RESEARCH ARTICLE

Do Healthy Monarchs Migrate Farther? Tracking Natal Origins of Parasitized vs. Uninfected Monarch Butterflies Overwintering in Mexico

Sonia Altizer1*, Keith A. Hobson2,3, Andrew K. Davis1, Jacobus C. De Roode4, Leonard I. Wassenaar2¤

1 Odum School of Ecology, University of Georgia, Athens, Georgia, United States of America, 2 Environment Canada, 11 Innovation Blvd., Saskatoon, Saskatchewan, Canada, 3 Department of Biology, University of Western Ontario, London, Ontario, Canada, 4 Department of Biology, Emory University, Atlanta, Georgia, United States of America

¤ Current Address: International Atomic Energy Agency, Vienna International Centre, Vienna, Austria
* saltizer@uga.edu

Abstract

Long-distance migration can lower parasite prevalence if strenuous journeys remove infected animals from wild populations. We examined wild monarch butterflies (Danaus plexippus) to investigate the potential costs of the protozoan Ophryocystis elektroscirrha on migratory success. We collected monarchs from two wintering sites in central Mexico to compare infection status with hydrogen isotope (δ2H) measurements as an indicator of latitude of origin at the start of fall migration. On average, uninfected monarchs had lower δ2H values than parasitized butterflies, indicating that uninfected butterflies originated from more northerly latitudes and travelled farther distances to reach Mexico. Within the infected class, monarchs with higher quantitative spore loads originated from more southerly latitudes, indicating that heavily infected monarchs originating from farther north are less likely to reach Mexico. We ruled out the alternative explanation that lower latitudes give rise to more infected monarchs prior to the onset of migration using citizen science data to examine regional differences in parasite prevalence during the summer breeding season. We also found a positive association between monarch wing area and estimated distance flown. Collectively, these results emphasize that seasonal migrations can help lower infection levels in wild animal populations. Our findings, combined with recent declines in the numbers of migratory monarchs wintering in Mexico and observations of sedentary (winter breeding) monarch populations in the southern U.S., suggest that shifts from migratory to sedentary behavior will likely lead to greater infection prevalence for North American monarchs.
Introduction

Many animals migrate long distances to follow seasonal changes in resources and suitable habitats [1,2]. For some species, these journeys span entire continents or hemispheres, can take several months to complete, and are accompanied by high energetic demands and extreme physiological changes [3,4]. Migration can have profound ecological and evolutionary consequences on a global scale [5], including effects on the spread and prevalence of infectious diseases [6]. For some species, migration increases pathogen exposure as animals move between and encounter different habitat types [7,8]. Long-distance migration can also have the opposite effect of reducing parasite prevalence (reviewed in [6]) if heavily infected animals migrate poorly (i.e., migratory culling), or if migration allows animals to escape from habitats where parasites have accumulated (i.e., migratory escape).

Interactions between monarch butterflies (*Danaus plexippus*) and a protozoan parasite (*Ophryocystis elektroscirrha*) have become a model system for studying the effects of seasonal migration on host-pathogen dynamics. Monarchs in eastern North America migrate up to 2500 km southwards each fall to discrete wintering sites in Central Mexico [9,10]. In spring, many of the same individuals migrate north to recolonize their breeding range [11,12]. Monarchs in western North America migrate shorter distances to winter along the coast of California [13], with increasing evidence that these monarchs intermix with eastern North American monarchs [14–16]. Monarchs also form non-migratory populations that breed year-round in locations such as southern Florida, Pacific and Caribbean Islands, and Central and South America [17,18].

The protozoan *O. elektroscirrha* (*OE*) is a specialist neogregarine sporozoan reported to infect monarch and queen (*D. gilippus*) butterflies [19,20]. Parasites are transmitted when infected adults scatter dormant spores onto milkweed leaves, especially during oviposition. Larvae ingest the spores, the parasites replicate within larval and pupal tissues, and butterflies emerge covered with millions of spores on the outsides of their bodies [19,20,21]. Previous research across multiple monarch populations suggested that parasite prevalence was lower in migratory compared to non-migratory populations [22], an effect that could stem from the combined effects of migratory culling and migratory escape. Infected monarchs were shown to have lower flight endurance and speed than healthy monarchs when flown in captivity [23]. Among monarchs in eastern North America, infection prevalence decreased as monarchs moved southwards during their fall migration, consistent with the prediction that infected monarchs migrate poorly [24]. However, more direct estimates of the relationship between parasite load and migration distance remain elusive, in part owing to difficulties in tracking migration success of healthy and infected butterflies in the field.

Here, we tested whether parasite infection predicts successful long-distance migration in eastern North American monarchs traveling different distances from natal to wintering grounds. We used stable-hydrogen isotope measurements of wings to link adults sampled at wintering sites in central Mexico to the natal grounds where they developed from larvae (following [25,26]). We hypothesized that the natal origins of parasitized monarchs that successfully reached Mexico would correspond more strongly to southerly latitudes (indicating that infected monarchs migrated shorter distances) compared to unparasitized butterflies, and we further hypothesized that estimated flight distances would decrease with increasing parasite loads within the subset of infected butterflies. We tested whether other factors (sex, wing area and measures of wing and body condition) predicted estimated migration distances, and used citizen science data from the previous summer to query whether variation in butterfly natal origins could be explained by more prevalent parasite infections in certain regions of eastern N. America.
Methods
Field sites and sampling wild monarchs
In February 2008, we collected adult monarchs at two discrete overwintering sites in the Neo-
volcanic Mountains of Central Mexico: (i) Sierra Chincua and (ii) Cerro Pelon. Both sites are
located in Michoacan, Mexico [27,28]. Butterflies were collected using standard aerial nets with
expandable poles. The prevalence of monarchs heavily infected with OE at each of these two
locations in 2008 was 14.4 and 13.6%, respectively, based on a total sample of 1686 individuals.
From ten replicate clusters (five clusters per site), we randomly selected healthy and infected
butterflies following preliminary screening of parasite samples in the field, for a total of 75
healthy and 100 heavily infected monarchs. Monarchs were sampled under collecting permits
from SEMARNAT (Secretaria de Medio Ambiente y Recursos Naturales, Permit # 08202) and
the Monarch Biosphere Reserve (Mariposa Monarcha Reserva de la Biosfera, Permit #
RBMM-DIRECT-0050.08), exported under permission from PROFEPA (Procuraduría Federal
de Protección al Ambiente, Permit # 23811 21/Enero/2008) and imported with permission
from the U.S. Fish and Wildlife Service and the US Department of Agriculture (P526P-06-
02137).

Measuring infection status
We initially used a non-destructive method to assess individual infection status by pressing
clear adhesive stickers on adult abdomens (described in [22]). Spores of OE were counted at
50X magnification, and samples with >100 spores were considered to be heavily infected. This
classification includes the two highest spore load categories as defined by Altizer et al. [22] and
excludes the majority of monarchs that acquired spores through horizontal transfer among
adults [29,30]. Monarchs with 0–20 spores per individual were considered in this analysis to be
uninfected. After samples were returned to our laboratory, we obtained a quantitative measure
of parasite infection for the subset of heavily infected monarchs. Monarch abdomens were vor-
texed at high speed in 5ml H2O for 5 mins and we estimated total spore loads per sample using
a haemocytometer slide as described in De Roode et al. [21].

Isotopic analysis and natal assignment
The right hind wing of each monarch was stored and processed for hydrogen isotope (δ2H)
alyses at the Stable Isotope Laboratory of Environment Canada, Saskatoon, Saskatchewan,
Canada. Wings were placed in glass vials, solvent cleaned with a 2:1 chloroform:methanol solu-
tion to remove lipid residues and air dried. For δ2H analysis, 0.35mg of wing tissue was loaded
into 4.0 x 3.2 mm silver capsules. The Comparative Equilibration method to determine the δ2H
of non-exchangeable H in the wing tissue was used as described in Wassenaar and Hobson
[26,31]. Lipid-free keratin standards (EC1—CBS, caribou hoof keratin, -197 ± 2 ‰; and EC2—
KHS, kudu horn keratin, -54 ± 1 ‰) were used to normalize the results, and are reported in
delta (δ) notation as parts per thousand (‰) deviation from the VSMOW–SLAP standard
scale (Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation).

The hydrogen isotopic analysis builds on previous work showing that, for the eastern breed-
ing population of North America, δ2H values in monarch wing chitin decrease linearly with
increasing latitude [32]. In particular, the δ2H in adult wing membranes is controlled by the
geospatial location of the larval host plant, and associated isotopic values of precipitation [31,
32]. As a result, δ2H has been used to estimate the latitudinal geographical origins of monarchs
overwintering in Mexico [25], during the fall in Cuba [33], and during the spring recoloniza-
tion in eastern N. America [12, 34]. The demonstrated regular pattern of depletion with
latitudes of $\delta^2$H in wing chitin of known-origin monarchs throughout their eastern range [25], a phenomenon also demonstrated experimentally with monarchs raised on milkweed of known isotopic compositions [33], makes wing $\delta^2$H a convenient proxy for latitude in our study. This phenomenon is well known and apparent also in other taxa [35,36].

Butterfly wing data

The dorsal sides of left and right monarch forewings were scanned with a flatbed scanner to obtain digital versions of their wings for measurement [37–40]. Using the FoveaPro plugin (Reindeer Graphics, Inc.) for Adobe Photoshop, we measured the area of each forewing in mm². Forewing area is a predictor of among-population variation in migratory behavior [38], and therefore could impact individual flight distances. Prior to analysis, measures of left and right wings were averaged, and we excluded data for monarchs with torn or damaged forewings. Prior analyses examined monarch wing color in relation to monarch migratory status and flight propensity, and found that deeper orange hue was associated with migratory status and flight performance [37,38]. However, because the monarchs we examined here were over 5 months old and many had faded wings with visible scale loss, we chose not to include wing color in our analyses as the scale loss would affect color measurements.

Citizen science data

To infer the geographic distribution of infection by *O. elektroscirrha* during the summer breeding period, we used Project Monarch Health (MH) data in which volunteers from across the US and Canada collected parasite samples from wild-caught monarchs by pressing transparent 1cm² stickers against adult monarch abdomens. Samples were returned to the University of Georgia and scored for the presence/absence of heavy infection based on the presence of > 100 parasite spores per sample. Protocols for this program are described online (www.monarchparasites.org) and in Bartel et al. [24] and Satterfield et al. [41]. Across 2006–2011, a total of 124 volunteers participated from 23 states and two Canadian provinces. To make inferences about the relative contribution of different geographic regions to monarch infection, we focused on data for monarchs sampled between Aug 15 and Oct 15, 2007, as these monarchs would be most likely to have contributed to the overwintering population in Central Mexico sampled in Feb 2008. We recorded the latitude of each observer based on the city and state (or province) where monarchs were sampled, and further divided observer locations into one of three regions based on the following categories: South = between 30 to 36.9° latitude, N = 190; Central = between 37 to 41.49° latitude, N = 542; and North = between 41.5 to 49° latitude, N = 354. We omitted samples collected below 30° latitude, as monarchs have been reported to breed year-round at these sites, and thus are unlikely to contribute to the overwintering population in Mexico [41–43]. To limit observer-induced contamination from volunteer-derived MH samples, we removed data from observers for which prevalence was $\geq 60\%$ based on five or more samples returned in a given year, as some observers in the early years of the project were less aware of the need to employ sterile rearing and sampling protocols, and thus unintentionally contaminated the majority of their samples. In total, we omitted 78 lines of data from 5 observers (out of an initial 1164 lines of data from 63 observers). One observer was omitted from the south, 3 observers were omitted from the central region, and 1 observer was omitted from the north.

Data analysis

Analyses were conducted in SPSS ver. 19.0 [44]. We used logistic regression to examine the relationship between monarch infection status (assigned as 0/1 based whether or not monarchs
were heavily infected or not) and the following dependent variables: δ²H values (as a proxy for latitudinal origin and distance migrated), sex, site (Cerro Pelon or Sierra Chincua) and cluster (as a random variable nested within site). Next, we used general linear models (GLM) to test the relationship between infection status and natal origin in a different way, using δ²H values as the dependent variable, and the following independent variables: infection status (assigned as 0/1 as before), site (Cerro Pelon or Sierra Chincua), cluster (as a random variable nested within site), sex, and forewing area. Using data for the subset of heavily infected monarchs only, we used linear regression to examine the relationship between δ²H values (as the dependent variable) and quantitative spore load and forewing area (as independent variables).

For the analysis of regional differences in infection patterns from citizen science observations during summer 2007, we used logistic regression to examine the effect of latitude (as a continuous variable) on OE infection probability, treating each sample as an independent observation. We also used logistic regression to test for the effects of region (North, Central, South) on OE infection probability, with state nested within region as a separate variable to control for uneven sampling among states.

**Results**

Logistic regression showed a strong positive relationship between infection probability and δ²H as a proxy for north-south distance migrated, with infected monarchs, on average, originating from more southerly latitudes (Wald $\chi^2 = 12.78$, d.f. = 1, $p < 0.0001$; Fig 1). No other variables were significant predictors of monarch infection status (Sex: Wald $\chi^2 = 0.451$, d.f. = 1, $p = 0.502$; Colony: Wald $\chi^2 = 0.019$, d.f. = 1, $p = 0.890$; Cluster: Wald $\chi^2 = 0.767$, d.f. = 4, $p = 0.943$). GLM analysis of δ²H values as the dependent variable showed that uninfected monarchs had significantly lower δ²H values than heavily infected monarchs, again indicating uninfected individuals originated from farther north (F$_{1,163} = 13.43$, $p < 0.0001$). This was true for monarchs sampled at the Sierra Chincua and Cerro Pelon overwintering sites, and we found no significant effects of colony, cluster, or sex on δ²H values (colony: F$_{1,163} = 1.073$, $p = 0.333$; cluster: F$_{6,163} = 1.892$, $p = 0.085$; sex: F$_{1,163} = 0.303$, $p = 0.583$). Monarchs with larger forewings also had lower δ²H values, indicating a positive relationship between the distance migrated and wing area (F$_{1,163} = 6.17$; $p = 0.014$). This relationship between wing area and δ²H was highly significant for the subset of uninfected monarchs (Fig 2) but was non-significant for infected monarchs.

Quantitative spore loads for heavily infected monarchs ranged from $5.01 \times 10^3$ to $6.31 \times 10^5$, representing a 125-fold difference in untransformed values. In a model focused on data for heavily infected monarchs only, we found a positive relationship between δ²H values and log$_{10}$-transformed spore load, indicating that monarchs with heavier parasite loads originated from more southerly latitudes ($\beta = 5.63$, d.f. = 99; $t = 2.30$, $p = 0.023$).

Our analysis of infection patterns among the late summer / early fall 2007 monarchs sampled by volunteer observers showed no significant effect of latitude as a continuous variable on infection probability (Wald $\chi^2 = 0.099$, d.f. = 1, $p = 0.753$). When treated as a categorical variable, we found a higher infection probability among summer breeding monarchs sampled in the Central region relative to the North and South regions, but this effect was not significant (Wald $\chi^2 = 0.000$, d.f. = 2, $p = 1.000$; Fig 3) in an analysis that also included the effect of state nested within region (Wald $\chi^2 = 18.10$, d.f. = 17, $p = 0.381$; Fig 3). Including samples that were omitted from the 5 observers with high prevalence caused infection prevalence to increase to 19% for the North (N = 375), 23% for the Central region (N = 559) and 21% for the South (N = 230) relative to levels shown in Fig 3, but the effect of region on infection prevalence remained non-significant (Wald $\chi^2 = 0.000$, d.f. = 2, $p = 1.000$).
Discussion

Our analysis showed that uninfected monarchs overwintering in Mexico travelled, on average, farther distances between their summer breeding and wintering sites than butterflies that were infected with the protozoan *O. elektroscirrha*. Moreover, within the class of infected hosts, monarchs with the heavier quantitative parasite loads originated from more southerly locations (closer to their wintering sites) compared to less heavily infected monarchs. One possible
explanation for these findings is that heavily parasitized monarchs that originate from more northern latitudes (farthest from their wintering sites) simply do not reach Mexico, whereas healthy monarchs are better able to travel the farthest distances. This explanation would be consistent with the general idea that long-distance migration can lower pathogen prevalence by removing infected animals from the population (i.e., ‘migratory culling’, [6,23,24,45]). In this scenario, diseased animals suffering from infection are less likely to successfully migrate long distances owing to the combined physiological demands of migration and infection.

All evidence to date indicates that the fall migration and wintering period is energetically costly to monarchs, such that even a small cost of infection could be the tipping point between successful migration and premature death. Adult butterflies emerging in the late summer in eastern N. America weigh only ~0.5 g, yet must travel up to 5000 km round trip [46]. Moreover, monarchs use lipids stored during the fall to fuel their long distance flight and also to maintain themselves during the five-month overwintering period in Mexico, during which nectar resources are extremely limited [46,47]. Because OE infection causes reduced adult body size, shorter adult life span [21,48], and reduced flight performance in captive monarchs [23], the demands of migration likely remove a substantial fraction of heavily infected individuals each year, which would be consistent with the isotopic results reported here. Moreover, a previous field study showed that *O. elektroscirrhra* prevalence decreased as monarchs moved southward along the east coast flyway during their annual fall migrations [24], consistent with the idea that infected animals migrate less successfully [45].

Evidence for hindered migratory ability among infected individuals has been reported for a few other animal species, including the fall armyworm moth (*Spodoptera frugiperda*) infected by an ectoparasitic nematode (*Noctuidonema guyanense*); in this case, adults appeared to have
reduced migratory ability because few or no parasites were detected in moths recolonizing sites as they returned north [49]. Other work on Bewick’s swans (Cygnus columbianus bewickii) showed that infection by low-pathogenic avian influenza (LPAI) viruses delayed departure dates for fall migration by over a month, and reduced the travel distances of infected birds compared with healthy individuals [50]. Studies of other species showed little or no effect of infection state on migration [51–53] suggesting that some species can better tolerate infection during long distance journeys. This raises the possibility that migration could select for high infection tolerance, owing to the high fitness costs of migrating while harboring a debilitating pathogen.

We used citizen science data to examine an alternative explanation for the negative relationship observed between monarch infection status and latitude of natal origin ($\delta^{2}H$) by testing whether lower latitudes late in the breeding season give rise to more infected monarchs. Although monarchs sampled in the central part of eastern N. America were more likely to be infected as compared to monarchs sampled in the north and south of their breeding range, this effect of region was not significant, and we further found no consistent effect of latitude as a continuous variable on late summer infection probability. It is also important to note that a recent analysis of OE infection probability across four separate years showed that regions with the highest prevalence differed among years [24], and in no years was late summer infection prevalence significantly higher at southern latitudes. Taken together, these results suggest that latitudinal differences in the natal origins of infected vs. uninfected monarchs that successfully reach Mexico are probably driven more by differences in migration ability than by differences in transmission among different breeding locations.

One possible confounding factor that could affect our analysis of quantitative spore loads within the infected class is that adult monarchs might lose spores from their bodies during the
journey south, thus causing a negative relationship between quantitative spore load and distance migrated. In fact, an analysis of captive monarchs held in outdoor enclosures during the summer indicated that reproductively active adults could lose up to 90% of spores between eclosion and death [30]. Thus, although we found a five-fold difference in average spore load between monarchs at the extreme distributions of the $\delta^{2}H$ range, some portion of this difference could be due to the loss of spores during migration. On the other hand, direct comparison between the results of De Roode et al. [30] and our study are difficult because mating and oviposition in caged monarchs could lead to the loss of higher numbers of spores relative to flight alone (i.e., monarchs in the caged study were actively landing on plants and laying eggs continuously, whereas migrating monarchs spend a great deal of time in gliding flight). In addition, the loss of spores during migratory flight would not account for the differences in infection status as a binomial variable, because heavily infected butterflies would not lose enough spores to be misclassified as an uninfected monarch.

Our analysis also found a positive association between monarch wing area and estimated distance flown based on $\delta^{2}H$. This same result was found in a prior (unpublished) investigation [54], and suggests that monarchs with larger wings are successful at migrating the farthest distances. While this conclusion seems intuitive, past evidence that wing size affects migration success (in monarchs) has only been circumstantial. For example, multiple studies showed that monarchs captured late in the fall migration season (which presumably fell behind) had smaller wings than those that migrated early [55–57]. This result could arise if monarchs with small wings fly more slowly and stay longer at stopover sites, resulting in a slower migration pace [58]. Slow-migrating monarchs are also at higher risk of mortality from extreme weather such as storms or falling temperatures [59]. In addition, long before the location of the overwintering sites were known, Beall [60] noted that monarchs found dead along the shore of Lake Erie were significantly smaller than those captured alive in this region during the fall, implying that small-winged monarchs are less successful at navigating water crossings within the flyway. Finally, monarchs from non-migratory populations tend to have smaller forewings than those from migratory populations [39,61]. Collectively, our results concerning monarch wing size are consistent with work on other migratory species showing that the demands of long-distance flight select for greater wing area to maximize powered and gliding flight [61–65].

We were surprised to find that the significant relationship between wing size and migration distance observed among uninfected monarchs did not appear in the infected group. We can only speculate as to the cause of this finding. It could be that the culling of smaller-winged monarchs is more likely over extremely long spans of travel, for monarchs that originate from the highest latitudes. Because the sample of infected monarchs successfully reaching Mexico is biased towards those that started out from more southerly origins, the culling of smaller monarchs might be less extreme at these shorter distances. In other words, it could be that infected monarchs that start out from more northerly locations simply do not reach Mexico, regardless of their wing size.

The monarchs’ fall migration in eastern North America is both unique and declining. Estimates of overwintering colony sizes fluctuate from year to year, but on average show evidence of a long-term decline of up to 90% during the past 20 years [66,67], with the last 3 consecutive years representing the lowest numbers of overwintering monarchs recorded in Mexico to date. The overall drop is thought to be due to loss of breeding habitat, especially within the Midwestern U.S. [68,69], which prior isotopic analysis identified as a major source of the Mexico overwintering population [25]. Over this same time period, some monarchs have become non-migratory and breed on exotic milkweed in the extreme southern US during the winter months rather than travel to Mexico [43,70]. A recent analysis of citizen science data on parasite infection showed that OE prevalence was markedly higher among winter breeding monarchs
compared with migratory monarchs [41], suggesting that diminished migration increases infection risk. In combination with this recent work, results presented here predict that human activities that threaten monarch migration and cause shifts towards sedentary status may increase parasite transmission, potentially leading to greater population-wide infection prevalence across eastern North America.

In summary, our study is consistent with a growing body of scientific knowledge suggesting that infected migratory animals are less likely to successfully traverse long distances, and thereby highlights the role that migration can play in lowering parasitism in wild animal populations [6]. Thus, in contrast to human populations, for which long-distance travel can allow pathogens to spread across the globe in a matter of hours [71,72], migratory animals undertake strenuous long-distance journeys on their own power, and heavily infected animals might not survive these costly journeys. Importantly, monarch migrations, like the migrations of many other animal species [73], are considered an endangered phenomenon [74]. Already, human activities that discourage long-distance animal movements and encourage the formation of local year-round populations have enhanced the emergence of zoonotic pathogens in wildlife and humans [75,76]. Thus, results of our study underscore the need to conserve long-distance animal migrations to mitigate infection processes in wild animal populations, with implications for future disease risks in humans and threatened species.

Supporting Information

S1 File. Raw data used in analyses. Data are provided on isotopic values for field collected monarchs from two overwintering sites in central Mexico, February 2008, and on Monarch Health citizen science data tracking infection by the protozoan parasite _Ophryocystis elektroscirra_ during late summer / early fall 2007. (DOCX)

Acknowledgments

We thank Rachel Rarick, Michael Maudsley, Adam Federman, Jeff Smith, Eduardo Rendon, and Isabella Ramirez for field assistance and Ernie Osburn, Samantha Burton and Michael Maudsley for laboratory assistance.

Author Contributions

Conceived and designed the experiments: SA. Performed the experiments: SA LW KH. Analyzed the data: SA AKD JCdR. Contributed reagents/materials/analysis tools: SA LW KH. Wrote the paper: SA KH AKD JCdR LW.

References

1. Johnson ML, Gaines MS. Evolution of dispersal: Theoretical models and empirical tests using birds and mammals. Annual Review of Ecology and Systematics. 1990; 21:449–80.
2. Dingle H. Migration: The Biology of Life on the Move. Oxford, UK: Oxford University Press. 2014.
3. Bowlin MS, Bisson IA, Shamoun-Baranes J, Reichard JD, Sapir N, Marra PP, et al. Grand challenges in migration biology. Integrative and Comparative Biology. 2010; 50: 261–79. doi:10.1093/icb/icq013 PMID: 21558203
4. Chapman JW, Reynolds DR, Wilson K. Long-range seasonal migration in insects: Mechanisms, evolutionary drivers and ecological consequences. Ecology Letters. 2015; 18: 287–302. doi:10.1111/ele.12407 PMID: 25611117
5. Bauer S, Hoye B. Migratory animals couple biodiversity and ecosystem functioning worldwide. Science. 2014; 344: 1242552. doi: 10.1126/science.1242552 PMID: 24700862
6. Altizer S, Han B, Bartel R. Animal migrations and infectious disease risk. Science. 2011; 331: 296–302. doi:10.1126/science.1194694 PMID: 21252339

7. Waldenström J, Bensch S, Kiboi S, Hasselquist D, Ottosson U. Cross-species infection of blood parasites between resident and migratory songbirds in africa. Molecular Ecology. 2002; 11: 1545–54. PMID: 12144673

8. Figuerola J, Green AJ. Haematozoan parasites and migratory behaviour in waterfowl. Evolutionary Ecology. 2000; 14: 143–53.

9. Urquhart F, Urquhart N. 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. Canadian Journal of Zoology. 1978; 56: 1759–64.

10. Calvert WH, Brower L. The location of monarch butterfly (Danaus plexippus l.) overwintering colonies in Mexico in relation to topography and climate. Journal of the Lepidopterists Society. 1986; 40: 164–87.

11. Malcolm SB, Cockrell BJ, Brower LP. Spring recolonization of eastern North America by the monarch butterfly: Successive brood or single sweep migration. In: Malcolm S and Zalucki M (eds) Biology and Conservation of the Monarch Butterfly. Natural History Museum of Los Angeles County. 1993.

12. Miller NG, Wassenaar LI, Hobson KA. Natal origins of migratory monarch butterflies at wintering colonies in Mexico: New isotopic evidence. Proceedings of the National Academy of Sciences. 1998; 95: 15436–41. doi:10.1126/science.1194694 PMID: 21252339

13. Nagano CD, Sakai WH, Malcolm SB, Cockrell BJ, Donahue JP, Brower LP. Spring migration of monarch butterflies in California. In: Malcolm SB, Zalucki MP (eds) Biology and Conservation of the Monarch Butterfly. Natural History Museum of Los Angeles County. 1993.

14. Brower L, Pyle R. The interchange of migratory monarchs between Mexico and the Western United States, and the importance of floral corridors to the fall and spring migrations. In: Nabhan (ed) Conservation of Migratory Pollinators and their Nectar Corridors in North America Arizona-Sonora Desert Museum, University of Arizona Press. 2004.

15. Lyons Ji, Pierce AA, Baribeau SM, Sternberg ED, Mongue AJ, de Roode JC. Lack of genetic differentiation between monarch butterflies with divergent migration destinations. Molecular Ecology. 2012; 21: 3433–44. doi:10.1111/j.1365-294X.2012.05613.x PMID: 22574833

16. Pierce A.A., Zalucki MP, Bangura M, Udayawatta M, Kronforst MR, Altizer S, et al. Serial founder effects and genetic differentiation during worldwide range expansion of monarch butterflies. Proceedings of the Royal Society of London B: Biological Sciences. 2014; 281: 20142230

17. Zhan S, Zhang W, Niitepöld K, Hsu J, Haeger JF, Zalucki MP, et al. The genetics of monarch butterfly migration and warning colouration. Nature. 2014; 514: 317–31. doi:10.1038/nature13812 PMID: 25274300

18. Ackery PR, Vane-Wright RI. Milkweed Butterflies: Their Cladistics and Biology. Ithaca, NY: Cornell University Press. 1984.

19. Leong KLH, Yoshimura MA, Kaya HK. Occurrence of a neogregarine protozoan, Ophryocystis elektroscirrha Mclaughlin and Myers, in populations of monarch and queen butterflies. Pan-Pacific Entomologist. 1997; 73: 49–51.

20. Mclaughlin RE, Myers J. Ophryocystis elektroscirra sp. N. A neogregarine pathogen of the monarch butterfly Danaus plexippus (L.) and the Florida queen butterfly Danaus gilippus berenice Cramer. Journal of Protozoology. 1970; 17: 300–5.

21. De Roode JC, Gold LR, Altizer S. Virulence determinants in a natural butterfly-parasite system. Parasitology. 2007; 134: 657–68. PMID: 17140464

22. Altizer SM, Oberhauser K, Brower LP. Associations between host migration and the prevalence of a protozoan parasite in natural populations of adult monarch butterflies. Ecological Entomology. 2000; 25: 125–39.

23. Bradley CA, Altizer SM. Parasites hinder monarch butterfly flight: Implications for disease spread in migratory hosts. Ecology Letters. 2005; 8: 290–300.

24. Bartel RA, Oberhauser KS, de Roode JC, Altizer SM. Monarch butterfly migration and parasite transmission in eastern North America. Ecology. 2011; 92: 342–51. PMID: 21618914

25. Wassenaar LI, Hobson KA. Natal origins of migratory monarch butterflies at wintering colonies in Mexico: New isotopic evidence. Proceedings of the National Academy of Sciences. 1998; 95: 15436–9.

26. Wassenaar LI, Hobson KA. Stable-hydrogen isotope heterogeneity in keratinous materials: Mass spectrometry and migratory wildlife tissue subsampling strategies. Rapid Communications in Mass Spectrometry. 2006; 20: 2505–10. PMID: 16862621

27. Urquhart FA. Found at last: The monarch's winter home. National Geographic. 1976; 150: 161–73.
28. Brower LP. Understanding and misunderstanding the migration of the monarch butterfly (Nymphalidae) in North America: 1857–1995. Journal of the Lepidopterists’ Society. 1995; 49: 304–85.
29. Altizer SM, Oberhauser K, Geurts KA. Transmission of the protozoan parasite, Ophryocystis elektroscirra, in monarch butterfly populations. In: Oberhauser K, Solensky M, editors. The Monarch Butterfly: Biology and Conservation. Ithaca, NY: Cornell University Press. 2004.
30. de Roode JC, Chi J, Rarick RM, Altizer S. Strength in numbers: High parasite burdens increase transmission of a protozoan parasite of monarch butterflies (Danaus plexippus). Oecology. 2009; 161: 67–75. doi: 10.1007/s00442-009-1361-6 PMID: 19418070
31. Wassenaar LI, Hobson KA. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. Isotopes in Environmental and Health Studies. 2003; 39: 211–17. PMID: 14521282
32. Hobson KA, Wassenaar LI, Taylor OR. Stable isotopes (δD and δ13C) are geographic indicators of natal origins of monarch butterflies in eastern North America. Oecologia. 1999; 120: 397–404.
33. Dockx C, Brower LP, Wassenaar LI, Hobson KA. Do North American monarch butterflies travel to Cuba? Stable isotope and chemical tracer techniques. Ecological Applications. 2004; 14: 1106–14.
34. Flockhart DT, Wassenaar LI, Martin TG, Hobson KA, Wunder MB, Norris DR. Tracking multi-generational colonization of the breeding grounds by monarch butterflies in eastern North America. Proceedings of the Royal Society of London B: Biological Sciences. 2013; 280: 20131087
35. Hobson KA, Wassenaar LI (eds). Tracking Animal Migration using Stable Isotopes. Handbook of Terrestrial Ecology Series, Academic Press / Elsevier, Amsterdam. 2008.
36. Hobson KA, Van Wilgenburg SL, Faaborg J, Toms JD, Rengifo C, Llanes Sosa A, et al. Connecting breeding and wintering grounds of Neotropical migrant songbirds using stable hydrogen isotopes: a call for an isotopic atlas of migratory connectivity. Journal of Field Ornithology. 2009; 85:237–257.
37. Davis AK. Wing color of monarch butterflies (Danaus plexippus) in eastern North America across life stages: Migrants are ‘redder’ than breeding and overwintering stages. Psyche 2009: doi: 10.1155/2009/705780
38. Davis AK, Chi J, Bradley CA, Altizer S. The redder the better: Wing color predicts flight performance in monarch butterflies. PloS One. 2009; 7: e41323.
39. Davis AK, Farrey B, Altizer S. Variation in thermally-induced melanism in monarch butterflies (Lepidoptera: Nymphalidae) from three North American populations. Journal of Thermal Biology. 2005; 30: 410–21.
40. Altizer S, Davis AK. Populations of monarch butterflies with different migratory behaviors show divergence in wing morphology. Evolution. 2010; 64: 1018–28. doi: 10.1111/j.1558-5646.2010.00946.x PMID: 20067519
41. Satterfield DA, Maerz JC, Altizer S. Loss of migratory behaviour increases infection risk for a butterfly host. Proceedings of the Royal Society of London B: Biological Sciences. 2015; 282: 20141734.
42. Knight A, Brower LP. The influence of eastern north american autumnal migrant monarch butterflies (Danaus plexippus L.) on continuously breeding resident monarch populations in southern Florida. Journal of Chemical Ecology. 2009; 35: 816–23. doi: 10.1007/s10886-009-9655-z PMID: 19579046
43. Howard E, Aschen H, Davis AK. Citizen science observations of monarch butterfly overwintering in the southern United States. Psyche. 2010:
44. IBM Corp. SPSS statistics for Windows, version 19.0. Armonk, NY: IBM Corp. 2010.
45. Hall RJ, Altizer S, Bartel RA. Greater migratory propensity in hosts lowers pathogen transmission and impacts. Journal of Animal Ecology. 2014; 83: 1068–77. doi: 10.1111/1365-2656.12204 PMID: 24460702
46. Brower LP. Monarch butterfly orientation: Missing pieces of a magnificent puzzle. Journal of Experimental Biology. 1996; 199: 93–103. PMID: 9317405
47. Alonso-Mejia A, Rendon-Salinas E, Montesinos-Patino E, Brower LP. Use of lipid reserves by monarch butterflies overwintering in Mexico: Implications for conservation. Ecological Applications. 1997; 7: 934–47.
48. de Roode JC, Yates AJ, Altizer S. Virulence-transmission trade-offs and population divergence in virulence in a naturally occurring butterfly parasite. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105: 7489–94. doi: 10.1073/pnas.0710901105 PMID: 18492806
49. Simmons AM, Rogers CE. Dispersal and seasonal occurrence of Noctuidonema guyanense, an ectoparasitic nematode of adult fall armyworm (Lepidoptera, Noctuidae), in the United States. Journal of Entomological Science. 1991; 26: 136–48.
50. van Gils JA, Munster VJ, Radersma R, Liefhebber D, Fouchier RAM, Klaassen M. Hampered foraging and migratory performance in swans infected with low-pathogenic avian influenza a virus. PloS One. 2007; 2: e184. PMID: 17264886
51. Cornelius E, Davis AK, Altizer S. How important are hemoparasites to migratory songbirds? Evaluating physiological measures and infection status in three neotropical migrants during stopover. Physiological and Biochemical Zoology. 2014; 87: 719–28. doi: 10.1086/677541 PMID: 25244383
52. Kleijn D, Munster VJ, Ebbinge BS, Jonkers DA, Musken GJDM, Van Randen Y, et al. Dynamics and ecological consequences of avian influenza virus infection in greater white-fronted geese in their winter staging areas. Proceedings of the Royal Society of London Series B. 2010; 277: 2041–8. doi: 10.1098/ rsb.2010.0026 PMID: 20200028
53. Gunnarsson G, Latorre-Margaléf N, Hobson KA, Van Wilgenburg SL, Elmberg J, Olsen B, et al. Disease dynamics and bird migration—linking mallards Anas platyrhynchos and subtype diversity of the influenza a virus in time and space. PLoS One. 2012; 4: e35679.
54. Becker C. A possible size-determined directional selection in Danaus plexippus (Lepidoptera: Danainae) with evidence from stable isotope analysis. Unpubl Masters Thesis University of Kansas. 2008. 119 pages.
55. Borland J, Johnson CC, Crampton TW III, Thomas M, Altizer S, Oberhauser K. Characteristics of fall migratory monarch butterflies, Danaus plexippus, in Minnesota and Texas. In: Oberhauser K, Solensky M, editors. The Monarch Butterfly: Biology and Conservation. Ithaca, NY: Cornell University Press. 2004. pp. 97–104.
56. Gibo DL, McCurdy JA. Evidence for use of water ballast by monarch butterflies, Danaus plexippus (Nymphalidae). Journal of the Lepidopterists’ Society. 1993; 47: 154–60.
57. McCord JW, Davis AK. Biological observations of monarch butterfly behavior at a migratory stopover site: Results from a long-term tagging study in coastal South Carolina. Journal of Insect Behavior. 2010; 23: 405–18.
58. McCord JW, Davis AK. Characteristics of monarch butterflies (Danaus plexippus) that stopover at a site in coastal South Carolina during fall migration. Journal of Research on the Lepidoptera. 2012; 45: 1–8.
59. Howard E, Davis AK. Mortality of migrating monarch butterflies from a wind storm on the shore of Lake Michigan, USA. Journal of Research on the Lepidoptera. 2012; 45: 49–54.
60. Beall G. Seasonal variation in sex proportion and wing length in the migrant butterfly, Danaus plexippus l. (lep. Danaidae). Transactions of the Royal Entomological society of London. 1946; 97: 337–53.
61. Dockx C. Directional and stabilizing selection on wing size and shape in migrant and resident monarch butterflies, Danaus plexippus (L.), in Cuba. Biological Journal of the Linnean Society. 2007; 92: 605–16.
62. Dingle H, Blakley NR, Miller ER. Variation in body size and flight performance in milkweed bugs (Oncopeltus). Evolution. 1980; 34: 371–385.
63. Calmaestra RG, Moreno E. A phylogenetically-based analysis on the relationship between wing morphology and migratory behaviour in Passeriformes. Ardea. 2001; 89: 407–16.
64. Monkkonen M. Do migrant birds have more pointed wings? A comparative study. Evolutionary Ecology. 1995; 9: 520–8.
65. Dingle H. Geographic variation and behavioral flexibility in milkweed bug life histories. In: Denno RF, Dingle H, editors. Insect Life History Patterns: Habitat and Geographic Variation. New York, NY: Springer-Verlag. 1981; pp. 57–73.
66. Vidal O, Rendón-Salinas E. Dynamics and trends of overwintering colonies of the monarch butterfly in Mexico. Biological Conservation. 2014; 180: 165–75.
67. Brower L. P., Taylor O. R., Williams E. H., Slayback D. A., Zubieta R. R., & Ramirez M. I. Decline of monarch butterflies overwintering in Mexico: is the migratory phenomenon at risk? Insect Conservation and Diversity. 2012; 5(2), 95.
68. Pleasant JM, Oberhauser KS. Milkweed loss in agricultural fields because of herbicide use: Effect on the monarch butterfly population. Insect Conservation and Diversity. 2013; 6: 135–44.
69. Flóchhart DT, Pichancourt JB, Norris DR, Martin TG. Unravelling the annual cycle in a migratory animal: breeding-season habitat loss drives population declines of monarch butterflies. Journal of Animal Ecology. 2015; 84:155–65. doi: 10.1111/1365-2656.12253 PMID: 24903083
70. Batalden RV, Oberhauser K. Potential changes in eastern North American monarch migration in response to an introduced milkweed, Asclepias curassavica. In: Oberhauser K, Nal K, Altizer S, editors. Monarchs in a Changing World: Biology and Conservation of an Iconic Insect. Ithaca, NY: Cornell University Press. 2015.
71. Tatem AJ, Rogers DJ, Hay S. Global transport networks and infectious disease spread. Advances in Parasitology. 2006; 62: 293–343. PMID: 16647974
72. Colizza V, Barrat A, Barthelemy M, Valleron A-J, Vespignani A. Modeling the worldwide spread of pandemic influenza: Baseline case and containment interventions. PLoS Medicine. 2007; 4: 95.

73. Wilcove DS, Wikelski M. Going, going, gone: Is animal migration disappearing? PLoS Biology. 2008; 6: doi: 10.1371/journal.pbio.0060188 PMID: 18666834

74. Brower LP, Malcolm SB. Animal migrations: Endangered phenomena. American Zoologist. 1991; 31: 265–76.

75. Krkošek M, Gottesfeld A., Proctor B., Rolston D., Carr-Harrins C., and Lewis M. A. Effects of host migration, diversity and aquaculture on sea lice threats to Pacific Salmon populations. Proceedings of the Royal Society of London Series B. 2007; 274: 3141–9. PMID: 17939989

76. Plowright RK, Foley P, Field HE, Dobson AP, Foley JE, Eby P, et al. Urban habituation, ecological connectivity and epidemic dampening: The emergence of Hendra virus from flying foxes (Pteropus spp.). Proceedings of the Royal Society of London B: Biological Sciences. 2011; 278: 3703–12.