



Supplementary Information for

Contemporary loss of migration in monarch butterflies

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Supplemental Materials and Methods

Animal Husbandry

For outdoor reared monarchs in 2016, we captured summer generation adult North American (NA) monarchs in Chicago and ordered adult monarchs from a commercial breeder in July. However, the number of wild monarchs in the Chicago area was very low in 2016, and so we mated some of our NA wild caught adults to commercially sourced as well as wild caught monarchs to produce our late summer generation. Our local late summer group contains 12 pure NA and 8 F1 hybrids (Fig. 1B). We also mated commercial monarchs to each other to produce our commercial late summer group (Fig. 1B). The summer generations emerged between August 9th and 31st, 2016; however, summer monarchs tested in the flight simulator and dissected for mature oocyte counts emerged between August 11th and August 22nd.

Since we had very few pure bred NA monarchs in our summer generation, we crossed the few remaining 100% NA monarchs to the F1 hybrids to create our NA autumn generation (roughly 75% NA to 25% commercial). We mated pure commercial individuals to each other to produce the commercial autumn generation. We reared the autumn generation outdoors, and they emerged between October 10th and 24th, 2016 (Fig. 1B). We then brought all remaining pupae indoors on Oct. 24th as the temperature was expected to drop below freezing that night. The remaining pupae emerged over the course of 3 to 4 days in an environmental chamber kept at 21°C with an 11-hour day (07:00-18:00). These monarchs are the indoor eclosion autumn group and siblings of the autumn NA monarchs (Fig. 3C).

We reared all monarchs in 1.83m³ outdoor cages with steel frames covered in screens with 0.63mm openings purchased from Bioquip. The eggs and larvae were

further contained inside 30.5cm³ mesh popup cages. The adult butterflies were also double contained in 91.5cm x 30.5cm² mesh popup cages. All larvae ate fresh *Asclepias syriaca*, the common milkweed, which is native to Illinois. Adults ate Birds Choice Butterfly nectar, replenished daily. We labeled each adult monarch with a unique ID number in permanent marker on the hindwing and we recorded the ID, population of origin, emergence date, and sex of each individual.

We followed the same protocol in 2018 for the outdoor rearing of our NA summer and autumn generations; however, we did not cross NA monarchs to the commercial line in 2018. The 2018 summer generation emerged between August 1st and 3rd, 2018 while the autumn generation emerged between September 7th and 19th, 2018. Additionally, we caught 24 wild adult monarchs as they migrated through Chicago on September 14th, 2018.

For monarchs reared in environmental chambers in 2018, we captured NA adults in Chicago in June 2018, mated the adults and housed them outdoors. We collected eggs from the adults in early July and then reared half of the eggs in a summer-like chamber kept at 25°C with a 16-hour day and half in an autumn-like chamber kept at 18°C with a 14-hour day. The environmental chambers were fitted with Zilla tropical 25 UVB fluorescent T8 bulbs, which emit low levels of UVA and UVB light, and Philips bright white light mercury Alto II grow lights. Larvae ate fresh *Asclepias syriaca* while adults ate Birds Choice Butterfly nectar. The summer chamber adults emerged between July 31st and August 3rd, 2018. The autumn chamber adults emerged between August 26th and 30th, 2018. We labeled each adult monarch with a unique ID number in permanent marker on

the hindwing as well as recorded the ID, population of origin, emergence date, and sex of each individual.

Flight Simulator Design

We performed all flight testing in a monarch flight simulator adapted from
5 Mouritsen and Frost (*I*). The simulator consists of four main components: a large tube, a rotary encoder embedded in a metal ring at the top of the tube, a metal base which the tube sits on top of and a fan. We purchased a white semi-translucent acrylic tube open on both ends with a diameter of 30.5cm and height of 50.8cm. The base is constructed from steel and consists of four 22.9cm tall legs holding up a 30.5cm square platform with a
10 15.24cm wide hole in the center for the fan. Air flows through parallel drinking straws contained by an aluminum tube. The straws sit just above a computer fan (Comair Rotron 19028254A, Digikey, Part number: CR136-ND) which is connected to a variable transformer to create laminar airflow (*I*). We record all flight tests with a Logitech B910 webcam mounted under the platform and a rotary optical encoder suspended from a steel
15 ring sitting on the tube. We purchased the optical encoder (Catalog # E5-360-250-NE-S-D-D-B) and the cable to connect the encoder to a USB port (Catalog # CA-FC5-SH-FC5-20) from US Digital.

Aspects of our design differ slightly from Mouritsen and Frost (*I*). Instead of using a plastic axle for our shaft and inserting it into Teflon cylinders, we milled an
20 aluminum axle and inserted it into two stainless steel ball bearings (Ultra-Precision mini stainless-steel ball bearings, McMaster-Carr, Catalog #3826T15, Dimensions: 1/4" Shaft Diameter, 3/8" OD, 1/8" W) to minimize friction and make the encoder easy for butterflies to turn. We disassembled the encoder and attached the 16.5cm long hollow

aluminum shaft using a small screw. The two ball bearings sit inside the metal ring and hold the axle steady making the shaft's rotation smooth. The bottom 2.5cm of the shaft extending down into the tube is milled to a narrower diameter so that flexible plastic tubing can be slid over the shaft. The butterflies are attached at this junction via a tungsten tether (A-M Systems, Dimensions: 0.020 x 6 inches). The tether is inserted into their thorax, and the small wound is sealed with beeswax. The end of the tether inserted into the butterfly has a small hook that holds the tether in place under the dorsal cuticle of the thorax. The tether should be the approximate length of the butterfly's forewing and have the top 0.5 cm of a P20 pipette tip super glued to the top. The tether's pipette tip "hat" ensures quick transition between samples in the simulator. The hat fits into the flexible plastic tubing junction of the simulator shaft. Butterflies can rotate 360 degrees while flying in place.

Flight Simulator Testing

We used methods consistent with previous experiments utilizing a monarch flight simulator (*I-5*). Outdoor reared monarchs remained outdoors for no less than 3 full days after emergence. Environmental chamber reared monarchs also remained in their respective environmental chambers for at least 3 days post eclosion. Once tethered, all butterflies spent at least 4 days resting in a glassine envelope in an environmental chamber before flight testing. In both 2016 and 2018, our tethered outdoor and environmental chamber reared autumn generation monarchs were stored in chambers at 21°C with a short 12-hour photoperiod. In the summer of 2016, we stored outdoor reared individuals at 26.7°C with a 16-hour day; however, we lowered the temperature to 25°C in 2018 which resulted in lower morality. We performed all tests under sunny skies, and a

successful test required that an individual fly continuously for 10 minutes. After each successful test, we froze the sample for future dissection and potential genetic analysis. Each test was video recorded to ensure continuous flight. The rotary encoder records every change of position (0-359°) along with the time difference in milliseconds between the previous and current angle.

Circular Data and Statistics

Using custom scripts and the packages “Circular” and “Plotrix” written in R, we converted degree counts into cartesian coordinates, X and Y (6-8). We then calculated the mean vector ($\sigma = 0 - 359^\circ$) and vector strength ($r = 0 - 1$) for each individual. Next, we calculated a weighted group mean vector (σ) and vector strength (r) by multiplying each individual’s mean Cartesian coordinates (X,Y) by their individual vector strength. We used the Rayleigh test (sample size* vector strength²) to determine whether the group mean was significantly directional. Given the strength of the NA autumn generation’s group direction ($r = 0.65$), a sample size of $n = 7$ is required for significance (SI Appendix, Fig. S7). We used the Wallraff test to determine whether the distribution of individual directions differed significantly from each other. The Wallraff test computes the angular distances from a reference angle, in each group, which are then compared with a rank sum test.

To determine whether our groups had significantly different distributions of their group vector strengths we applied a bootstrap analysis. We randomly sampled, with replacement, each dataset with a sample size set to the smaller of the two groups, 1000 times. We then took the 1000 subsampled datasets and calculated a group mean and

vector strength. We calculated 95% confidence intervals around each mean (SI Appendix, Fig. S1).

Mature Oocyte Counts

We dissected females who completed a flight test by making a longitudinal cut
5 down the abdomen to remove ovaries and eggs. We then counted each mature oocyte. Mature oocytes have ridges that form prior to oviposition and run from the top to the bottom of the egg. We used the Mann-Whitney U test to determine whether mature oocyte counts differed significantly between groups. Age and mating status of the female affects the numbers of mature oocytes (9). Our females were never mated, but the
10 females were not dissected at the same age. On average, females were 15 days old and varied between 7 and 27 days old at time of dissection. We noticed a positive trend between age of female and number of mature oocytes in both summer and autumn generations (Summer's $R^2 = 0.599$ & Autumn's $R^2 = 0.368$).

Geometric Morphometrics

15 Forewings of dead monarchs were detached from the thorax using forceps. We placed the forewings on grid paper (0.05×0.05 cm squares) and took photos of the ventral side with a Canon EOS 70d camera. Using tpsDig232, a software designed for digitizing landmarks and outlines for geometric morphometric analyses (10), we put 13 landmarks on each forewing (SI Appendix, Fig. S2B). Each landmark, either at vein
20 intersections or ends, was an anatomically homologous point between individuals. We loaded landmarks coordinates into R and performed principal component analysis to examine variation in wing size. To examine variation in shape, we used Generalized Procrustes Analysis (GPA) in the R “Shapes” package to exclude effects unrelated to

shape including reflection, position, scale, and orientation (11). After the Procrustes analysis, we performed principal component analysis. We included 14 commercial and 15 NA individuals raised in a summer-like environmental chamber, and 11 commercial and 16 NA individuals raised outdoors in the summer in the forewing shape analysis
5 (commercial n = 29 & NA n = 31). We found no difference in forewing shape by rearing condition (SI Appendix, Fig. S8). These commercial and NA samples were pure bred. We tested for differences in group means using the Mann-Whitney U test.

Population Genetics and Phylogenetics

We extracted DNA from the thorax of fifteen autumn generation commercial
10 monarchs raised outdoors during autumn 2016, 14 of which successfully completed flight testing. We used the modified VDRC *Drosophila* genomic extraction, a chloroform/phenol method (12). After extraction, we checked the quality of the extracted DNA with an Agilent bioanalyzer. We used a KAPA Hyper Prep kit from KAPA Biosystems along with 15 custom Illumina barcodes to generate paired-end 75bp
15 libraries. We checked the quality of the libraries with an Agilent bioanalyzer and then pooled them for sequencing on the NextSeq500 Illumina platform. Raw reads for each individual were trimmed using Trimmomatic (13) to remove barcode adapters, and quality checked using FastQC (14). To establish the ancestry and degree of genetic change in the commercial monarchs, we downloaded whole genome sequencing data
20 from 72 monarch and 9 outgroup samples from NCBI SRA (Table S2). These genome data originate from monarchs collected from around the world (15). The previously sequenced samples were collected from North American (California, North Florida, South Florida, Massachusetts, Mexico, New Jersey, Texas), Central/South American

(Aruba, Belize, Bermuda, Costa Rica, Ecuador), Pacific (Australia, Hawaii, Fiji, New Caledonia, New Zealand, American Samoa), and Atlantic (Morocco, Portugal, and Spain) populations. Coverage for all 96 genomes, including the commercial and wild populations, averaged 13.74X. We mapped all sequences using Burrows-Wheeler aligner (16) to the North American monarch reference genome (version 3, repeat masked) (17) and converted file formats using SAMtools (18). The average percent of reads mapped for all samples was 81.7%. We marked duplicates with Picard (19) and assigned sample genotypes using Genome Analysis Toolkit's (GATK) HaplotypeCaller command with heterozygosity set to sample pi, 0.0127, and all other settings at default values (20). We then jointly called single nucleotide polymorphisms (SNPs/variants) in all samples using GATK's GenotypeGVCFs command with default settings, with the exception that heterozygosity which was set at sample pi, 0.0127 (20). We removed all insertions, deletions, and all variants with a quality score lower than 30 and kept only biallelic sites leaving 23,058,661 variants with a genotyping rate of 0.9612. We then filtered each site by a genotyping rate of at least 90% and removed variants not in Hardy-Weinberg equilibrium. With all samples merged, we then removed variants with minor allele frequencies less than 0.01. Finally, we pruned variants in high linkage disequilibrium (LD); such that any pair of SNPs in a 1kb window with a correlation of 0.95 or greater were removed until no such pairs remained. These further filtering steps left 4,593,379 variants with an overall genotyping rate of 0.9954 for analysis. We filtered using a combination of the programs Plink version 1.90 and VCFtools version 0.1.14 (21,22). We performed Principal Component Analysis (PCA) using Plink version 1.90 on the 4,593,379 variants left after filtering and pruning. We then randomly pruned the

4,593,379 variants to 1 million, and estimated population identity from 2 to 8 distinct populations (K=2 to K=8) using Frappe, version 1.1 (23) (Fig. S9). We averaged nucleotide diversity across 10kb windows 20 generated by VCFtools (Table S1). We used FastMe to build a neighbor-joining tree based on the 4,593,379 SNPs used for the population genetic analyses (24). We used the BioNJ method with subtree pruning and regrafting (SPR) tree refinement and performed 500 rapid bootstrap replicates (24). We then used Phylip's consense program to draw a tree with branches proportional to the number of bootstraps supporting each branch (25) (Fig. 2C). Using the same SNP set, we ran TreeMix, which infers a maximum likelihood tree, to investigate historical relationships and gene flow among monarch butterfly populations (26) (Fig. S5).

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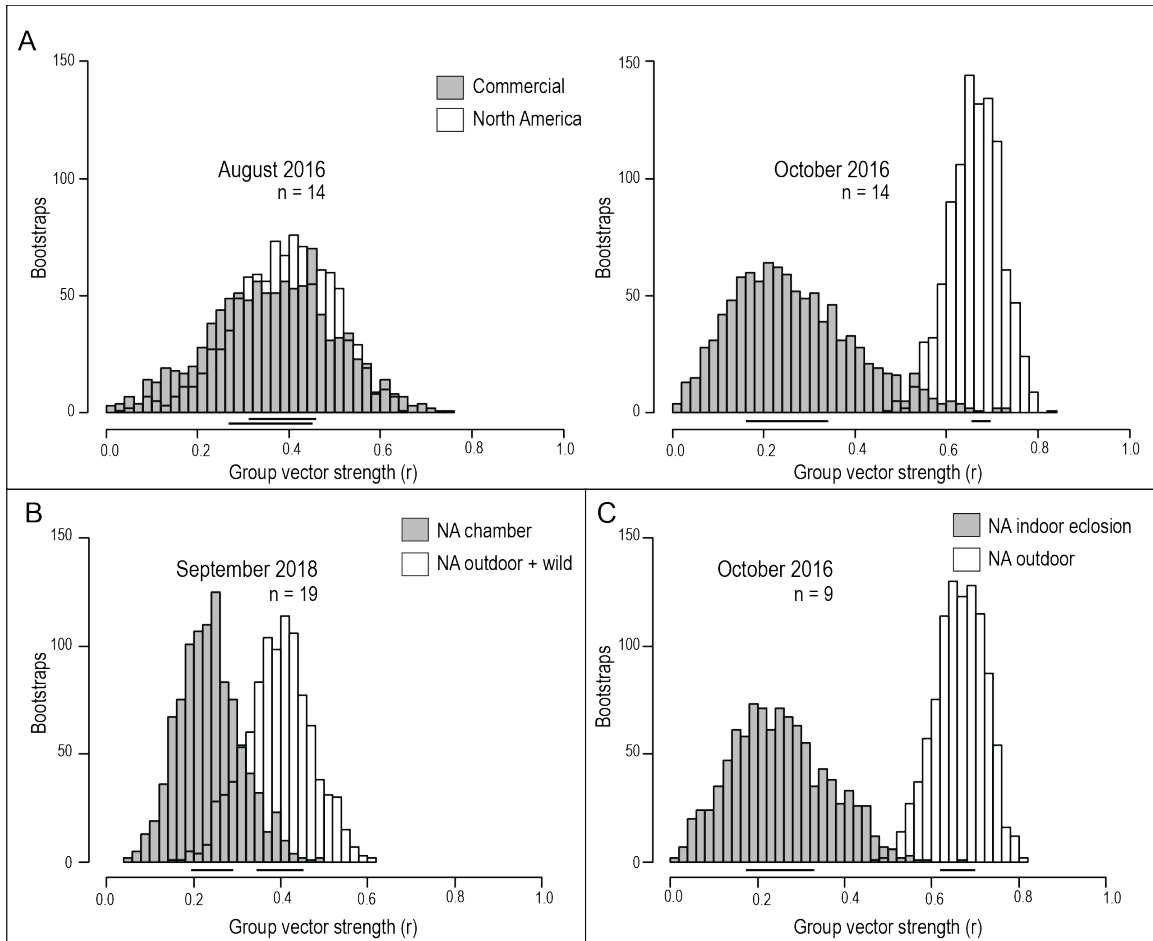


Fig S1. Distribution of group vector strengths (r) generated from subsampled orientation data. Black bars under histograms are 95% confidence intervals. A) In August 2016, NA: 95% CI [0.316, 0.460] and commercial: 95% CI [0.274, 0.452]. In October NA: 95% CI [0.659, 0.700] and commercial: 95% CI [0.177, 0.352]. B) In September 2018, NA autumn chamber: 95% CI [0.190, 0.284] and combined NA outdoor + wild: 95% CI [0.345, 0.445]. C) In October 2016, indoor eclosion: 95% CI [0.180, 0.325] and outdoor eclosion: 95% CI [0.625, 0.701].

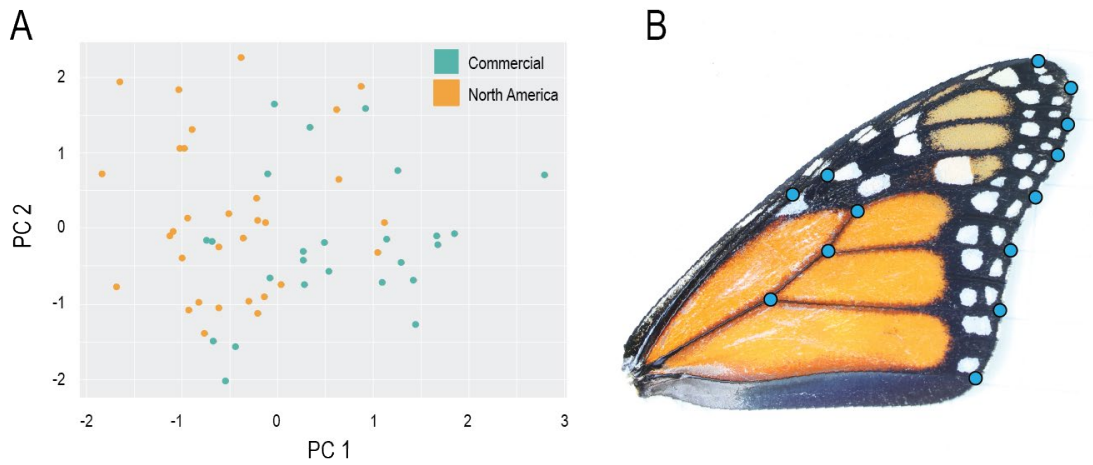


Fig. S2. Commercial monarchs and North American (NA) monarchs have differently shaped forewings. A) PC1 and PC2 represent the top two vectors of a principle component analysis (PCA) explaining 55.53% and 10.53% of the variation in shape respectively. B) A representative monarch forewing, blue dots indicate the placement of landmarks.

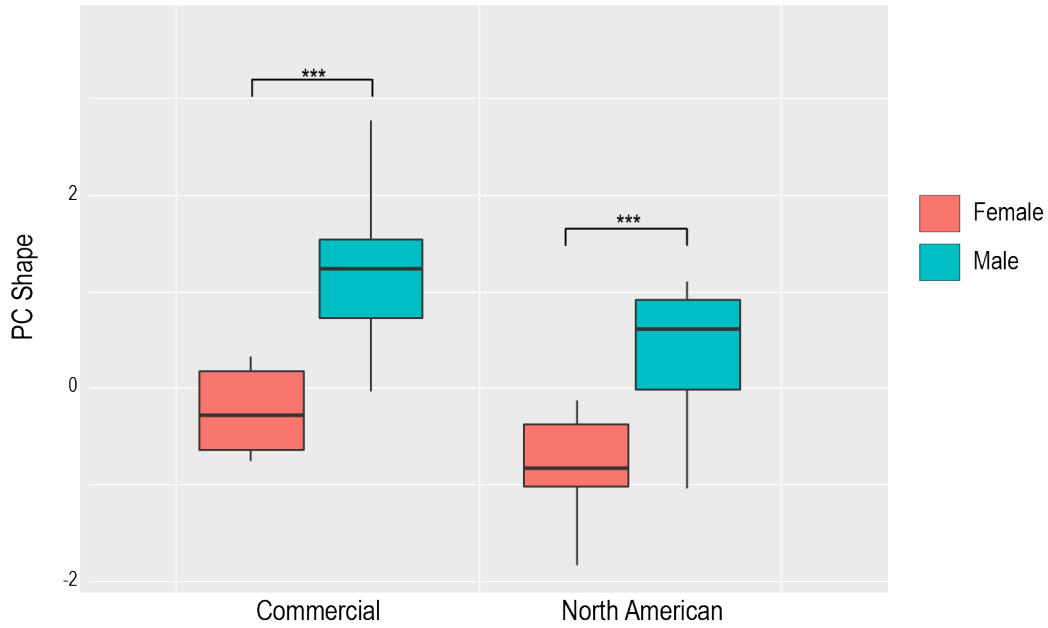


Fig. S3. There is sexual dimorphism in the shape of monarch forewings in both commercial and North American (NA) populations. Males (blue) have rounder forewings than the females (red) in both populations. PC Shape refers to the first vector of the principle component analysis (PCA).

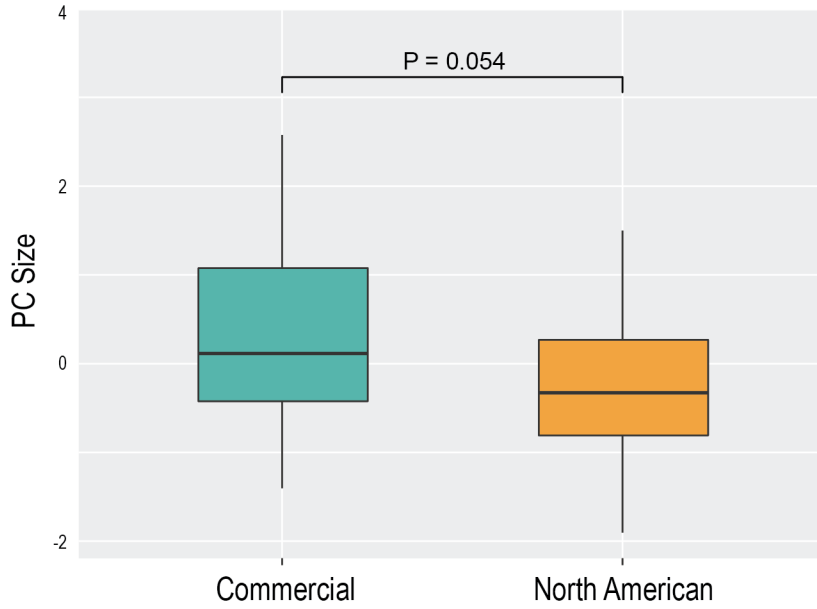


Fig. S4. Commercial monarch forewings tend to be smaller than North American (NA) monarchs though the difference is not significant. PC Size refers to the first vector of the principle component analysis (PCA) and explains 61.73% of the variation. Higher PC

5 values correspond to a smaller forewing.

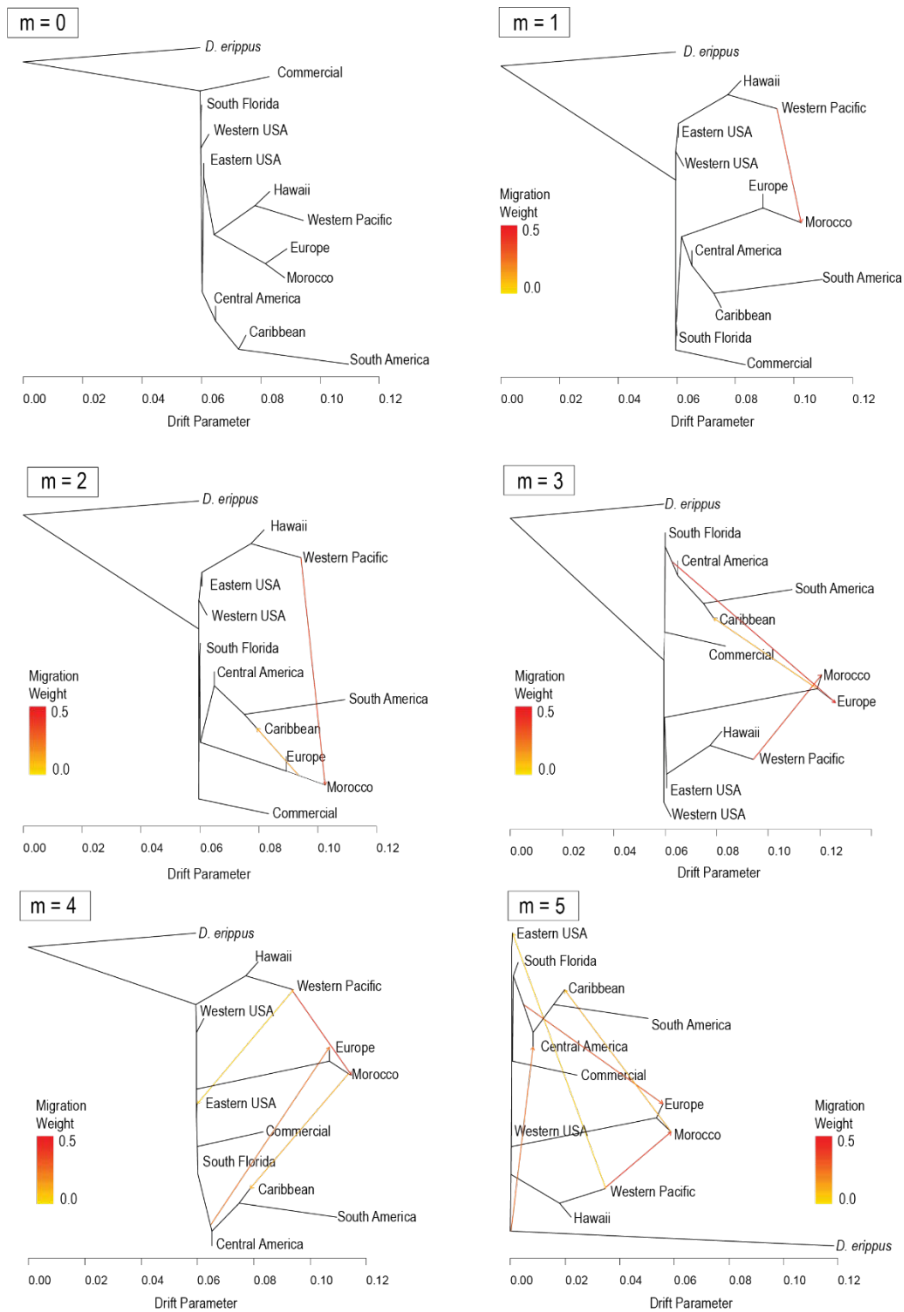


Fig. S5. Results of TreeMix analysis for commercial, North American (NA), and non-migratory monarch populations reveal no evidence of migration between commercial and other monarch butterfly populations. *Danaus erippus* is the outgroup. Arrows indicate

direction of migration given a migration event ($m= 0, 1, 2, 3, 4, 5$). Colors of the arrows represent migration weights which are correlated to admixture proportions.

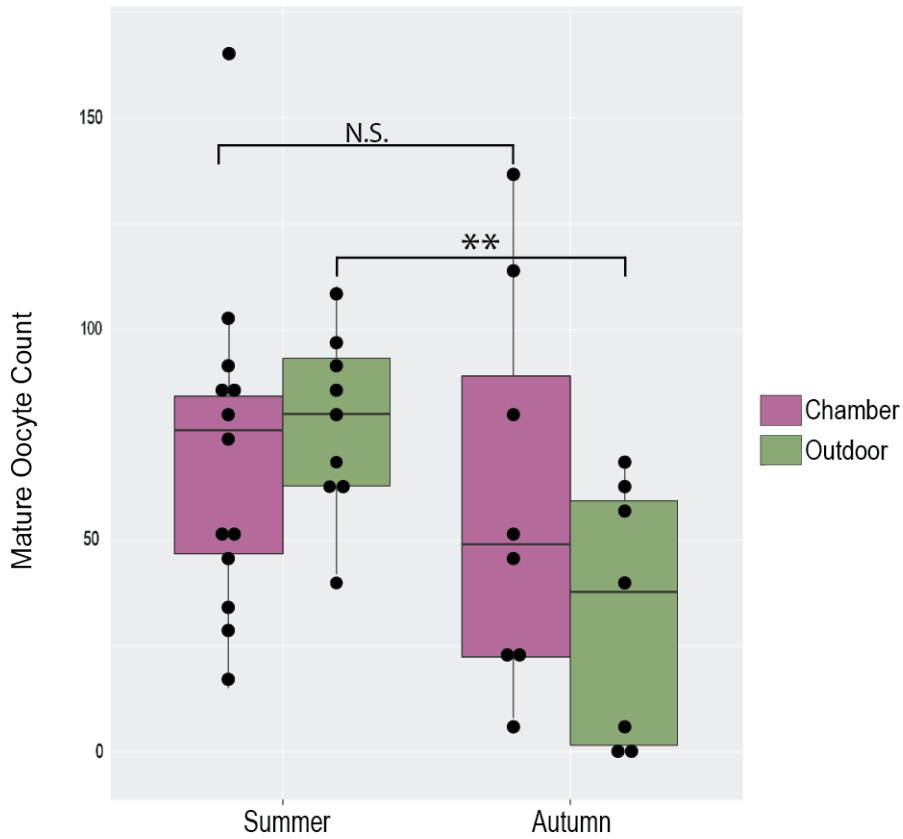


Fig. S6. Mature oocyte counts for environmental chamber reared monarchs. The number of mature oocytes decreases between the summer and autumn generations of outdoor reared North American monarchs (green), but there is no difference in oocyte counts between the autumn and summer-like environmental chamber treatments (purple).

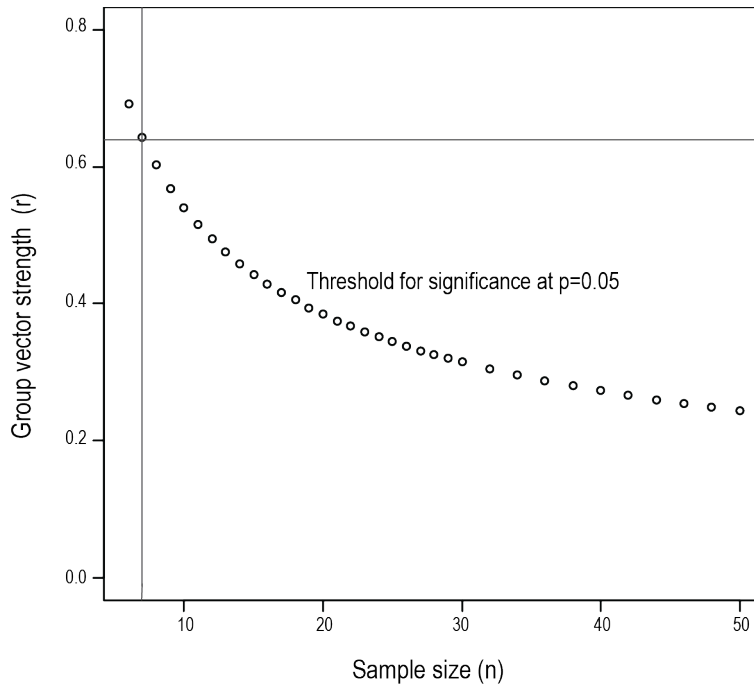


Fig. S7. Relationship between group vector strength (r) and sample size. Circles are the sample size required for significance ($p = 0.05$) at a given vector strength. The vertical and horizontal grey lines show that an $r = 0.64$ requires a sample size of $n = 7$ to detect significant directionality.

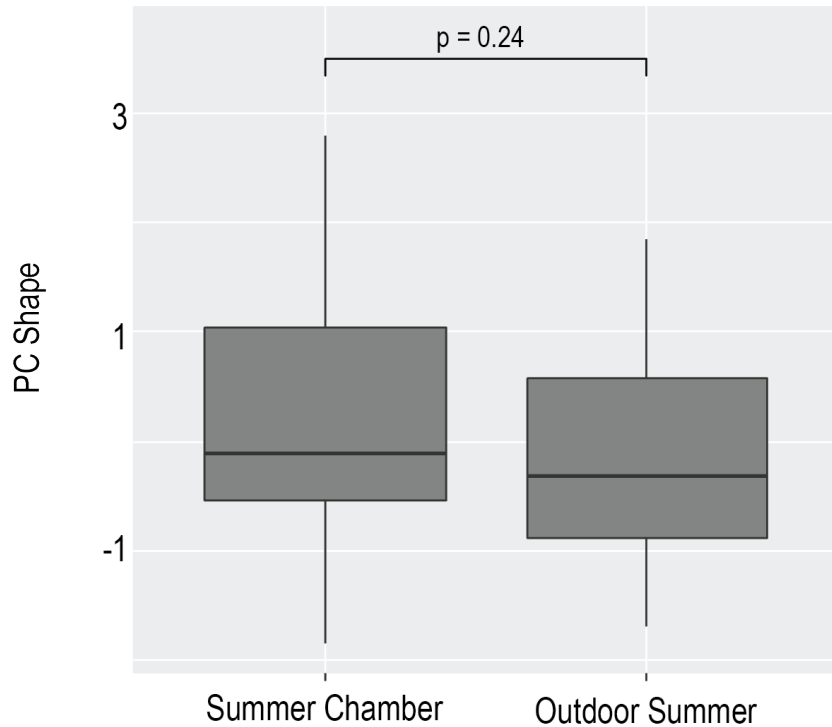


Fig. S8. There is no difference in wing shape between monarchs raised outdoors in the summer versus in a summer-like environmental chamber. Of the total 25 commercial monarchs used in the geometric morphometric analyses, 11 were reared outdoors in the summer while 14 were reared in an environmental chamber. Of the total 31 North American monarchs, 16 were raised outdoors and 15 in a summer-like environmental chamber. PC Shape refers to the first vector of the principle component analysis (PCA).

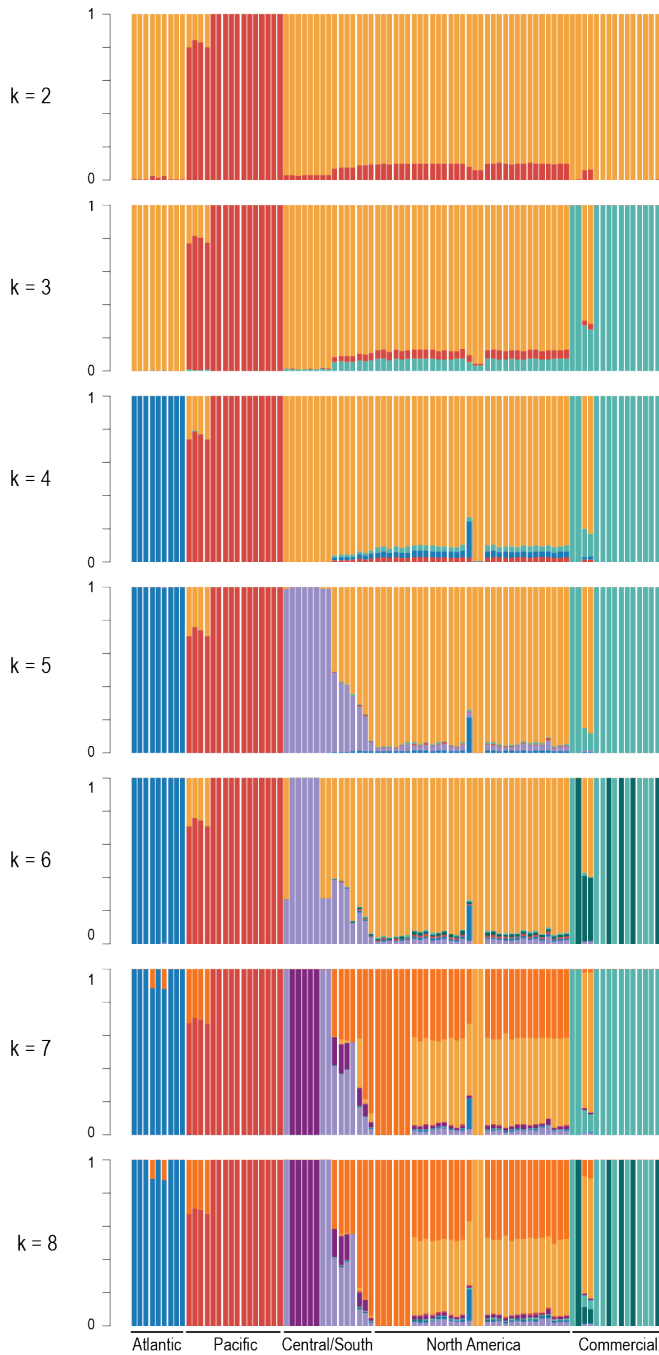


Fig. S9. Analysis of single nucleotide polymorphisms (SNPs) using Frappe reveals the commercial lineage is a distinct population with recent North American (NA) admixture.

Each column represents an individual sample and colors in each column the proportion of ancestry over population sizes ranging from $K=2$ to $K=8$.

Table S1. Nucleotide diversity (π) in the commercial lineage is less than North American (NA) wild populations and comparable to non-migratory populations of monarchs worldwide. SD stands for standard deviation.

Populations	π	SD
Atlantic	0.00805	0.00487 5
Pacific	0.00812	0.00490
Commercial	0.00892	0.00525
Central/South American	0.00990	0.00577
North American	0.01019	0.00596
South Florida	0.01050	0.0060910
All Samples	0.01273	0.00730

Table S2. Monarch and outgroup species sample information and statistics.

Accession #	Collection Location	Source	Raw (Mb)	Post QC (Mb)	Depth	Mapped %
SAMN10576418	Commercial Breeder, USA	This study	2029	1628	10.02	79.23
SAMN10576419	Commercial Breeder, USA	This study	3781	3714	22.04	80.40
SAMN10576420	Commercial Breeder, USA	This study	4378	4095	21.99	88.66
SAMN10576421	Commercial Breeder, USA	This study	2336	2058	18.32	88.23
SAMN10576422	Commercial Breeder, USA	This study	3350	2767	18.50	79.40
SAMN10576423	Commercial Breeder, USA	This study	4533	4237	23.53	75.90
SAMN10576424	Commercial Breeder, USA	This study	3632	3451	18.57	79.44
SAMN10576425	Commercial Breeder, USA	This study	3081	2654	17.97	80.32
SAMN10576426	Commercial Breeder, USA	This study	3031	2295	21.52	77.93
SAMN10576427	Commercial Breeder, USA	This study	4265	3318	11.42	78.76
SAMN10576428	Commercial Breeder, USA	This study	3889	3383	17.06	80.23
SAMN10576429	Commercial Breeder, USA	This study	3264	2865	23.02	79.99
SAMN10576430	Commercial Breeder, USA	This study	3734	3614	17.73	78.31
SAMN10576431	Commercial Breeder, USA	This study	4871	4438	15.39	80.13
SAMN10576432	Commercial Breeder, USA	This study	3704	3290	15.12	79.55
SRR1549530	Spain	Zhan et al. 2014			8.43	88.59
SRR1549531	Spain	Zhan et al. 2014			14.12	82.04
SRR1549532	Spain	Zhan et al. 2014			16.72	89.68
SRR1551990	Portugal	Zhan et al. 2014			12.44	76.14
SRR1551991	Portugal	Zhan et al. 2014			5.99	71.42
SRR1551992	Portugal	Zhan et al. 2014			24.02	89.30
SRR1552005	Morocco	Zhan et al. 2014			10.05	83.92
SRR1552006	Morocco	Zhan et al. 2014			7.68	88.67
SRR1552007	Morocco	Zhan et al. 2014			7.28	88.30
SRR1551994	Belize	Zhan et al. 2014			5.86	70.47
SRR1551996	Costa Rica	Zhan et al. 2014			10.83	90.80
SRR1552000	Puerto Rico, USA	Zhan et al. 2014			10.02	91.40
SRR1552102	Aruba	Zhan et al. 2014			20.55	78.98

SRR1552103	Aruba	Zhan et al. 2014	21.04	89.11
SRR1552107	Bermuda	Zhan et al. 2014	9.74	86.59
SRR1552228	Belize	Zhan et al. 2014	6.69	87.96
SRR1552229	Belize	Zhan et al. 2014	8.40	85.80
SRR1552230	Costa Rica	Zhan et al. 2014	18.53	91.32
SRR1552231	Costa Rica	Zhan et al. 2014	10.41	91.22
SRR1552310	Ecuador	Zhan et al. 2014	16.76	90.89
SRR1552311	Ecuador	Zhan et al. 2014	15.77	90.15
SRR1552312	Ecuador	Zhan et al. 2014	8.69	87.72
SRR1552313	Puerto Rico, USA	Zhan et al. 2014	14.04	91.90
SRR1552314	Puerto Rico, USA	Zhan et al. 2014	8.61	91.52
SRR1548504	Massachusetts, USA	Zhan et al. 2014	22.74	88.39
SRR1548506	Massachusetts, USA	Zhan et al. 2014	18.29	85.56
SRR1548571	Massachusetts, USA	Zhan et al. 2014	11.46	92.20
SRR1548572	New Jersey, USA	Zhan et al. 2014	9.42	85.38
SRR1548573	New Jersey, USA	Zhan et al. 2014	12.17	77.49
SRR1548574	New Jersey, USA	Zhan et al. 2014	9.95	82.47
SRR1548575	Massachusetts, USA	Zhan et al. 2014	7.66	80.86
SRR1548576	Massachusetts, USA	Zhan et al. 2014	6.83	88.69
SRR1548578	Massachusetts, USA	Zhan et al. 2014	23.35	68.64
SRR1549524	Saint Marks, Florida, USA	Zhan et al. 2014	13.75	84.60
SRR1549525	Saint Marks, Florida, USA	Zhan et al. 2014	13.20	88.40
SRR1549526	Saint Marks, Florida, USA	Zhan et al. 2014	21.10	83.34
SRR1549527	Texas, USA	Zhan et al. 2014	10.67	87.72
SRR1549528	Texas, USA	Zhan et al. 2014	9.54	87.72
SRR1549529	Texas, USA	Zhan et al. 2014	7.68	79.87
SRR1551995	California, USA	Zhan et al. 2014	9.22	87.00
SRR1552204	Mexico	Zhan et al. 2014	7.05	88.35
SRR1552205	Mexico	Zhan et al. 2014	13.03	81.09
SRR1552206	Mexico	Zhan et al. 2014	9.39	89.20
SRR1552207	Mexico	Zhan et al. 2014	9.41	80.45
SRR1552208	Mexico	Zhan et al. 2014	10.73	89.70
SRR1552209	Mexico	Zhan et al. 2014	9.31	88.73
SRR1552222	Saint Marks, Florida, USA	Zhan et al. 2014	21.51	89.03
SRR1552223	Saint Marks, Florida, USA	Zhan et al. 2014	6.47	65.12
SRR1552224	California, USA	Zhan et al. 2014	9.19	50.50
SRR1552225	California, USA	Zhan et al. 2014	12.24	89.85
SRR1548393	Hawaii, USA	Zhan et al. 2014	20.46	79.17
SRR1549537	Western Samoa	Zhan et al. 2014	38.95	86.12

SRR1551989	Western Samoa	Zhan et al. 2014	23.59	54.28
SRR1551993	Australia	Zhan et al. 2014	7.24	87.74
SRR1551997	Hawaii, USA	Zhan et al. 2014	6.78	90.62
SRR1551999	New Zealand	Zhan et al. 2014	8.99	90.11
SRR1552002	New Caledonia	Zhan et al. 2014	9.98	69.63
SRR1552004	New Caledonia	Zhan et al. 2014	6.58	50.39
SRR1552111	Fiji	Zhan et al. 2014	16.08	89.55
SRR1552113	Fiji	Zhan et al. 2014	13.13	65.72
SRR1552232	Hawaii, USA	Zhan et al. 2014	10.44	89.37
SRR1552233	Hawaii, USA	Zhan et al. 2014	18.92	88.32
SRR1552234	Australia	Zhan et al. 2014	11.98	90.48
SRR1552235	Australia	Zhan et al. 2014	16.70	90.92
SRR1552236	New Zealand	Zhan et al. 2014	15.29	93.22
SRR1552237	New Zealand	Zhan et al. 2014	18.39	84.88
SRR1551998	Miami, Florida, USA	Zhan et al. 2014	11.41	88.58
SRR1552211	Miami, Florida, USA	Zhan et al. 2014	12.32	86.52
SRR1552213	Miami, Florida, USA	Zhan et al. 2014	7.86	70.07
SRR1552214	Miami, Florida, USA	Zhan et al. 2014	14.07	63.74
SRR1552216	Miami, Florida, USA	Zhan et al. 2014	13.96	87.92
SRR1552226	Miami, Florida, USA	Zhan et al. 2014	14.38	91.27
SRR1552518	Brazil	Zhan et al. 2014	11.01	58.61
SRR1552519	Brazil	Zhan et al. 2014	6.17	55.19
SRR1552522	Costa Rica	Zhan et al. 2014	15.47	65.60
SRR1552523	Florida, USA	Zhan et al. 2014	13.84	60.53
SRR1552520	Australia	Zhan et al. 2014	16.73	88.77
SRR1552521	Australia	Zhan et al. 2014	24.28	92.58
SRR1552524	Costa Rica	Zhan et al. 2014	14.44	60.71
SRR1552525	Florida, USA	Zhan et al. 2014	5.47	89.85
SRR1980588	Texas, USA	Zhan et al. 2014	13.41	42.24

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