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Running head: Monarch natal origins and wing morphology

Title: Intra-population variation in the natal origins and wing morphology of overwintering western monarch butterflies (*Danaus plexippus*)

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Abstract

Understanding the natal origins of migratory animals is critical for understanding their population dynamics and conservation. However, quantitative estimates of population recruitment from different natal habitats can be difficult to assess for many species, especially those with large geographic ranges. These limitations hinder the evaluation of alternative hypotheses about the key movements and ecological interactions of migratory species. Here, we quantitatively investigated intra-population variation in the natal origins of western North American monarch butterflies (*Danaus plexippus*) using spatial analyses of stable isotope ratios and correlations with wing morphology. A map of hydrogen isotope values in western monarch butterfly wings ($\delta^2\text{H}_m$) was estimated using a transfer function that relates the $\delta^2\text{H}_m$ values of monarch butterfly wing keratin to a long-term dataset of precipitation isotope ($\delta^2\text{H}_p$) values across the western United States. Isotopic analyses of 114 monarch butterfly wings collected at four California overwintering locations indicated substantial individual variation in natal origins, with most recruitment coming from broad regions along the Pacific coast, the southwestern US and the northern intermountain region. These observed patterns may partially resolve and reconcile several past hypotheses about the natal origins of western monarch butterflies, while also raising new questions. More negative $\delta^2\text{H}_m$ values (associated with longer migratory distance) were significantly correlated with larger forewing sizes, consistent with expectations based on the aerodynamic and energetic costs of long-distance migration, while analyses of wing shape suggest potential differences in the movement behaviors and constraints observed in the western range, compared with previous observations in eastern North America. Taken together, the results of this study indicate substantial individual variation in the natal origins of overwintering western monarch butterflies, suggesting both local and long-distance movement to overwintering sites.

Keywords: *Asclepias*, hydrogen/deuterium isotopes, migration

Introduction

Studying the natal origins of migratory species can provide key insights into their population dynamics and inform effective strategies for their conservation. However, because migratory species often travel between habitats that are distant and distinct, identifying the natal origins of these species can be challenging. Moreover, the observation of substantial intra-population variation in migratory behavior (Bêty et al. 2004, Thorup et al. 2007, Brodersen et al. 2012, Gill et al. 2014) suggests that different individuals within a single population may commonly have widely different natal origins. Thus, characterizing the quantitative contributions of different natal habitats to populations of migratory species requires an assessment of individual variation, and offers the opportunity to illuminate a key component of their ecology.

The monarch butterflies (*Danaus plexippus*) of North America include both eastern and western populations (Urquhart and Urquhart 1977). The traditional model of monarch butterfly migration in North America suggests that the individuals which overwinter in central Mexico developed in North America east of the Rocky Mountains (e.g., Malcolm et al. 1993, Wassenaar and Hobson 1998, Miller et al. 2010, 2012, Flockhart et al. 2013), while those that overwinter along the coast of California emerged from natal habitats west of the Rocky Mountains (Urquhart and Urquhart 1977, Nagano et al. 1993, Wenner and Harris 1993, Dingle et al. 2005, Stevens and Frey 2010). However, recent studies suggest that there may be significant interchange between the eastern and western populations (Brower and Pyle 2004, Dingle et al. 2005), consistent with the observation that these two populations are genetically panmictic at all tested loci (Shephard et al. 2002, Lyons et al. 2012). Although these populations have geographically distant (albeit climatically similar) overwintering grounds, the degree of overlap in their natal ranges remains unclear.

Compared to the eastern population (Wassenaar and Hobson 1998, Miller et al. 2010, Flockhart et al. 2013), the natal origins of the western overwintering population remain surprisingly uncertain (Nagano et al. 1993, Wenner and Harris 1993, Dingle et al. 2005, Stevens and Frey 2010). Previous mark-recapture studies have documented the movement of wing-tagged monarch butterflies from distant locations throughout western North America to various recapture locations at overwintering sites along the California coast (Urquhart and Urquhart 1977), as well as the inland movement of post-overwintering butterflies to recapture locations throughout California (Nagano et al. 1993). The conclusions of these studies are broadly consistent with the month-by-month pattern of museum collection localities across a broad area of the North American west observed by Dingle et al. (2005), suggesting that the overwintering populations of western monarch butterflies are likely to originate from natal habitats across a broad extent of the North American west. A more recent study considering host plant availability and late summer temperatures in the western United States estimated that 55% of the land area across seven western states could provide suitable conditions for the development of this overwintering generation, with the climatic conditions within a large inland region of California showing a particularly strong association with monarch butterfly overwintering abundances (Stevens and Frey 2010). The capacity for monarch butterflies to show such long-distance migration to their overwintering sites is further supported by studies that have illuminated the remarkable navigational neuro-mechanisms that would allow for such directed long-distance movement (Reppert et al. 2010, Guerra and Reppert 2013).

Despite these findings, questions about the natal origins of the overwintering monarch butterflies in western North America persist. For example, Wenner and Harris (1993) proposed the “range expansion and contraction hypothesis” (or “local recruitment hypothesis” *sensu* Frey and Schaffner 2004, Stevens and Frey 2010) which suggested that a relatively large proportion of overwintering western monarch butterflies are recruited from year-round breeding in habitats near the coastal overwintering sites, with

the broader observation of monarch butterflies throughout western North America resulting from seasonal population expansion and contraction. A component of this hypothesis (i.e., the relatively large contribution of recruitment from proximate habitats) is supported by the consistent and widespread observation of year-round monarch butterfly reproduction in Santa Barbara County (CA, USA) on multiple milkweed host species (Harris 1986, Wenner and Harris 1993). While it now seems clear that many western overwintering monarch butterflies do migrate from distant locations throughout the western range, this hypothesis suggests a broader, quantitative question which has remained difficult to answer: What are the relative contributions of different natal habitats to the overwintering population of western monarch butterflies? For example, what proportion of the overwintering population originates from distant natal habitats, compared to more local natal sites?

Isotopic ($\delta^2\text{H}$) analyses of precipitation and monarch butterflies have the potential to address many of these persistent questions (Hobson et al. 1999, Vander Zanden et al. 2014). This approach is based on a strong and consistent continental-scale gradient of $\delta^2\text{H}$ values in precipitation which results from the preferential precipitation of heavy isotopes as water vapor is transported northward and inland from the Pacific Ocean to the Rocky Mountains in North America (i.e., “the rain out effect”, Welker 2000, 2012, Hobson 2007, Bowen 2009, Winnick et al. 2014). This resulting geographical pattern of isotope values (Dutton et al. 2005, Vachon et al. 2010) in precipitation can provide informative isotopic landscape maps (hereafter, “isoscapes” *sensu* West et al. 2009) for studying the large-scale movement patterns of animals (Hobson 2007). While isotopic studies have already helped to clarify the migratory patterns of the eastern population of monarch butterflies (Wassenaar and Hobson 1998, Hobson et al. 1999, Miller et al. 2010, 2012, Flockhart et al. 2013), we are not aware of any previously published isotopic studies investigating the western population. However, such studies are rapidly becoming more accessible, building on foundational work in the eastern population and the continued development of an isotopic data infrastructure in the western range. In particular, past field and lab studies have

developed species- and tissue-specific transfer functions to estimate the relationship between the $\delta^2\text{H}$ values of precipitation and the $\delta^2\text{H}$ values of monarch butterfly wings (Hobson et al. 1999). Further technical advances in isotope ecology now allow for comparable laboratory analyses of butterfly wings using calibrated keratin standards and comparative ambient equilibration methods (Wassenaar and Hobson 2000, 2003). Finally, an ongoing program of systematic rainfall collections (Lamb and Bowersox 2000) and analysis across the United States (e.g., Welker 2000, 2012, Dutton et al. 2005, Vachon et al. 2007) and all of North America (Delavau et al. 2015) is now able to inform models that generate continental-scale isoscapes of $\delta^2\text{H}$ in precipitation. In combination, these advances have dramatically improved our ability to use precipitation isotopic methods to understand the recruitment of western monarch butterflies from their natal habitats.

In addition to isotopic signature, there is also evidence that intraspecific variation in the movement behaviors of monarch butterflies may be correlated with measurable variation in several other aspects of the butterfly phenotype (Altizer et al. 2000, Dockx 2007, Altizer and Davis 2010, Davis et al. 2012, Zhan et al. 2014). In particular, previous studies have suggested that monarch butterflies from different populations show variation in wing morphology which is correlated with their migratory behavior (Beall and Williams 1945, Dockx 2007, 2012, Altizer and Davis 2010). For example, Dockx (2007, 2012) observed larger and higher aspect ratio wings in migratory monarch butterflies compared to resident monarch butterflies from Cuba, and Altizer and Davis (2010) showed that monarch butterflies from eastern and western migratory populations had larger wings than non-migratory populations in southern Florida, Puerto Rico, Costa Rica and Hawaii, with the most migratory (eastern) population also showing particularly high body mass, high wing loading and high aspect ratio forewings. These inter-population differences in wing morphology are generally consistent with adaptive expectations based on the energetics and aerodynamics of powered and soaring long-distance flight (Gibo and Pallett 1979, Dingle 2014). Although adaptive morphological differences have been previously observed in

comparisons between migratory and resident populations of monarch butterflies, it remains unknown whether similar correlations between wing morphology and migratory distance also occur at the individual level, within populations (but see Davis et al. 2012).

In this study, we ask two key questions: 1) How do broad geographic areas of potential natal habitat contribute to the overwintering population of western monarch butterflies in California? and 2) How does individual variation in the wing morphology of overwintering western monarch butterflies correlate with estimated migratory distance from their natal origins? To begin to address these questions, we first compared the δH_m of monarch butterfly wings collected from four overwintering sites with a continental-scale monarch butterfly wing isoscape derived from the spatially and temporally rich United States Network for Isotopes in Precipitation (USNIP) database of δ^2H values in precipitation. This comparison allowed us to quantitatively estimate the contributions from broad regions of natal habitat throughout western North America to the overwintering population of California monarch butterflies. Second, we examined correlations between the observed δ^2H_m and multivariate measures of monarch butterfly forewing size and shape in order to determine if individual variation within the western monarch butterfly population is consistent with previously observed patterns.

Methods

Monarch butterfly collection

Monarch butterflies were collected from four overwintering locations along the coast of California, United States during December 4-6, 2009 by sweeping with a fully extended 5.4 m long aerial net. All butterflies were randomly sampled from overwintering aggregations in trees. In total, 114 live monarch butterflies were collected, including 19 males and 9 females from the Coronado Butterfly Preserve in Ellwood, CA (34°25'21.71"N 119°53'27.56"W), 22 males and 8 females from Pismo State Beach (35°

164 7°45.45"N 120°37'58.83"W), 20 males and 10 females from Moran Lake (36°57'33.14"N
165 121°58'31.52"W) and 18 males and 8 females from Lighthouse Field State Beach (36°57'13.97"N 122°
166 1°43.13"W). No significant infection of the protozoan parasite *Ophyrocystis elektroscirrha* was detected
167 among any of monarch butterflies included in this study (see Supplementary material, Appendix 1).

168 *Morphological measurements*

169 We measured the dry mass of all monarchs with their wings attached (*total mass*) and with wings
170 removed (*wingless mass*). Forewing morphology was measured from high-resolution wing scans using
171 ImageJ image analysis software (Rasband 1997) following the methods of Altizer and Davis (2010).
172 Briefly, we measured 1) the length of the longest axis of each forewing from the point of thoracic
173 attachment to the apex of the wing (*wing length*), 2) the width of each wing as the longest
174 measurement perpendicular to the wing length axis (*wing width*), 3) the perimeter of the entire
175 forewing (*wing perimeter*), and 4) the area of the wing (*wing area*). We averaged right and left forewing
176 measurements in order to calculate the *wing aspect ratio* as the mean wing length divided by the mean
177 wing width, and calculated *wing roundness* as $4\pi * (\text{mean wing area}) / (\text{mean wing perimeter})^2$. As in
178 Altizer and Davis (2010), we used Principal Components Analysis (PCA) to reduce wing area, length and
179 width to their first principle component (*PC-size*) which explained 95.4% of the total variation. In this
180 analysis, high values of PC-size correspond with larger forewings. The first principal component of wing
181 aspect ratio and roundness (*PC-shape*) explained 73.5% of the total variance; high values of PC-shape
182 corresponded with higher aspect ratio (lower roundness) wings.

183 *Isotope analysis*

184 We analyzed the stable hydrogen ($\delta^2\text{H}$) values in these monarch butterfly wing samples following the
185 methods of Wassenaar and Hobson (2000). Wings were washed with a solution of chloroform and

methanol in a 2:1 ratio by volume to remove surface lipids, then air-dried in a fume hood for at least 24 h. These wings were then milled to a fine powder under liquid nitrogen using a stainless steel cryogrinder (SPEX 6770 Freezer/Mill, SPEX SamplePrep, Metuchen, NJ, USA). Samples of powdered wing (1.875 ± 0.2 mg) and calibrated laboratory keratin standards were allowed to equilibrate for 96 h in order to estimate the non-exchangeable hydrogen fraction of the samples, following the comparative equilibration methods described by Wassenaar and Hobson (2003). These samples were loaded in 5 x 9 mm pressed sliver capsules (Costech 41067, Valencia, CA, USA) and analyzed by the UC Davis Stable Isotope Facility using an isotope ratio mass spectrometer (a Hekatech HT Oxygen Analyzer interfaced to a PDZ Europa 20-20, Sercon Ltd., Cheshire, UK). Reported $\delta^2\text{H}$ values are expressed in per mil (‰) relative to the V-SMOW (Vienna Standard Mean Ocean Water) international standard. The distribution of site-specific monarch butterfly $\delta^2\text{H}_m$ values was visualized using Gaussian kernel density estimation using a standard bandwidth based on Silverman's rule-of-thumb (Silverman 1986) in R 3.1.1 (R Core Team 2014).

GIS analysis

We used the USNIP data base (1989-2005) which includes weekly precipitation $\delta^2\text{H}_p$ values from 75 sites across the contiguous United States, including 35 sites in the western United States (see Supplementary material Appendix 1, Table A1, Welker 2000, Vachon et al. 2010) to calculate monthly amount-weighted $\delta^2\text{H}_p$ averages for the months of April to September (i.e., the "growing season period" during which the average monthly temperature was greater than 0°C for each station) (Cormie et al. 1994, Hobson and Wassenaar 1996, Hobson et al. 1999). A mean of these amount-weighted monthly averages was further calculated to represent the mean hydrogen isotope ratio of precipitation over the entire growing season at each station. We used a previously published (Hobson et al. 1999) linear regression relating the $\delta^2\text{H}_p$ of precipitation to the $\delta^2\text{H}_m$ of monarch butterfly wing keratin ($\delta^2\text{H}_m = 0.62 \delta^2\text{H}_p - 79$) in order determine

the expected isotope ratio of monarch butterfly wing keratin that would develop at each site. These expected $\delta^2\text{H}_m$ values were mapped and interpolated by ordinary kriging using ArcGIS (Environmental Systems Research Institute, Redlands, CA, USA) and then placed into four bins of $\delta^2\text{H}_m$ values for interpretation by region. These four bins were selected to provide informative sub-regional definition to previously postulated natal origins in the western range of monarch butterflies.

Statistical analyses

Continuous probability density functions describing the distribution of $\delta^2\text{H}_m$ values observed in monarch butterfly wings from four overwintering sites were estimated using Gaussian kernel density estimation and presented in combination with the underlying data distribution (Figure 1). This nonparametric density estimation approach is appropriate for the analysis and interpretation of continuous probability density functions based on observed data (Silverman 1986). After inspection, we conducted a post-hoc Kolmogorov–Smirnov test to determine if the observed distribution of the two northern sites differed significantly from that of the two southern sites. The relationships among morphological and isotopic data (including PC-size, PC-shape, wing loading, and $\delta^2\text{H}_m$ values) were examined using partial correlations to control for sex and site in the analysis across all sites, or to control for sex in site-specific analyses. Because the fall migration to the coastal overwintering sites generally ascends a continental isoscape gradient in the western range, we used our isotopic measurements of butterfly wings as a proxy of migration distance from natal habitat in these correlation analyses. The underlying assumption that natal habitats associated with more negative isotopic values are farther from the coastal overwintering sites is generally supported by the $\delta^2\text{H}_m$ isoscape (Figure 2). The effects of site and sex on body mass, PC-size and wing loading were assessed using analysis of variance to compare linear models with and without the term of interest; assumptions of residual normality and homoscedasticity were met for all linear models. All analyses were conducted in R 3.1.1 (R Core Team 2014).

Results

Each of the four overwintering sites sampled showed substantial variation in wing $\delta^2\text{H}_\text{m}$ values (site: *mean* [min to max] $\delta^2\text{H}_\text{m}$; Lighthouse Field: -109‰ [-160‰ to -64‰]; Moran Lake: -114‰ [-161‰ to -64‰]; Pismo Beach: -125‰ [-163‰ to -66‰]; Coronado Preserve: -121‰ [-173‰ to -72‰]; Figure 1). Unexpectedly, data from Lighthouse Field, Moran Lake and Pismo Beach suggested a bimodal distribution of $\delta^2\text{H}_\text{m}$ values, separated by a region of generally lower density in the approximate region of -130‰ to -100‰ $\delta^2\text{H}_\text{m}$ (Figure 1). This pattern was not apparent from monarch butterflies collected at the Coronado Preserve.

Hydrogen isotopes in precipitation varied widely across the western range of the monarch butterfly, showing $\delta^2\text{H}_\text{p}$ values that generally declined while moving inland and northward from the Pacific coast. This pattern is broadly consistent with expectations from previous isoscape studies in western North America (Welker 2000, Fry 2006, Hobson and Wassenaar 2008, West et al. 2009, and references therein). This broad geographic pattern was reflected in our isoscape of estimated $\delta^2\text{H}_\text{m}$ values for the wings of monarch butterflies across this natal range (Figure 2; standard deviation map, Supplementary material, Figure A1).

The observed distributions of $\delta^2\text{H}_\text{m}$ values of overwintering monarch butterflies suggested natal origins across a broad extent of the western range. Butterflies that showed $\delta^2\text{H}_\text{m}$ values $> -100\text{‰}$ suggest a natal origin in the coastal part of this range, particularly in the southern coastal ranges of California (the “southern coastal range”, Figure 2). The individuals with $\delta^2\text{H}_\text{m}$ values between -100‰ and -115‰ suggest a natal range that combines the northern coastal regions of California, Oregon and Washington with and the southwestern regions of Arizona and New Mexico (the “northern coast and southern inland range”, Figure 2). $\delta^2\text{H}_\text{m}$ values between -115‰ and -130‰ suggest natal origins in a broad sweep of northeastern California, western Oregon, western Washington, the Nevada Great Basin and the

southern Intermountain West (the “central range”, Figure 2). In contrast, $\delta^2\text{H}_m$ values $< -130\text{‰}$ are consistent with natal origins in the northern region of the intermountain west including Idaho, eastern Oregon, eastern Washington, Montana and Wyoming (the “northern inland range”, Figure 2). An analysis of the overall probability density function for all overwintering monarch butterflies in the study suggests that approximately 30% of these individuals developed in the southern coastal range, 12% developed in the northern coast and southern inland range, 16% developed in the central range, and 40% developed in the northern inland range.

Isotope values indicated that the natal origins of butterflies collected from the two northern sites in this study were significantly different from those collected at the two southern overwintering sites (two-sample, two-sided Kolmogorov–Smirnov test, $D = 0.2611$, $P = 0.03245$). Interestingly, the two most northern overwintering sites in this study showed the largest contributions from the southern coastal range (Lighthouse Field: 45%, Moran Lake: 37%, Pismo Beach: 22%, Coronado Reserve: 24%, Figure 2), while the two most southern overwintering sites showed the largest contributions from the northern inland range (Lighthouse Field: 30%, Moran Lake: 35%, Pismo Beach: 53%, Coronado Reserve: 39%, Figure 2).

Several patterns were observed in a broad analysis of butterfly morphological traits across all four overwintering sites. Male monarch butterflies showed mean total masses that were 5.8% larger than those of females ($F_{1,110}=3.37$, $P=0.069$) in analyses controlling for site, and mean forewing areas that were 4.2% larger than those of females ($F_{1,110}=6.23$, $P=0.014$), consistent with previous observations of sexual dimorphism in monarch butterflies (Altizer and Davis 2010). Total mass varied significantly by site in analyses controlling for sex (*site*: mean [standard error]; Lighthouse: 247.8 [6.8] mg; Moran: 269.4 [9.4] mg; Pismo: 246.4 [8.3] mg; Coronado: 270.8 [8.1] mg; $F_{1,112}=2.7904$, $P=0.04395$). In partial correlation analyses controlling for the effects of sex and site, isotope ratios were not significantly

correlated with forewing shape or wing loading across all sites (PC-shape vs. δ^2H_m , $r=0.09$, $t_{110}=0.92$, $P=0.36$; wing loading vs. δ^2H_m , $r=-0.019$, $t_{110}=-0.20$, $P=0.83$, Figure 3). However, more depleted (i.e., negative) δ^2H_m values (associated with longer migratory distance) were significantly correlated with larger forewing sizes (PC-size vs. δ^2H_m , $r=-0.42$, $t_{110}=-4.88$, $p<0.001$) overall (Figure 3a).

Site-specific partial correlation analyses (controlling for sex) suggested significant negative correlations between δ^2H_m values and forewing size consistent with the overall pattern at three overwintering sites (PC-size vs. δ^2H_m ; Lighthouse Field: $r=-0.65$, $t_{23}=-4.10$, $p<0.001$; Moran Lake: $r=-0.42$, $t_{27}=-2.44$, $P=0.021$; Pismo Beach: $r=-0.38$, $t_{27}=-2.11$, $P=0.04$), but not at the fourth (Coronado Preserve: $r=-0.066$, $t_{25}=-0.33$, $P=0.74$; Supplementary material Appendix 1, Figure A2). Similar analyses found a significant correlation between δ^2H_m values and forewing shape only from the Pismo Beach population (PC-shape vs. δ^2H_m ; Pismo Beach: $r=0.48$, $t_{27}=2.91$, $P=0.007$); significant correlations were not observed at the other sites (Lighthouse Field: $r=-0.11$, $t_{23}=-0.52$, $P=0.61$; Moran Lake: $r=-0.25$, $t_{27}=-1.32$, $P=0.20$; Coronado Preserve: $r=0.036$, $t_{25}=0.18$, $P=0.85$; Supplementary material Appendix 1, Figure A2).

Discussion

The broad distribution of wing hydrogen isotope values observed in this study suggests that the western monarch butterfly overwintering population likely includes individuals with varied and expansive natal origins across the western United States (Figures 1 and 2). Quantitatively, these data suggest that the largest contributions to the California overwintering population come from natal habitats in the “southern coastal” and “northern inland” parts of this range, although there were substantial contributions from all regions (Figure 2). More generally, these results suggest significant intra-population variation in the movement behavior of western monarch butterflies, with some individuals traveling from distant natal habitats to arrive at their overwintering sites, while other individuals arrive at their overwintering sites from relatively proximate natal habitats.

301 The findings of this study build upon the results of previous studies in the western monarch butterfly
302 range. For example, the disproportionately large contribution from the southern coastal range is
303 consistent with the breeding observations of Wenner and Harris (1993), suggesting that a considerable
304 proportion of the overwintering population may develop in the coastal ranges of California. The
305 suggested pattern of population recruitment from the northern inland range is also consistent with the
306 observed distribution of collection localities in an extensive dataset of monarch butterfly museum
307 specimens shown in Dingle et al. (2005), although collection records in Idaho are notably sparse during
308 the late summer and early fall months that likely produce most of the overwintering generation. It is
309 unclear whether this sparseness reflects the actual distribution of monarch butterflies or some
310 collection bias. However, Dingle et al. (2005) did assemble considerable collection records from eastern
311 Oregon, eastern Washington and Canada which would fall within the northern inland isoscape region,
312 and these natal habitats would contribute to the observed pattern of overwintering monarch butterfly
313 $\delta^2\text{H}_m$ values. Interestingly, Stevens and Frey's (2010) geographic analysis of potential monarch butterfly
314 breeding areas in the western range predicted some, but comparatively limited, breeding in the
315 northern inland range, with a particularly strong correlation between inter-annual climatic conditions in
316 central California and overwintering monarch butterfly abundance. Our current analysis seems to
317 suggest a somewhat larger contribution from the northern inland range than would be expected based
318 on this analysis, although recruitment from the other isoscape regions are generally consistent with the
319 suitable natal habitat zones suggested by Stevens and Frey (2010).

320 There are several possible hypotheses for the larger northern contributions suggested in this study. One
321 possible explanation building on both Stevens and Frey (2010) and Dingle (2005) is that a substantial
322 component of the overwintering monarch butterfly population may develop on milkweeds which use
323 water transported from the upper basin of the Colorado River, where the isotopic values of precipitation
324 are more depleted (Dutton et al. 2005). However, given that a large fraction of this upper basin is

actually in the central range, this explanation seems unlikely to explain the large proportion of northern range $\delta^2\text{H}_m$ values observed. Another possibility is that the contribution of the northern inland region could vary from year to year, and the year of butterfly sampling in this study could differ from expectations based on longer-term climatic constraints. Similarly, the apparent differences could result from differences between breeding abundance and survivorship to the overwintering stages; given sufficiently high survivorship, it might be possible for a relatively small breeding population to contribute disproportionately to the overwintering population. Finally, it seems important to note that these studies do suggest generally consistent patterns of recruitment for the western range overall, despite fundamentally different approaches and underlying assumptions.

While the broad outlines of these findings are consistent with expected patterns of continental-scale movement in monarch butterflies, these data also suggest some unexpected quantitative patterns. For example, the findings of the current study suggest relatively large contributions from the southern coastal ranges to overwintering sites in the north, combined with relatively large contributions from the northern inland ranges to overwintering sites in the south. A corollary of this observation is the unexpectedly limited contribution of individuals from the middle range of the isoscape (and the resulting bimodal distribution of observed $\delta^2\text{H}_m$ values, Figure 1). These patterns are counter-intuitive and raise new questions about the seasonal movements of western monarch butterflies. Interestingly, Nagano et al.'s (1993) wing-tagging study of spring migration in western monarch butterflies observed an intriguing bimodal distribution of departure headings from 14 California overwintering sites (all south of 35.4° N), with some spring monarch butterflies seeming to travel northwest along the coast, while others were recaptured at sites more directly inland to the east. Monarch butterflies have also been observed to move among western overwintering sites (Urquhart et al. 1965, Griffiths 2014), although the potential role of this intersite movement in the currently observed pattern remains unknown. In

addition, the observed patterns at northern overwintering sites could possibly reflect a greater role of isotopically heavy fog inputs for coastal milkweeds at more northern latitudes (Corbin et al. 2005).

A key assumption of the current analysis was our choice of the transfer (i.e., rescaling) function which estimates the isoscape of monarch butterfly wing $\delta^2\text{H}_\text{m}$ values from the $\delta^2\text{H}_\text{p}$ values. We chose to use the field-based transfer function from Hobson et al. (1999), which reflects the largest available dataset. This function is based on isotopic measurements from monarch butterflies field-reared on native milkweeds in the eastern range, while a lab-based rescaling function in the same study was determined from monarch butterflies reared on non-native tropical milkweeds (*A. curassavica*) that were given isotopically distinct water, but allowed to freely exchange water vapor in the lab. A third rescaling function suggested by Vander Zanden et al. (2014) was developed using a subset of the same underlying field-based dataset from Hobson et al. (1999) with the goal of methods development and cross-validation in order to assess the potential utility of year-specific isoscapes. The reduced spatial sampling underlying this dataset makes the resulting rescaling function less appropriate for this analysis, and isoscapes generated for the western range using this rescaling function actually place the northern inland region ($<-130\text{‰}$ $\delta^2\text{H}_\text{m}$ values) north of the United States-Canadian border, in contrast with expectations based on previous studies in the western range (Dingle et al. 2005, Stevens and Frey 2010).

Future studies may be able to offer additional insights into the natal origins of overwintering western monarch butterflies by applying improved isotopic datasets or integrating multiple kinds of geospatial data. For example, increasing the spatial resolution of the underlying precipitation dataset, especially in California, could potentially allow for clearer isotopic separation of monarch butterflies that developed along the Pacific coast compared to those that developed in the southern inland regions of Arizona and New Mexico, as well as clarifying the quantitative contributions from northern inland regions. Increased temporal resolution, perhaps as year- and month-specific isoscape data, could potentially improve the

371 assignment of individual natal origins, especially if this increased temporal resolution could be achieved
372 while maintaining spatial resolution (Vander Zanden et al. 2014). Studies integrating multiple
373 approaches – many of which have already been informative in the eastern range - could offer a
374 particularly promising avenue to substantially improve our understanding of monarch butterfly
375 movement and natal origins in the western range by cross-referencing independent spatial assignments.
376 For example, developing methods to combine evidence from direct breeding observations (Wenner and
377 Harris 1993), wing wear estimates (Miller et al. 2012, Flockhart et al. 2013), wing morphology (Dockx
378 2007, Altizer and Davis 2010), museum collections (Dingle et al. 2005), multiple isotopic markers
379 (Hobson et al. 1999, Flockhart et al. 2013), cardenolide fingerprints (Brower et al. 1984, Knight and
380 Brower 2009), species distribution modeling (Stevens and Frey 2010, Flockhart et al. 2013) and wing
381 tagging (Urquhart and Urquhart 1977, Nagano et al. 1993) could provide an efficient framework to
382 resolve persistent questions about the movement of western monarch butterflies.

383 The analysis of wing morphology in this study showed significant negative correlations between $\delta^2\text{H}_m$
384 values and forewing size at three of four overwintering sites, and a strongly significant correlation
385 between these $\delta^2\text{H}_m$ values and forewing size in the overall analysis (Figure 3 and Supplementary
386 material Appendix 1, Figure A2). These observed patterns suggest that individual variation in migration
387 distance is correlated with wing size: longer distance migrants generally had larger wings. This finding is
388 consistent with previous inter-population comparative studies in monarch butterflies (Dockx 2007, 2012,
389 Altizer and Davis 2010), and with general adaptive expectations of adaptive morphology for long
390 distance flight in other butterflies (e.g., Betts and Wootton 1988, Dudley and Srygley 1994, Dudley
391 2002), other insects (e.g., Dingle et al. 1980, Dingle 1981, 2014), and other winged animals (e.g.,
392 Norberg 1994, Marchetti et al. 1995, O'Hara et al. 2006, Dingle 2014).

The correlation between wing size and migration distance could reflect the associated correlation between wing size and total mass (Figure 3f); in studies of other species, total mass often shows a stronger correlation with flight speed than wing size (Dudley and Srygley 1994, Dudley 2002). While the behavior and aerodynamics of insect flight are undoubtedly complex, larger wings may allow for more efficient long-distance flight by maintaining optimal wing loading as overall body size increases to meet energetic demands (Figure 3). In this study, forewing size and total mass showed the expected positive correlation (Figure 3f), but this correlation was not significant when body mass was measured with the wings removed. This pattern suggests that larger wing sizes provide aerodynamic advantages for long distance flight apart from the energetic benefits of larger body size, or it might reflect the relatively depleted lipid stores in these post-migration, overwintering individuals. Separating the aerodynamic and energetic constraints on wing morphology is difficult, given inherent correlations and trade-offs between wing size, overall body size, thoracic musculature and lipid storage (Dingle et al. 1980, Dudley and Srygley 1994, 2008), and the combination of powered and soaring flight observed in monarch butterflies (Gibo and Pallett 1979, Gibo 1986). However, the strong correlation between wing size and isotopic ratios in this study does suggest that there is adaptive morphological variation within the western population associated with migratory distance, raising new questions about the timing of selection and maintenance of this observed variation. It remains unclear whether larger-winged individuals are more likely to initiate movements over longer distances, or if selection favoring larger wings is stronger for longer distance migrants.

These data did not show the expected correlation between more negative $\delta^2\text{H}_m$ values and higher aspect ratio wings; no significant correlation was observed in three of four overwintering sites, and the observed correlation at Pismo Beach was in the opposite of the predicted direction (i.e., more negative $\delta^2\text{H}_m$ values were correlated with rounder wings, Supplementary material Appendix 1, Figure A2). Higher aspect ratio wings are generally thought to increase soaring efficiency by reducing wing tip vortices and

417 associated drag forces (Dudley 2002, Dingle 2014), although significant correlations between higher
418 aspect ratio wings and reduced flight speed have been observed in other butterflies under powered
419 flight (Chai and Srygley 1990, Dudley and Srygley 1994, Dingle 2014), and higher aspect ratio wings are
420 generally expected to decrease maneuverability (Dingle 2014). Altizer and Davis (2010) noted that the
421 western monarch butterfly population generally had lower aspect ratios (rounder wings) and smaller
422 body sizes than the eastern population, and suggested that the shorter distances involved in the
423 western population might select for different morphological adaptations and trade-offs. The observed
424 patterns in the current study are consistent with the interpretation that longer distance migrants in the
425 western population may experience weaker selection pressure for soaring efficiency, and somewhat
426 stronger selection for powered flight speed and/or maneuverability compared to the eastern
427 population.

428 Taken together, the findings of this study may contribute to resolving some persistent questions about
429 the movement behavior of western monarch butterflies, while simultaneously suggesting new ones. In
430 particular, this study indicates that there is significant intra-population variation within the western
431 population, both in isotopic estimates of natal origin and in morphological traits correlated with
432 migratory distance. This individual variation suggests the possibility that a significant component of the
433 overwintering population develops in natal habitats relatively proximate to the overwintering sites,
434 while another significant component of the overwintering population engages in long-distance
435 migration from much more distant natal habitats. Such individual variation would be consistent with
436 (and partially reconcile) varied observations from previous studies in the western range (Wenner and
437 Harris 1993, Dingle et al. 2005, Stevens and Frey 2010). This conclusion would suggest that while the
438 western population certainly engages in directed long-distance migration, there is also likely to be
439 considerable recruitment from relatively local natal habitats represented in the overwintering
440 population. One (not mutually exclusive) alternative to this interpretation is that the fall migration could

involve multiple generations, resulting from the interruption of reproductive diapause as monarch butterflies encounter suitable temperatures and host plants along the migratory route (Perez and Taylor 2004, Baum and Sharber 2012). If this pattern were common, the observed $\delta^2\text{H}_m$ values of the overwintering population would not reflect the maximal inland extent of the migration, but rather the most recent breeding site along the return migration. The recent widespread observation of fall monarch butterfly breeding on native milkweeds at Coast Range and Central Valley California sites (*pers. obs.*; Art Shapiro, *pers. comm.*) is consistent with this hypothesis, although it doesn't appear to have been historically common in northern California (Art Shapiro, *pers. comm.*). The ecological implications of these observations remain to be determined.

Many key questions of both basic and applied interest depend on identifying the natal origin of overwintering monarch butterflies in the western population. Fundamentally, improving our understanding of these natal origins could suggest additional insights into the spatial distribution and seasonal movements of the population, and inform our understanding of factors that maintain the observed intraspecific variation in natal habitat and morphology. At a more applied level, this information could improve our understanding of the local environmental drivers that influence patterns in western monarch butterfly abundance while informing efforts at milkweed conservation and habitat restoration.

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466 **References**

- 467 Altizer, S., and A. K. Davis. 2010. Populations of monarch butterflies with different migratory behaviors
468 show divergence in wing morphology. *Evolution* 64:1018–1028.
- 469 Altizer, S. M., K. S. Oberhauser, and L. P. Brower. 2000. Associations between host migration and the
470 prevalence of a protozoan parasite in natural populations of adult monarch butterflies.
471 *Ecological Entomology* 25:125–139.
- 472 Baum, K. A., and W. V. Sharber. 2012. Fire creates host plant patches for monarch butterflies. *Biology*
473 *Letters* 8:968–971.
- 474 Beall, G., and C. B. Williams. 1945. Geographical variation in the wing length of *Danaus plexippus* (Lep.
475 Rhopalocera). Pages 65–76 *Proceedings of the Royal Entomological Society of London. Series A,*
476 *General Entomology.* Wiley Online Library.
- 477 Betts, C. R., and R. J. Wootton. 1988. Wing Shape and Flight Behaviour in Butterflies (Lepidoptera:
478 Papilionoidea and Hesperioidea): A Preliminary Analysis. *Journal of Experimental Biology*
479 138:271–288.
- 480 Bêty, J., J.-F. Giroux, and G. Gauthier. 2004. Individual variation in timing of Migration: causes and
481 reproductive consequences in greater snow geese (*Anser caerulescens atlanticus*). *Behavioral*
482 *Ecology and Sociobiology* 57:1–8.
- 483 Bowen, G. J. 2009. Statistical and geostatistical mapping of precipitation water isotope ratios. *Isoscapes:*
484 *Understanding movement, pattern, and process on Earth through isotope mapping.* 1st Edition.
485 Springer.
- 486 Brodersen, J., P. A. Nilsson, B. B. Chapman, C. Skov, L.-A. Hansson, and C. Brönmark. 2012. Variable
487 individual consistency in timing and destination of winter migrating fish. *Biology Letters* 8:21–
488 23.
- 489 Brower, L. P., and R. M. Pyle. 2004. The interchange of migratory monarchs between Mexico and the
490 western United States, and the importance of floral corridors to the fall and spring migrations.
491 Conservation of migratory pollinators and their nectar corridors in North America. Arizona-
492 Sonora Desert Museum, Natural History of the Sonoran Desert Region:144–166.
- 493 Brower, L. P., J. N. Seiber, C. J. Nelson, S. P. Lynch, and M. M. Holland. 1984. Plant-determined variation
494 in the cardenolide content, thin-layer chromatography profiles, and emetic potency of monarch
495 butterflies, *Danaus plexippus* L. Reared on milkweed plants in California: 2. *Asclepias speciosa*.
496 *Journal of chemical ecology* 10:601–639.
- 497 Chai, P., and R. B. Srygley. 1990. Predation and the flight, morphology, and temperature of neotropical
498 rain-forest butterflies. *American Naturalist*:748–765.

499 Corbin, J. D., M. A. Thomsen, T. E. Dawson, and C. M. D'Antonio. 2005. Summer water use by California
500 coastal prairie grasses: fog, drought, and community composition. *Oecologia* 145:511–521.

501 Cormie, A. B., H. P. Schwarcz, and J. Gray. 1994. Relation between hydrogen isotopic ratios of bone
502 collagen and rain. *Geochimica et Cosmochimica Acta* 58:377–391.

503 Davis, A. K., S. Altizer, and E. Friedle. 2004. A Non-Destructive, Automated Method of Counting Spores of
504 *Ophryocystis elektroscirrha* (Neogregarinorida: Ophryocystidae) in Infected Monarch Butterflies
505 (Lepidoptera: Nymphalidae). *Florida Entomologist* 87:231–234.

506 Davis, A. K., J. Chi, C. Bradley, and S. Altizer. 2012. The redder the better: wing color predicts flight
507 performance in monarch butterflies. *PloS one* 7:e41323.

508 Delavau, C., K. P. Chun, T. Stadnyk, S. J. Birks, and J. M. Welker. 2015. North American precipitation
509 isotope ($\delta^{18}\text{O}$) zones revealed in time series modeling across Canada and northern United
510 States. *Water Resources Research*.

511 Dingle, H. 1981. Geographic Variation and Behavioral Flexibility in Milkweed Bug Life Histories. Pages
512 57–73 in R. F. Denno and H. Dingle, editors. *Insect Life History Patterns*. Springer New York.

513 Dingle, H. 2014. *Migration: The Biology of Life on the Move*. Oxford University Press.

514 Dingle, H., N. R. Blakley, and E. R. Miller. 1980. Variation in body size and flight performance in milkweed
515 bugs (*Oncopeltus*). *Evolution*:371–385.

516 Dingle, H., M. R. Zalucki, W. A. Rochester, and T. Armijo-Prewitt. 2005. Distribution of the monarch
517 butterfly, *Danaus plexippus* (L.) (Lepidoptera: Nymphalidae), in western North America.
518 *Biological Journal of the Linnean Society* 85:491–500.

519 Dockx, C. 2007. Directional and stabilizing selection on wing size and shape in migrant and resident
520 monarch butterflies, *Danaus plexippus* (L.), in Cuba. *Biological Journal of the Linnean Society*
521 92:605–616.

522 Dockx, C. 2012. Differences in phenotypic traits and migratory strategies between eastern North
523 American monarch butterflies, *Danaus plexippus* (L.). *Biological Journal of the Linnean Society*
524 106:717–736.

525 Dudley, R. 2002. *The Biomechanics of Insect Flight: Form, Function, Evolution*. Princeton University
526 Press.

527 Dudley, R., and R. Srygley. 1994. Flight physiology of neotropical butterflies: allometry of airspeeds
528 during natural free flight. *Journal of Experimental Biology* 191:125–139.

529 Dudley, R., and R. B. Srygley. 2008. Airspeed adjustment and lipid reserves in migratory Neotropical
530 butterflies. *Functional Ecology* 22:264–270.

531 Dutton, A., B. H. Wilkinson, J. M. Welker, G. J. Bowen, and K. C. Lohmann. 2005. Spatial distribution and
532 seasonal variation in $^{18}\text{O}/^{16}\text{O}$ of modern precipitation and river water across the conterminous
533 USA. *Hydrological Processes* 19:4121–4146.

534 Flockhart, D. T., L. I. Wassenaar, T. G. Martin, K. A. Hobson, M. B. Wunder, and D. R. Norris. 2013.
535 Tracking multi-generational colonization of the breeding grounds by monarch butterflies in
536 eastern North America. *Proceedings of the Royal Society B: Biological Sciences* 280.

537 Frey, D., and A. Schaffner. 2004. Spatial and temporal pattern of monarch overwintering abundance in
538 western North America. Pages 167–176 *The Monarch Butterfly: Biology and Conservation*.
539 Cornell University Press.

540 Fry, B. 2006. *Stable Isotope Ecology*. Springer, New York.

541 Gibo, D. L. 1986. Flight strategies of migrating monarch butterflies (*Danaus plexippus* L.) in southern
542 Ontario. Pages 172–184 *Insect Flight*. Springer.

543 Gibo, D. L., and M. J. Pallett. 1979. Soaring flight of monarch butterflies, *Danaus plexippus* (Lepidoptera:
544 Danaidae), during the late summer migration in southern Ontario. *Canadian Journal of Zoology*
545 57:1393–1401.

546 Gill, J. A., J. A. Alves, W. J. Sutherland, G. F. Appleton, P. M. Potts, and T. G. Gunnarsson. 2014. Why is
547 timing of bird migration advancing when individuals are not? *Proceedings of the Royal Society of*
548 *London B: Biological Sciences* 281:20132161.

549 Griffiths, J. 2014. Monarch butterfly (*Danaus plexippus*) tree preference and intersite movement at
550 California overwintering sites. Master's Theses and Project Reports.

551 Guerra, P. A., and S. M. Reppert. 2013. Coldness triggers northward flight in remigrant monarch
552 butterflies. *Current Biology* 23:419–423.

553 Harris, A. M. 1986. Natural history of the monarch butterfly (*Danaus plexippus*) in the Santa Barbara
554 region. M.A., University of California, Santa Barbara.

555 Hobson, K. A. 2007. Isotopic tracking of migrant wildlife. in R. Michener and K. Lajtha, editors. *Stable*
556 *Isotopes in Ecology and Environmental Science*. Second edition. Wiley-Blackwell.

557 Hobson, K. A., and L. I. Wassenaar. 1996. Linking breeding and wintering grounds of neotropical migrant
558 songbirds using stable hydrogen isotopic analysis of feathers. *Oecologia* 109:142–148.

559 Hobson, K. A., and L. I. Wassenaar. 2008. *Tracking Animal Migration with Stable Isotopes*, Volume 2. First
560 edition. Academic Press.

561 Hobson, K. A., L. I. Wassenaar, and O. R. Taylor. 1999. Stable isotopes (δD and $\delta^{13}\text{C}$) are
562 geographic indicators of natal origins of monarch butterflies in eastern North America.
563 *Oecologia* 120:397–404.

564 Knight, A., and L. P. Brower. 2009. The Influence of Eastern North American Autumnal Migrant Monarch
565 Butterflies (*Danaus plexippus* L.) on Continuously Breeding Resident Monarch Populations in
566 Southern Florida. *Journal of Chemical Ecology* 35:816–823.

567 Lamb, D., and V. Bowersox. 2000. The national atmospheric deposition program: an overview.
568 *Atmospheric Environment* 34:1661–1663.

569 Lyons, J. I., A. A. Pierce, S. M. Barribeau, E. D. Sternberg, A. J. Mongue, D. Roode, and C. Jacobus. 2012.
570 Lack of genetic differentiation between monarch butterflies with divergent migration
571 destinations. *Molecular Ecology* 21:3433–3444.

572 Malcolm, S. B., B. J. Cockrell, and L. P. Brower. 1993. Spring recolonization of eastern North America by
573 the monarch butterfly: successive brood or single sweep migration. *Biology and conservation of*
574 *the monarch butterfly*:253.

575 Marchetti, K., T. Price, and A. Richman. 1995. Correlates of Wing Morphology with Foraging Behaviour
576 and Migration Distance in the Genus *Phylloscopus*. *Journal of Avian Biology* 26:177–181.

577 Miller, N. G., L. I. Wassenaar, K. A. Hobson, and D. R. Norris. 2010. Monarch butterflies cross the
578 Appalachians from the west to recolonize the east coast of North America. *Biology Letters*.

579 Miller, N. G., L. I. Wassenaar, K. A. Hobson, and D. R. Norris. 2012. Migratory Connectivity of the
580 Monarch Butterfly (*Danaus plexippus*): Patterns of Spring Re-Colonization in Eastern North
581 America. *PLoS ONE* 7:e31891.

582 Nagano, C., W. H. Sakai, S. B. Malcolm, B. J. Cockrell, J. P. Donahue, and L. P. Brower. 1993. Spring
583 migration of monarch butterflies in California. Pages 219–232 *Biology and Conservation of the*
584 *Monarch Butterfly*. Natural History Museum of Los Angeles County and Allen Press, Inc.

585 Norberg, U. 1994. Wing design, flight performance, and habitat use in bats. *Ecological Morphology:*
586 *Integrative Organismal Biology*. University of Chicago Press.

587 O’Hara, P. D., G. Fernández, B. Haase, H. de la Cueva, and D. B. Lank. 2006. Differential Migration in
588 Western Sandpipers with Respect to Body Size and Wing Length. *The Condor* 108:225–232.

589 Perez, S., and O. Taylor. 2004. Effects of female age, female mass and nutrients from males on monarch
590 egg mass. Pages 21–26 *The Monarch Butterfly: Biology and Conservation*. Cornell University
591 Press.

592 Rasband, W. S. 1997. ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA.

593 R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical
594 Computing, Vienna, Austria.

595 Reppert, S. M., R. J. Gegear, and C. Merlin. 2010. Navigational mechanisms of migrating monarch
596 butterflies. *Trends in Neurosciences* 33:399–406.

597 Shephard, J. M., J. M. Hughes, and M. P. Zalucki. 2002. Genetic differentiation between Australian and
598 North American populations of the monarch butterfly *Danaus plexippus* (L.) (Lepidoptera:
599 Nymphalidae): an exploration using allozyme electrophoresis. *Biological Journal of the Linnean*
600 *Society* 75:437–452.

601 Silverman, B. W. 1986. *Density Estimation for Statistics and Data Analysis*. CRC Press.

602 Stevens, S. R., and D. F. Frey. 2010. Host plant pattern and variation in climate predict the location of
603 natal grounds for migratory monarch butterflies in western North America. *Journal of Insect*
604 *Conservation* 14:1–14.

605 Thorup, K., J. Rabøl, and B. Erni. 2007. Estimating variation among individuals in migration direction.
606 *Journal of Avian Biology* 38:182–189.

607 Urquhart, F. A., P. Beard, and R. Brownlee. 1965. A population study of a hibernial roosting colony of the
608 monarch butterfly (*D. plexippus*) in northern California. *Journal of Research on the Lepidoptera*
609 4:221–226.

610 Urquhart, F. A., and N. R. Urquhart. 1977. Overwintering Areas and Migratory Routes of Monarch
611 Butterfly (*Danaus plexippus*, Lepidoptera Danaidae) in North-America, With Special Reference to
612 Western Population. *Canadian Entomologist* 109:1583–1589.

613 Vachon, R. W., J. M. Welker, J. W. C. White, and B. H. Vaughn. 2010. Monthly precipitation isoscapes
614 ($\delta^{18}\text{O}$) of the United States: Connections with surface temperatures, moisture source
615 conditions, and air mass trajectories. *Journal of Geophysical Research: Atmospheres* (1984–
616 2012) 115.

617 Vachon, R. W., J. W. C. White, E. Gutmann, and J. M. Welker. 2007. Amount-weighted annual isotopic
618 ($\delta^{18}\text{O}$) values are affected by the seasonality of precipitation: A sensitivity study. *Geophysical*
619 *Research Letters* 34.

620 Vander Zanden, H. B., M. B. Wunder, K. A. Hobson, S. L. Van Wilgenburg, L. I. Wassenaar, J. M. Welker,
621 and G. J. Bowen. 2014. Contrasting assignment of migratory organisms to geographic origins
622 using long-term versus year-specific precipitation isotope maps. *Methods in Ecology and*
623 *Evolution* 5:891–900.

624 Wassenaar, L. I., and K. A. Hobson. 1998. Natal origins of migratory monarch butterflies at wintering
625 colonies in Mexico: new isotopic evidence. *Proceedings of the National Academy of Sciences of*
626 *the United States of America* 95:15436–15439.

627 Wassenaar, L. I., and K. A. Hobson. 2000. Improved method for determining the stable-hydrogen
628 isotopic composition (δD) of complex organic materials of environmental interest.
629 *Environmental Science & Technology* 34:2354–2360.

630 Wassenaar, L. I., and K. A. Hobson. 2003. Comparative equilibration and online technique for
631 determination of non-exchangeable hydrogen of keratins for use in animal migration studies.
632 *Isotopes in Environmental and Health Studies* 39:211–217.

- 633 Welker, J. M. 2000. Isotopic ($\delta^{18}\text{O}$) characteristics of weekly precipitation collected across the USA: an
634 initial analysis with application to water source studies. *Hydrological Processes* 14:1449–1464.
- 635 Welker, J. M. 2012. ENSO effects on $\delta^{18}\text{O}$, $\delta^2\text{H}$ and d-excess values in precipitation across the US using a
636 high-density, long-term network (USNIP). *Rapid Communications in Mass Spectrometry*
637 26:1893–1898.
- 638 Wenner, A. M., and A. M. Harris. 1993. Do California monarchs undergo long-distance directed
639 migration? Pages 209–218 *Biology and Conservation of the Monarch Butterfly*. Natural History
640 Museum of Los Angeles County.
- 641 West, J. B., G. J. Bowen, T. E. Dawson, and K. P. Tu. 2009. *Isoscapes: Understanding movement, pattern,*
642 *and process on Earth through isotope mapping*. 1st Edition. Springer.
- 643 Winnick, M. J., C. P. Chamberlain, J. K. Caves, and J. M. Welker. 2014. Quantifying the isotopic
644 “continental effect.” *Earth and Planetary Science Letters* 406:123–133.
- 645 Zhan, S., W. Zhang, K. Niitepöld, J. Hsu, J. F. Haeger, M. P. Zalucki, S. Altizer, J. C. de Roode, S. M.
646 Reppert, and M. R. Kronforst. 2014. The genetics of monarch butterfly migration and warning
647 colouration. *Nature* 514:317–321.
- 648
- 649 Supplementary material (Appendix EXXXXXX at <www.oikosoffice.lu.se/appendix>). Appendix 1.

Fig. 1 Estimated probability density functions of wing keratin δD_m values from overwintering monarchs collected at four overwintering sites along the California coast: a) Lighthouse Field State Park (n=26), b) Moran Lake (n=30), c) Pismo Beach (n=30) and d) the Coronado Butterfly Preserve (n=28). Points represent rug plots of the underlying data. Dashed lines indicate $\delta D_m = -130\text{‰}$, dash-dot lines indicate $\delta D_m = -115\text{‰}$, and dotted lines indicate $\delta D_m = -100\text{‰}$.

Fig. 2 Isoscape of estimated δD_m values for the wings of monarchs originating throughout western North America, based on a weighted average of precipitation δD_p values throughout the growing season (see Methods). Isoclines are shown at $\delta D_m = -130$, -115 and -100‰ to show four broad regions; estimated δD_m values become increasingly negative moving inland and northward. Pie charts show the proportion of individual monarchs from a) Lighthouse Field, b) Moran Lake, c) Pismo Beach and d) Coronado Preserve with δD_m values that correspond with the four isoscape regions. Filled points represent precipitation collection sites in the USNIP database; unfilled points represent overwintering sites.

Fig. 3 Partial correlations of for the overall dataset, controlling for sex and site; males are represented as blue points, females are represented as orange points. In these figures, high PC-size values represent larger wings, and high PC-shape values represent wings with higher aspect ratios (i.e., lower roundness). Significant correlations (assessed at $P < 0.05$ with Pearson's partial correlation statistic) are shown with a bivariate normal ellipse representing 95% of the total probability area. The significant correlation between PC-size and δD_m (panel a) is consistent with the expectation that larger wings are associated with greater migratory distance. The significant correlation between total mass and δD_m (panel c) appears to be largely driven by the strong positive correlation between total mass and wing size (panel f); the correlation between δD_m and wingless mass (panel d) is not significant. Shape and wing loading are also not significantly correlated with δD_m (panels b and e).

Fig. 1

Fig. 2

Fig. 3

Supplementary material Appendix 1

Survey for the protozoan parasite *Ophryocystis elektroscirrha*

Two separate surveys were conducted to assess *O. elektroscirrha* prevalence among the 114 monarch butterfly specimens included in this study. The first survey was conducted in 2010 using visual inspection of posterior abdominal scales swabbed from each specimen, placed on dry microscope slides and inspected under 40 to 100X magnification, with 12.2 megapixel digital images archived (combining and modifying methods described in Altizer *et al.* 2000 and Davis *et al.* 2004). No infected butterflies were identified from this survey. Because the prevalence of *O. elektroscirrha* in this population was unexpectedly low, a second survey of these same specimens was conducted in 2015 using a visual inspection of new abdominal swab samples taken from the original butterfly specimens which had been stored in glassine envelopes at -20 C, this time archiving the physical microscope slide samples. This second survey found two butterflies with a single spore. Because previous studies (e.g., Altizer and Davis 2010) of monarch butterfly wing morphology only excluded specimens with much higher spore loads (>1000 spores in a standard assay), these extremely low spore loads seem unlikely to have had any significant effect on the conclusions of this study. A further re-analysis of the dataset excluding the two butterflies with a single spore showed no qualitative changes in the conclusions of this study, and analyses of the complete dataset are presented here.

Altizer, S. M., K. S. Oberhauser, and L. P. Brower. 2000. Associations between host migration and the prevalence of a protozoan parasite in natural populations of adult monarch butterflies. *Ecological Entomology* 25:125–139.

Altizer, S., and A. K. Davis. 2010. Populations of monarch butterflies with different migratory behaviors show divergence in wing morphology. *Evolution* 64:1018–1028.

Davis, A. K., S. Altizer, and E. Friedle. 2004. A non-destructive, automated method of counting spores of *Ophryocystis elektroscirrha* (Neogregarinorida: Ophryocystidae) in infected monarch butterflies (Lepidoptera: Nymphalidae). *Florida Entomologist* 87:231–234.

Table A1. Table of 35 precipitation collection sites in western North America, drawn from the USNIP data base of weekly precipitation $\delta^2\text{H}$ values from 75 sites across the contiguous US.

<i>USNIP Code</i>	<i>Site Name</i>	<i>State</i>	<i>Latitude</i>	<i>Longitude</i>	<i>Elevation (m)</i>
AZ03	Grand Canyon National Park	AZ	33.07	-109.86	1173
AZ06	Organ Pipe Cactus National Monument	AZ	36.07	-112.15	2152
AZ99	Oliver Knoll	AZ	31.95	-112.80	506
CA42	Tanbark Flat	CA	34.21	-117.76	853
CA45	Hopland	CA	39.00	-123.08	253
CA50	Sagehen Creek	CA	39.43	-120.24	1931
CA66	Pinnacles National Monument-Bear Valley	CA	36.48	-121.16	317
CA67	Joshua Tree National Park-Black Rock	CA	34.07	-116.39	1239
CA75	Sequoia National Park-Giant Forest	CA	36.57	-118.78	1921
CA99	Yosemite National Park	CA	37.79	-119.85	1408
CAMR	Santa Maria	CA	34.20	-120.45	66
CO02	Niwot Saddle	CO	40.05	-105.59	3520
CO15	Sand Spring	CO	40.50	-107.70	1998
CO91	Wolf Creek Pass	CO	37.47	-106.79	3292
CO99	Mesa Verde National Park	CO	37.19	-108.49	2172
ID03	Craters of the Moon National Monument	ID	43.46	-113.55	1807
ID15	Smith's Ferry	ID	44.29	-116.06	1442
MT00	Little Bighorn Battlefield National Monument	MT	45.57	-107.43	957
MT05	Glacier National Park - Fire Weather Station	MT	48.51	-113.10	980
MT97	Lost Trail Pass	MT	45.69	-113.97	2414
NM01	Gila Cliff Dwellings National Monument	NM	33.22	-108.23	1772
NM08	Mayhill	NM	32.91	-105.47	2009
NV00	Red Rock Canyon	NV	36.13	-115.42	1137
NV03	Smith Valley	NV	38.79	-119.25	1501
NV05	Great Basin National Park - Lehman Caves	NV	39.00	-114.21	2067
OR02	Alsea Guard Ranger Station	OR	44.38	-123.61	104
OR10	H.J. Andrews Experimental Forest	OR	44.21	-122.25	436
OR18	Starkey Experimental Forest	OR	45.22	-118.51	1253
UT01	Logan	UT	41.66	-111.90	1370
UT99	Bryce Canyon National Park	UT	37.62	-112.17	2477
WA14	Olympic National Park - Hoh Ranger Station	WA	47.856	-123.93	176
WA24	Palouse Conservation Farm	WA	46.76	-117.18	766
WY06	Pinedale	WY	42.92	-109.79	2388
WY08	Yellowstone National Park Tower Falls	WY	44.92	-110.42	1912
WY95	Brooklyn Lake	WY	41.37	-106.24	3212

Figure A1. Map of standard deviations from kriging interpolation of the monarch wing hydrogen isoscape ($\delta^2\text{H}_m$). Mean SD across the map was 5.5 ‰.

Figure A2. Partial correlations of PC-size and PC-shape by δD_m for each site controlling for sex; males are represented as blue points, females are represented as orange points. In these figures, high PC-size values represent larger wings, and high PC-shape values represent wings with higher aspect ratios (i.e., lower roundness). Significant correlations (assessed at $P < 0.05$ with Pearson's partial correlation statistic) are shown with a bivariate normal ellipse representing 95% of the total probability area.