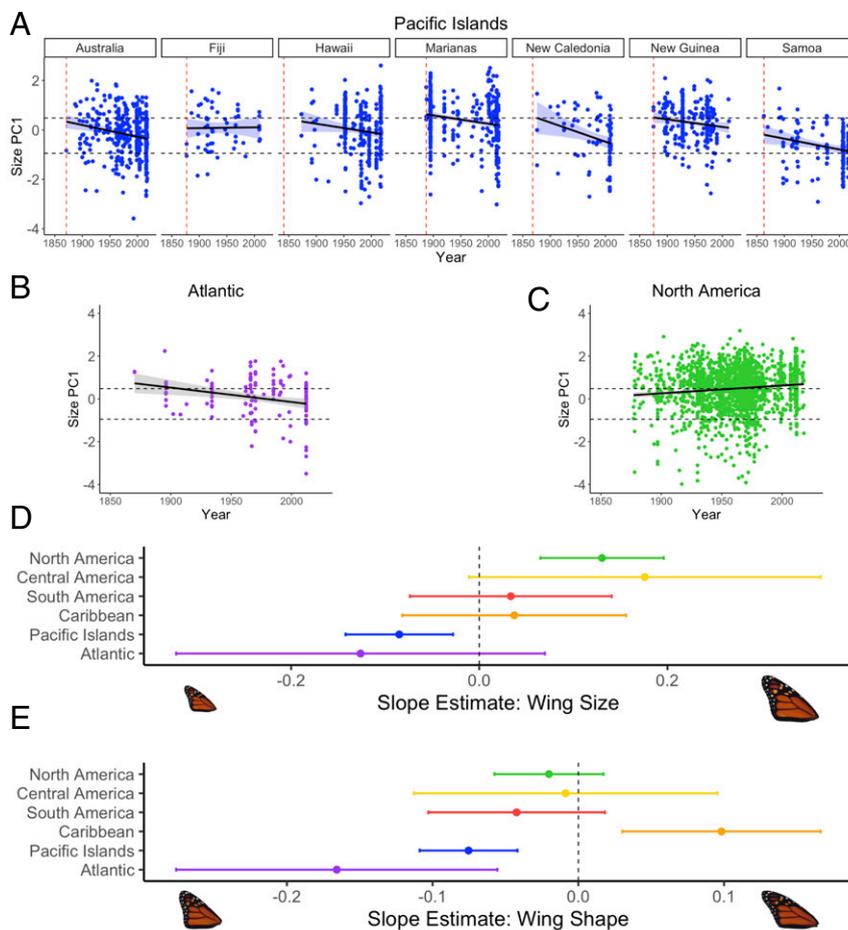


Fig. 4. (A) Time series showing change in wing size for Pacific island populations. Of the seven best-sampled Pacific island populations, five show significant decreases in forewing size through time. Top horizontal line corresponds to the historical migratory North American mean; bottom horizontal line corresponds to the historical nonmigratory Caribbean mean. Dashed red vertical lines are the earliest known establishment dates of monarchs in each location, based on museum records or historical accounts (14). Note that most Pacific populations begin at or above the mean for North American forewing size. (B) Atlantic specimens also show a significant decrease in forewing size through time. (C) North American monarchs show an increase in forewing size through time. (D) Model-estimated coefficients describing wing size evolution through time across all six regions of interest. Error bars correspond to the 95% confidence intervals. Note the contrast between nonmigratory populations from Central/South America and the Caribbean versus recently established nonmigratory populations from the Pacific and Atlantic. (E) Model-estimated coefficients for wing shape evolution also show reductions in forewing elongation for Pacific and Atlantic specimens. Model summaries are provided in *SI Appendix, Table S1*. Panel C reprinted with permission from ref. 39.

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 ($4\pi \cdot \text{area}/\text{perimeter}^2$) 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:

$$\text{response} \sim \text{region} \cdot \text{year} + \text{sex} + \text{latitude} + \text{image type} + (1|\text{collection ID}) + (1|\text{archipelago})$$

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 \log_{10} 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

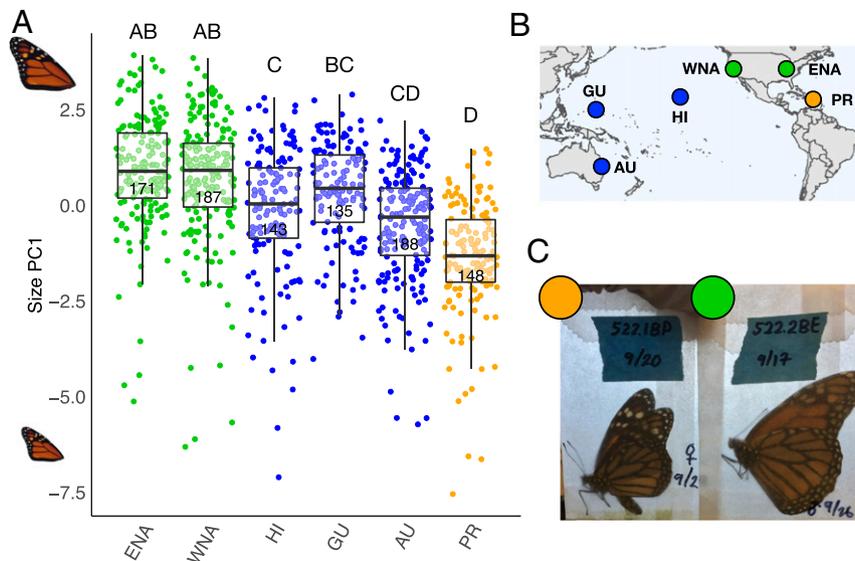


Fig. 5. (A) Wing size of common-garden reared monarchs from six populations around the world. Abbreviations correspond to map at right. Numbers inside of boxplots correspond to total number of measured adults for each population. Measured offspring were the F1 progeny of wild-caught females from 11 to 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 *cvqc* 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/B8157C>) (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.

ACKNOWLEDGMENTS. We thank the following museums and their curators for providing assistance and access to monarch collections: The American Museum of Natural History, the Australian National Insect Collection, The Australian National Museum, the Bishop Museum of Hawaii, the Berlin Museum of Natural History, the Bohart Museum (University of California [UC], Davis), the Museum of Natural History (London), the California Academy of Sciences, the Cornell University Insect Collection, the Essig Museum (UC Berkeley), the Harvard Museum of Comparative Zoology, the

Iowa State University Insect Collection, the Los Angeles County Museum of Natural History, the McGuire Center for Lepidoptera and Biodiversity, the Michigan State Arthropod Collection, the Norfolk Island Insect Collection, the Peabody Museum (Yale University), the Queensland Department of Agriculture and Forestry, the Queensland Museum (Brisbane), the UC Riverside Entomology Research Museum, the Smithsonian Museum of Natural History, the University of Guam Insect Collection, and a number of private collections and online databases. Myron Zalucki, Louie Yang, Riccardo Papa, Elizabeth Evans, Will Haines, and many others contributed live monarchs used in rearing experiments. Asia Jones, Sue-Ling Choquette, Evan Jordan, Jessica Liaudat, Jacob Dehkordi, and Christopher Jason helped with monarch rearing and wing measurements. Will Hemstrom contributed to bioinformatic analysis. Butterflies and host plant material were collected under U.S. Department of Agriculture permits P37-15-00580, P526P-18-02061, and P526P-17-02696. This work was funded through awards from the NSF Graduate Research Fellowship Program, the NSF East Asia Pacific Sciences Initiative (EAPSI) program, the UC Davis Center for Population Biology, and the National Geographic Society to M.G.F., as well as a Dickson Emeritus Professor Award to H.D., a California Agricultural Experiment Station grant to S.Y.S., and a David and Lucille Packard Fellowship to S.R.R.

- H. Dingle, "Migration: Definition and scope" in *Migration: The Biology of Life on the Move*, H. Dingle, Ed. (Oxford University Press, 2014), ed. 2, pp. 13–23.
- R. Lockwood, J. P. Swaddle, J. M. V. Rayner, Avian wingtip shape reconsidered: Wingtip shape indices and morphological adaptations to migration. *J. Avian Biol.* **29**, 273–292 (1998).
- L. Jenni, S. Jenni-Eiermann, Fuel supply and metabolic constraints in migrating birds. *J. Avian Biol.* **29**, 521–528 (1998).
- C. Merlin, R. J. Gegear, S. M. Reppert, Antennal circadian clocks coordinate sun compass orientation in migratory monarch butterflies. *Science* **325**, 1700–1704 (2009).
- J. Rolland, F. Jiguet, K. A. Jönsson, F. L. Condamine, H. Morlon, Settling down of seasonal migrants promotes bird diversification. *Proc. Biol. Sci.* **281**, 20140473 (2014).
- V. Gómez-Bahamón *et al.*, Speciation associated with shifts in migratory behavior in an avian radiation. *Curr. Biol.* **30**, 1312–1321.e6 (2020).
- R. S. Waples, R. W. Zabel, M. D. Scheuerell, B. L. Sanderson, Evolutionary responses by native species to major anthropogenic changes to their ecosystems: Pacific salmon in the Columbia River hydropower system. *Mol. Ecol.* **17**, 84–96 (2008).
- N. I. Gilbert *et al.*, Are white storks addicted to junk food? Impacts of landfill use on the movement and behaviour of resident white storks (*Ciconia ciconia*) from a partially migratory population. *Mov. Ecol.* **4**, 7 (2016).
- B. J. Crespi, R. Teo, Comparative phylogenetic analysis of the evolution of semelparity and life history in salmonid fishes. *Evolution* **56**, 1008–1020 (2002).
- C. I. Vágási *et al.*, Morphological adaptations to migration in birds. *Evol. Biol.* **43**, 48–59 (2016).
- H. Dingle, N. R. Blakley, E. R. Miller, Variation in body size and flight performance in milkweed bugs (*Oncopeltus*). *Evolution* **34**, 371–385 (1980).
- M. W. Baldwin, H. Winkler, C. L. Organ, B. Helm, Wing pointedness associated with migratory distance in common-garden and comparative studies of stonechats (*Saxicola torquata*). *J. Evol. Biol.* **23**, 1050–1063 (2010).
- B. B. Chapman, C. Brönmark, J.-Å. Nilsson, L.-A. Hansson, The ecology and evolution of partial migration. *Oikos* **120**, 1764–1775 (2011).
- S. Zhan *et al.*, The genetics of monarch butterfly migration and warning coloration. *Nature* **514**, 317–321 (2014).
- F. A. Urquhart, N. R. Urquhart, Autumnal migration routes of the eastern population of the monarch butterfly (*Danaus p. plexippus* L.; Danaidae; Lepidoptera) in North America to the overwintering site in the Neovolcanic Plateau of Mexico. *Can. J. Zool.* **56**, 1759–1764 (1978).
- P. R. Ackery, R. I. Vane-Wright, *Milkweed Butterflies: Their Cladistics and Biology* (Cornell University Press, 1984).
- R. I. Vane-Wright, "The Columbus Hypothesis: An explanation for the dramatic 19th century range expansion of the monarch butterfly" in *Biology and Conservation of the Monarch Butterfly*, S. B. Malcolm, M. P. Zalucki, Eds. (Natural History Museum of Los Angeles County, 1993), pp. 179–187.
- M. P. Zalucki, A. R. Clarke, Monarchs across the Pacific: The Columbus hypothesis revisited. *Biol. J. Linn. Soc. Lond.* **82**, 111–121 (2004).
- B. L. Phillips, G. P. Brown, J. K. Webb, R. Shine, Invasion and the evolution of speed in toads. *Nature* **439**, 803 (2006).

20. R. Shine, G. P. Brown, B. L. Phillips, An evolutionary process that assembles phenotypes through space rather than through time. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 5708–5711 (2011).
21. B. L. Phillips, G. P. Brown, R. Shine, Evolutionarily accelerated invasions: The rate of dispersal evolves upwards during the range advance of cane toads. *J. Evol. Biol.* **23**, 2595–2601 (2010).
22. B. M. Ochocki, T. E. X. Miller, Rapid evolution of dispersal ability makes biological invasions faster and more variable. *Nat. Commun.* **8**, 14315 (2017).
23. M. Szűcs *et al.*, Rapid adaptive evolution in novel environments acts as an architect of population range expansion. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 13501–13506 (2017).
24. C. Weiss-Lehman, R. A. Hufbauer, B. A. Melbourne, Rapid trait evolution drives increased speed and variance in experimental range expansions. *Nat. Commun.* **8**, 14303 (2017).
25. A. A. Pierce *et al.*, Serial founder effects and genetic differentiation during worldwide range expansion of monarch butterflies. *Proc. Biol. Sci.* **281**, 20142230 (2014).
26. M. Slatkin, L. Excoffier, Serial founder effects during range expansion: A spatial analog of genetic drift. *Genetics* **191**, 171–181 (2012).
27. D. C. Lahti *et al.*, Relaxed selection in the wild. *Trends Ecol. Evol.* **24**, 487–496 (2009).
28. S. Altizer, A. K. Davis, Populations of Monarch butterflies with different migratory behaviors show divergence in wing morphology. *Evolution* **64**, 1018–1028 (2010).
29. G. Beall, C. B. Williams, Geographical variation in the wing length of *Danaus plexippus* (Lep. Rhopalocera). *Proc. R. Entomol. Soc. Lond.* **20**, 65–76 (1945).
30. C. Dockx, Directional and stabilizing selection on wing size and shape in migrant and resident monarch butterflies, *Danaus plexippus* (L.), in Cuba. *Biol. J. Linn. Soc. Lond.* **92**, 605–616 (2007).
31. Y. Li, A. A. Pierce, J. C. de Roode, Variation in forewing size linked to migratory status in monarch butterflies. *Anim. Migr.* **3**, 49 (2016).
32. L. H. Yang, D. Ostrovsky, M. C. Rogers, J. M. Welker, Intra-population variation in the natal origins and wing morphology of overwintering western monarch butterflies *Danaus plexippus*. *Ecography* **39**, 998–1007 (2016).
33. D. T. T. Flockhart *et al.*, Migration distance as a selective episode for wing morphology in a migratory insect. *Mov. Ecol.* **5**, 7 (2017).
34. L. P. Brower, Understanding and misunderstanding the migration of the monarch butterfly (Nymphalidae) in North America: 1857–1995. *J. Lepid. Soc.* **49**, 304–385 (1995).
35. J. Asher *et al.*, Eds. *The Millennium Atlas of Butterflies in Britain and Ireland* (Oxford University Press, 2001), pp. 319–323.
36. D. A. Satterfield, A. K. Davis, Variation in wing characteristics of monarch butterflies during migration: Earlier migrants have redder and more elongated wings. *Anim. Migr.* **2**, 290 (2014).
37. M. L. Cody, J. McC. Overton, Short-term evolution of reduced dispersal in island plant populations. *J. Ecol.* **84**, 53–61 (1996).
38. K. A. Jönsson *et al.*, Evidence of taxon cycles in an Indo-Pacific passerine bird radiation (Aves: Pachycephala). *Proc. Biol. Sci.* **281**, 20131727 (2014).
39. M. G. Freedman, H. Dingle, Wing morphology in migratory North American monarchs: Characterizing sources of variation and understanding changes through time. *Anim. Migr.* **5**, 61–73 (2018).
40. E. Pfeiler *et al.*, Population genetics of overwintering monarch butterflies, *Danaus plexippus* (Linnaeus), from central Mexico inferred from mitochondrial DNA and microsatellite markers. *J. Hered.* **108**, 163–175 (2017).
41. J. I. Lyons *et al.*, Lack of genetic differentiation between monarch butterflies with divergent migration destinations. *Mol. Ecol.* **21**, 3433–3444 (2012).
42. C. Le Roy, V. Debat, V. Llaurens, Adaptive evolution of butterfly wing shape: From morphology to behaviour. *Biol. Rev. Camb. Philos. Soc.* **94**, 1261–1281 (2019).
43. O. Ovaskainen, M. Karhunen, C. Zheng, J. M. C. Arias, J. Merilä, A new method to uncover signatures of divergent and stabilizing selection in quantitative traits. *Genetics* **189**, 621–632 (2011).
44. M. Karhunen, J. Merilä, T. Leinonen, J. M. Cano, O. Ovaskainen, DRIFTSEL: An R package for detecting signals of natural selection in quantitative traits. *Mol. Ecol. Resour.* **13**, 746–754 (2013).
45. S. E. McGaugh *et al.*, The cavefish genome reveals candidate genes for eye loss. *Nat. Commun.* **5**, 5307 (2014).
46. M. D. Shapiro *et al.*, Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature* **428**, 717–723 (2004).
47. A. Burga *et al.*, A genetic signature of the evolution of loss of flight in the Galapagos cormorant. *Science* **356**, eaal3345 (2017).
48. C. Parmesan, G. Yohe, A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37–42 (2003).
49. O. A. Ali *et al.*, RAD Capture (Rapture): Flexible and efficient sequence-based genotyping. *Genetics* **202**, 389–400 (2016).
50. S. Zhan, S. M. Reppert, MonarchBase: The monarch butterfly genome database. *Nucleic Acids Res.* **41**, D758–D763 (2013).
51. H. Li *et al.*, 1000 Genome Project Data Processing Subgroup, The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
52. T. S. Korneliussen, A. Albrechtsen, R. Nielsen, ANGSD: Analysis of next generation sequencing data. *BMC Bioinformatics* **15**, 356 (2014).
53. E. Paradis, K. Schliep, Ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**, 526–528 (2019).
54. C. A. Schneider, W. S. Rasband, K. W. Eliceiri, NIH image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671–675 (2012).
55. M. G. Freedman, C. Jason, S. R. Ramirez, S. Y. Strauss, Host plant adaptation during contemporary range expansion in the monarch butterfly. *Evolution* **74**, 377–391 (2020).
56. D. Bates, M. Mächler, B. Bolker, S. Walker, Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* **67**, 1–48 (2015).
57. R Core Team, *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, 2020).
58. S. M. Altizer, K. S. Oberhauser, Effects of the protozoan parasite ophryocystis elektroscirrha on the fitness of monarch butterflies (*Danaus plexippus*). *J. Invertebr. Pathol.* **74**, 76–88 (1999).
59. M. Karhunen, O. Ovaskainen, Estimating population-level coancestry coefficients by an admixture F model. *Genetics* **192**, 609–617 (2012).
60. M. Beigy, cvcqv: Coefficient of Variation (CV) with Confidence Intervals (CI). R package version 1.0.0. <https://CRAN.R-project.org/package=cvcqv> (2019).
61. M. Freedman, Monarch global wing morphology. Dryad Data Repository. <https://doi.org/10.25338/B8157C>. Deposited 19 October 2020.