**Migration behaviour of commercial monarchs reared outdoors and wild-derived monarchs reared indoors**

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**Abstract**

Captive rearing of monarch butterflies is a commercial and personal pursuit enjoyed by many different groups and individuals. However, the practice remains controversial especially after new evidence showed that both a group of commercially-derived monarchs reared outdoors and a group of wild-derived but indoor-reared monarchs failed to orient south, unlike wild-derived monarchs reared outdoors. To more fully characterize the mechanisms responsible for the loss of orientation in both commercial and indoor-reared monarchs, we performed flight simulator experiments to determine: 1) whether any fraction of commercial monarchs maintains a southern heading over multiple tests, and 2) whether indoor conditions with the addition of sunlight can induce southern flight in wild-derived monarchs. Commercial monarchs changed their flight direction more often over the course of multiple tests than wild-derived monarchs. While as a group the commercial monarchs did not fly south on average, a subset of individuals did orient south over multiple tests, potentially explaining the discordance between flight simulator assays and the recovery of tagged commercial monarchs at overwintering locations. We also show that even when raised indoors with sunlight, wild-derived monarchs did not consistently orient south in the flight simulator, though wild-derived monarchs reared outdoors did orient south.

**Keywords**

*Danaus plexippus*, migration, captive rearing, commercial breeding, monarch flight behaviour, directional orientation

**Introduction**

Captive-reared monarch butterflies are reared and released at schools, weddings, conservation events, fairs, and by individual enthusiasts. However, the term ‘captive reared’ represents a spectrum of practices, including 1) raising wild-collected eggs and caterpillars in non-natural environments for eventual release 2) breeding wild-collected individuals for a few generations and releasing them into the wild and 3) raising eggs and caterpillars bought from a commercial source for release. Captive breeding can affect reared individuals’ behavior, morphology, and physiology in two distinct ways: changes to the genetic background of the population through inbreeding and adaptation to captive environments and exposure to and development in non-natural conditions1,2.

Long-term breeding in captivity is known to alter behaviour in fishes, mice, drosophila, and toads3-7. In monarchs, we have previously identified a population of commercially bred individuals that are genetically divergent from North American wild-type monarchs that no longer orient south as a group even when reared in conditions known to induce directional orientation in wild-derived individuals8. While the orientation of the commercial monarchs was non-directional as a group8, other tagged commercial monarchs have been found at Mexican overwintering sites, prompting the question of whether some fraction of the commercial individuals can, in fact, migrate. To assess the individual directionality of commercial monarchs, we assessed directional orientation of individuals from a known ‘non-directional’ North American commercial population8 and North American wild-derived population (from here on referred to as commercial and wild type respectively) multiple times to establish whether an individual would repeatedly fly south. Previous work, including our own, concluded testing after a single, successful orientation flight trial per individual8-11. By testing an individual repeatedly, we aim to determine whether specific individuals within the larger population exhibit directional orientation.

While rearing wild-collected monarch eggs will not change the genetic background of the individuals, artificial captive environments induce fitness differences in numerous fish species and monarch butterflies reared captively for a single generation12-18. In general, artificial rearing environments produce individuals that fare worse than wild individuals when released2. In both migratory fish and monarchs, changes to rearing environment affect migratory behavior8,17. Specifically, rearing migratory wild-type North American monarchs in an autumn-like environmental chamber (short day length and cool temperature) resulted in a group that oriented in random directions, while rearing wild types outdoors resulted in a group that oriented south8.

Since the environmental chamber does not replicate natural sunlight, we reared wild-type monarchs indoors with access to sunlight as filtered through glass windows during autumn and tested their directional orientation. Changes in photoperiod and declination of the sun during the transition between summer and autumn are hypothesized to be important environmental cues to induce migratory monarch development19-21. Monarchs are known to use a time-compensated sun compass to navigate; in fact, shifting their circadian clock with different light entrainment shifts orientation in migrating individuals9-11. While the position of the sun throughout the day plus light entrainment is critical for navigation, we do not know how important natural sunlight is for the development and triggering of directional orientation.

**Methods**

Animal Husbandry

In late July 2019, we caught approximately 20 wild monarchs in Hyde Park, Chicago, Illinois and ordered 20 commercial monarchs from the same source of commercial monarchs documented in Tenger-Trolander *et al.*8 We then checked the abdomens of each monarch for signs of *Ophryocystis elektroscirrha* (OE) spores and froze individuals with apparent infection. We housed the uninfected male and female monarchs from their respective populations in medium size (91.5cm x 30.5cm2) mesh pop-up cages outdoors with access to their host plant, *Asclepias syriaca*. Once females laid eggs, we washed and transferred the eggs to small (30.5cm3) mesh pop-up cages. We reared caterpillars in groups rather than individually. We fed caterpillars a diet of wild-collected *Asclepias syriaca* that we replenished daily. We washed the milkweeds in a 1% bleach solution and then in water before offering them to the larvae. Upon emergence, we labeled each adult with a unique identification number in permanent marker on the hindwing. Adults were housed in medium size (91.5cm x 30.5cm2) mesh pop-up cages before directional orientation testing and fed a diet of Birds Choice butterfly nectar.

Treatments

We housed the developing wild-type monarchs in one of three treatment groups: (1) outdoors, (2) indoors in a glass-top greenhouse, and (3) indoors in our laboratory (lab) next to a south-facing window. The commercial monarchs developed outdoors and had no other treatments. For the outdoor treatment, pop-up cages were contained within a large outdoor 1.83cm3 mesh cage. The greenhouse received only natural light, and we kept the temperature at 23⁰C during the day and 18⁰C at night. Temperatures in the lab remained fairly consistent between 22-23⁰C, 24 hours a day. Both indoor groups emerged between September 24th and October 28th of 2019. Commercial monarchs reared outdoors emerged between September 12th and October 1st 2019, and wild-type monarchs reared outdoors emerged between September 8th and September 16th 2019.

Unfortunately, we experienced a suspected outbreak of nuclear polyhedrosis virus (NPV) which reduced expected sample sizes and pushed back the dates of emergence of our wild types reared indoors as we attempted to control spread of the virus. We found the wild-type population was particularly susceptible; however, final sample sizes were sufficient to determine directional orientation.

Flight Simulator and Testing

After four days in their respective rearing conditions (outdoor, indoor greenhouse, or indoor lab), we tethered the monarch adults following the protocol outlined in Tenger-Trolander *et al*8. All tethered monarchs then spent five days recovering in glassine envelopes stored in a 12:12 hour light-dark cycle, 21⁰C environmental chamber before testing. Directly before testing, all monarchs spent at least a full hour in an outdoor cage free to move.

We tested all individuals in the monarch flight simulator developed by Mouritsen and Frost11 (Fig. 1A, see Tenger-Trolander *et al.*8 for description of modified flight simulator). All testing occurred outdoors in sunny conditions between the hours of 10am and 2:30pm. We counted the orientation test as successful if the individual flew continuously for 10 minutes as confirmed by video recording (See TengerTrolander\_Video\_S1.mp4 for an example of a non-directional monarch and TengerTrolander\_Video\_S2.mp4 for a southern-oriented monarch). We only tested individuals once per day whether the test was successful or not. Due to changing weather conditions, time restrictions on testing, and variability in emergence dates, every tethered monarch could not be tested each day of testing. We focused testing on the outdoor wild type and commercial individuals to determine individual preferences in directional orientation in these groups. Table S1 details the number of orientation tests and successful tests of each individual by treatment and population.

In total, we tethered 83 monarchs. 74 survived long enough to be tested, including 15 wild types reared outdoors, 18 wild types reared in the greenhouse, 4 wild types reared in the lab, and 37 commercials reared outdoors. Of these 74 tested, 65% (N = 48) flew at least once, including 8 wild types reared outdoors, 12 wild types reared in the greenhouse, 3 wild types reared in the lab, and 25 commercials reared outdoors. Of the 48 individuals that flew at least once, 56% (N = 27) completed at least one additional test, including 6 wild types reared outdoors, 4 wild types reared in the greenhouse, 1 wild type reared in the lab, and 16 commercials reared outdoors. The number of repeated tests in the indoor-reared group was small (N = 5); however, we were not attempting to determine whether a portion of these individuals was migratory, but rather if the group as a whole (N=15) headed south on average. The number of successful tests per individual ranged between one and seven (Table S1). Flight headings were recorded using a US Digital optical rotary encoder and captured on video (Fig. 1A). Since orientation data and video were recorded autonomously, testing was not conducted blind.

Data Analysis and Circular Statistics

In our flight simulator assays, tethered monarchs were attached to a rotary encoder and placed inside the simulator. As the monarch changed position, the rotary encoder recorded the new position (in degrees, 0 - 359°) and the amount of time elapsed (in milliseconds) between each change. We used circular statistics packages, Circular and Plotrix in R, to analyze and plot flight simulator data22-24. After converting degrees to cartesian coordinates, we found the mean vector direction (𝞼 = 0 - 359°) and mean resultant vector magnitude (r = 0 - 1) of each test. The mean vector direction is the average heading and the vector magnitude is a measure of consistency of the heading (r = 1 - variance). We also calculated a weighted group mean vector and magnitude and used the Rayleigh test to determine whether the group mean was directional.

Additionally, we calculated overall vector mean direction and vector magnitude for each monarch with between two and seven independent orientation tests. For example, an individual monarch with three tests with strong vector magnitudes (r>0.5) could still have a weak overall vector magnitude if it headed in vastly different directions (e.g. 0, 90⁰, 180⁰) for each test. The weak overall vector magnitude indicates the monarch chose a different direction for each of the three tests. We then took each individual’s overall heading and subtracted that from the individual’s first heading. We compared the difference in degrees between commercial and wild type with a Welch’s t-test. We also calculated each individual’s vector magnitude variance for all tests and compared commercial and wild type means with a Welch’s t-test.

Random Re-sampling of Migratory Flight Data

To determine whether any commercial monarchs with multiple orientation tests were likely migrators, we assessed the probability of finding each individual’s multiple flight headings within a distribution of known migrators using a random re-sampling approach. Including data from the autumns of 2016, 2018, and 2019, we have orientation data for 55 wild-type North American monarchs raised outdoors. Directional orientation data from the outdoor-reared wild types tested in 2016 and 2018 are available in Dataset\_S01.xlsx file of Tenger-Trolander *et al*.8. Data from 2019 are available in the supplementary file TengerTrolander\_Data\_S1.xlsx of this paper. Monarchs from 2016, 2018, and 2019 were all reared outdoors in the same conditions, but with variability in eclosion dates. Monarchs reared in 2016 eclosed between October 7th – 20th, those reared in 2018 eclosed between Sept 7th-18th, and those reared in 2019 eclosed between September 8th – 16th.

We binned the 55 orientation tests into either north (270-89⁰) or south (90-269⁰) bins, resulting in 51 southern binned and four northern binned tests. From those 55 binned wild-type migratory tests, we randomly sampled, with replacement, the number of tests an individual completed (between 2-7), 5,000 times. Each random sample had several possible orientation patterns going north and south. For instance, in the case of 5,000 random samples of three tests, the possible patterns encountered are SSS, SSN, SNN, or NNN (where S was south and N was north and order was not considered). We then counted how many of those 5,000 random patterns were SSS, SSN, SNN, and NNN. In the case of SSN, we found it appeared 350 times out of 5000 trials or 7% of the time in the known migratory group. An individual with 3 orientation tests that oriented south twice and north once has a 7% probability of being a migrator.

The number of bins and degree cutoffs for each bin was arbitrary and could be changed. We also analyzed the data in 90⁰ bins with the following degree cutoffs: northeast (0-89⁰), southeast (90-179⁰), southwest (180-269⁰), and northwest (270-359⁰). While the specific probabilities changed, which individuals are least and most likely to be migratory did not; exceptions are highlighted in white in Table 1. In our dataset, degree cut-offs affected the probabilities of two individuals (E101 & E103) described in detail in the results.

Outdoor Exposure and Southern Orientation

After the conclusion of our study, new work suggested that indoor-reared monarchs could re-orient when released outdoors25. We were interested in whether increasing outdoor exposure would potentially correlate with southern orientation. Since tethered monarchs were brought outdoors during each testing session and remained outdoors for the full testing period, which lasts several hours, most individuals spent many hours outside over the course of days. Using flight records from each test day, we calculated the minimum time spent outdoors by each of the indoor-reared monarchs. We tested whether there was any correlation with directional orientation south using a non-parametric Kruskal-Wallis H test.

**Results**

Multiple Directional Orientation Tests in Wild-type and Commercial Monarchs Reared Outdoors

For the orientation tests comparing wild type and commercial, we reared monarchs outdoors during autumn. We tested eight wild-type and 27 commercial monarchs. Six of the wild types and 16 of the commercial yielded multiple orientation tests (Fig. 1B wild type: 24 total flights = 8 first flights + 16 additional flights from the six individuals with multiple tests, commercial: 61 total flights = 27 first flights + 34 additional flights from the 16 individuals with multiple tests). Wild-type monarchs flew with an average heading south (𝞼 =143⁰) and a vector magnitude of r = 0.35 (Fig. 1B, Rayleigh test, z-score = 2.88, 0.05 < p < 0.10). Commercial monarchs’ average heading was also south (𝞼 =155⁰), but with a much weaker magnitude, r = 0.11 (Fig. 1B, Rayleigh test, z-score = 0.68, p > 0.50).

We then determined overall orientation headings for each of the monarchs with multiple orientation tests (Fig. 1C). Five of the six (83.33%) wild types had overall vector magnitudes > 0.4 with overall headings south (90-270⁰), while the 6th individual’s overall direction was 89⁰ with a relatively weak vector magnitude, 0.22 (Fig 1C & Table 1, wild type). Six of the 16 (37.5%) commercial individuals had overall headings south with vector magnitudes > 0.4 while the remaining 10 individuals’ overall headings were north and/or with magnitudes < 0.4 (Fig. 1C & Table 1, commercial). The difference in degrees between an individual’s first flight and the mean of all their flights showed wild-type monarchs chose more similar headings over multiple tests than commercial (t-test, t = 1.64, df = 18.88, p-value = 0.058, Fig. 2A). Additionally, we compared the variance of vector strengths in each individual’s multiple tests between the two groups. Commercial monarch vector magnitudes varied significantly more around an individual’s mean than wild type (t-test, t = 2.29, df = 19.33, p-value = 0.016, Fig. 2B), indicating that commercial monarchs were sometimes very directional during a test and then much less directional for the subsequent test whereas the wild types maintained similar vector strengths over multiple tests.

We next determined whether commercial monarchs with multiple orientation tests were possible migrators, by assessing the probability of finding each individual’s multiple flight headings within a distribution of known migrators. 37.5% (six of 16) of commercial and 83.33% (five of six) of wild-type monarchs had orientation test patterns consistent with the known migratory distribution of orientations (Table 1). Panels A and B of figure 3 are examples of four individuals whose test patterns suggest a strong probability of southern orientation. Panel C of figure 3 shows the patterns of two individuals with low probability.

We noted that though E103 and E101 (wild types reared outdoors) had low probabilities of being part of the migratory distribution in the 180⁰ binning procedure, both have overall southern headings with strong vector magnitudes (Table 1, Fig. 3D). E103 headed north on two out of five tests, but one of those flights was within 10⁰ of being binned as south (Fig. 3D). This is in contrast to the low probability commercial individuals, which all had northern mean headings or weak southern vector magnitudes (Table 1). In total, only 6 of 16 commercial monarchs showed signs of directional orientation south (Table 1).

Directional Orientation in Wild-type Monarchs Reared Indoors

We reared wild-type monarchs with natural light (as filtered through glass windows) during autumn in both a glass-top greenhouse and near a south-facing window in our laboratory and compared them to the outdoor reared group. We tested 15 indoor-reared monarchs, five of which produced multiple orientation tests (Fig. 4, indoor wild type: 26 total flights = 15 first flights + 11 additional flights from five individuals with multiple tests). The mean heading for those reared indoors was west, 𝞼 = 259⁰ with a weak vector magnitude, r = 0.12 (Fig. 4A, Rayleigh test, z-score = 0.40, p > 0.50). 11 of the 26 flights (42.3%) (from nine distinct individuals) had northern headings (Fig. 4A) compared to six (from three distinct individuals) of 24 (25%) flights in wild type reared outdoors (Fig. 1B). The five wild types reared indoors with multiple tests had overall means both south and north with strong and weak vector magnitudes (Fig. 4B). Even with the addition of autumnal sunlight through windows, we found outdoor wild-type flight behaviour was not completely recapitulated in the indoor-reared group.

New work has suggested that monarchs reared indoors, but then released are capable of re-orienting outdoors25. In light of this work, we used our testing records to calculate the total amount of time that each indoor-reared monarch spent outdoors prior to their test and found no correlation with directional orientation – more outdoor time did not increase the likelihood of southern orientation (Kruskal-Wallis chi-squared = 25, df = 25, p-value = 0.4624, Table S2).

**Discussion**

While a great deal is known about inducing diapause19,26,27 as well as how the monarch utilizes its circadian clock to navigate9-11, how monarchs develop and maintain directional orientation is less clear. The southern directional orientation phenotype requires a yet unknown combination of environmental conditions and genetics. Our earlier work suggested changes in long-term selection pressures and short-term developmental conditions can affect whether monarchs orient south in a flight simulator8. Here, we looked more closely at the behaviour of individual commercially sourced monarchs and investigated the effects of indoor rearing conditions with sunlight exposure on directional orientation.

We found that the commercial monarchs are a mix of southern-orienting and non-southern orienting individuals, suggesting that the directional orientation phenotype is not fixed in this population. Migration imposes a strong selective pressure on migratory monarchs as only successful migrators will pass on their genes in the coming spring. In commercial facilities, the difficulties of flying thousands of kilometers, finding the overwintering ground, and surviving until spring are no longer barriers to successful breeding. Add to that small population sizes inherent to commercial breeding and long-term captivity could lead to stochastic increase in the frequency of non-migratory alleles that do not respond to the correct environmental cues or alter the reaction norm of the population making responses to the environment more variable. While this study is limited to a single population of commercial monarchs, the mechanism of loss may be relevant to all long-term captive breeding populations.

While the effect of commercial releases on the North American monarch population is currently unknown, it may be ultimately inconsequential if natural selection purges the wild population of non-migratory individuals. After all, any non-migratory individuals would simply die in winter, their alleles never passed on to the next generation. However, this argument ignores two things, 1) the presence of new resident populations in the southern U.S. that can offer refuge to poor migrators and 2) the likely recessive8 and polygenic nature of migration genetics. In fact, crosses of the commercial and wild-type monarchs resulted in offspring that oriented south in autumn8. Non-migratory alleles could persist in the genetic background of a migratory individual. Releasing these commercial individuals may result in more monarchs in Mexican overwintering grounds in the short term, but have unintended consequences on their genetics in the long term. Additionally, the introduction of non-migratory alleles into the wild population may actually increase the number of individuals that breed year-round in the southern U.S.28-30 which has implications for the increased transmission of the monarch parasite OE. Resident populations have higher rates of OE infections31, and having more resident populations could lead to increased infection in the migratory population as it travels between the overwintering grounds and summer habitat32. Beneficial, neutral, or detrimental, the release of non-migratory alleles into a wild migratory population is worth discussing critically.

The effect of rearing environment should also be considered. Wild-type monarchs reared indoors with full exposure to natural autumn sun did not consistently orient south, though their genetic background is identical to the wild types reared outdoors. That being said, our results do not fully answer the question of what degree of “naturalness” is required to rear a directional adult. As we have only 5 indoor-reared individuals with multiple tests, we do not know if some proportion of the indoor-reared individuals are directional. However, placing captive-reared monarch larvae/pupae near a window does not result in as many directionally oriented monarchs as full outdoor exposure. Scientists have long speculated about the potential environmental variables that “turn on” the migration developmental program including photoperiod changes, temperature variation, sun declination, and host plant quality19-21,33,34. While we do not know which cue or combination of factors is responsible or the critical development times, we do know that the following conditions did not result in adults with consistent southern orientation: 1) rearing in an autumn-like environmental chamber 2) rearing in a room with sunlight and autumn-like temperatures during autumn and 3) eclosing in an environmental chamber after almost complete juvenile development outdoors. So far, in our flight simulator experiments, only wild adult monarchs caught in autumn and wild-type monarchs reared outdoors in autumn fly consistently south. And once oriented south, storing monarchs in an environmental chamber does not affect their southern orientation35 unless the temperature is dropped. Exposure to very cool temperatures in an environmental chamber causes re-orientation north36 in preparation for the spring re-migration.

New work from Wilcox *et al.*25 suggests that monarchs reared indoors may recover southern orientation after release. In their study, Wilcox *et al.*25 used a flight simulator to find the headings of a group of indoor-reared monarchs and found they did not orient south, consistent with our flight results of monarchs reared indoors. They also released groups of radio transmitter tagged monarchs reared indoors and found that the individuals flew an average of 37.4 km south 25. These results imply that regardless of rearing conditions experienced during development, adults given sufficient time outdoors in autumn would eventually fly south, suggesting monarchs are capable of re-orienting.

Currently, we cannot directly compare the flight simulator or radio-tracking data from Wilcox *et al.*25 to wild-caught or wild-type monarchs reared outdoors, which are known to fly south in autumn, because Wilcox *et al.*25 did not employ positive or negative controls. While our results and those of Wilcox *et al*.25 do not give us a completely clear understanding of the development of southern orientation in autumn in monarchs, together they suggest southern directional flight behaviour could be engaged in adulthood. In light of this possibility, we calculated the amount of time each indoor-reared monarch spent outdoors prior to each test but found no correlation between increased time spent outdoors and propensity to fly south.

In addition to radio-tracking data, mark-recapture studies of indoor-reared monarchs do recover a number of individuals at overwintering sites37,38. However, a study that tagged and released groups of both wild-caught and captive-reared eastern monarchs showed the recovery rate of captive-reared monarchs was significantly lower than that of the wild monarchs38. 56 of 11,333 wild-caught monarchs were recovered in Mexico whereas only 2 of 3,056 captive-reared monarchs were recovered (χ2 = 10.96, p = 0.00093)38. The same study also re-captured monarchs as they traveled south. While only 3 reared and 5 wild recoveries are reported in the paper38, a total of 10 indoor-reared and 6 wild-caught individuals were eventually recovered39. The captive-reared traveled an average distance of 120km and the wild-caught an average of 560 km (Mann-Whitney U, p-value = 0.002997)38,39. Even in the case that monarchs reared indoors re-orient upon release, captive-reared monarchs were less successful in reaching Mexico than wild monarchs38.

While many people hope that captive rearing is helping a declining population, the cumulative data available suggest that captive breeding of monarchs has negative consequences for migration behaviour and that monarchs reared indoors are not as well equipped to survive migration as those left in the wild8,13,38. We also know that rearing monarchs at home and in educational settings inspires new generations of conservationists, nature-lovers, and scientists. For those who love rearing monarchs, we advise the following: rear caterpillars individually in clean enclosures, rear outdoors when possible (especially in late summer and autumn), , limit the total number reared, avoid purchasing, and participate in citizen science projects. The non-profit, Monarch Joint Venture (monarchjointventure.org/get-involved/study-monarchs-citizen-science-opportunities), lists links to many on-going studies which have contributed vastly to our understanding of monarch biology. Finally, if we want to ensure the future of migratory monarch populations, we must promote longer-term solutions, like protecting and restoring habitat and addressing climate change.

**Data Availability**

All directional orientation data are included in supplemental file TengerTrolander\_Data\_S1.xslx.

**Acknowledgements**

We thank Jack Chaillet for help with catching and rearing the monarch butterflies used in this study. We thank the many monarch enthusiasts and biologists with whom we have discussed these and previous results. Their passion and insight have helped fuel this research. We also thank our reviewers who provided valuable feedback which greatly improved the paper.

**Funding**

This work was supported by the Graduate Research Fellowship Program, National Institute of Health Genetics and Regulation Training Grant [grant number T32 GM07197], US Fish and Wildlife Service Award [award number F17AC01222], National Science Foundation Grant [grant number IOS-1452648], and National Institute of Health Grant [grant numberGM131828].

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**Figures and Tables**

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