Are eastern and western monarch butterflies distinct populations? A review of evidence for ecological, phenotypic, and genetic differentiation and implications for conservation

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Abstract

Monarch butterflies are a species of conservation priority due to declining overwintering populations in both eastern and western North America. Declines in western overwintering monarchs—more than 99% since monitoring began—are especially acute. However, the degree to which the western monarch is a distinct biological entity is uncertain. In this review, we focus on phenotypic and genetic differentiation between eastern and western monarchs, with the goal of informing researchers and policy-makers who are interested in monarch conservation. Eastern and western monarchs occupy distinct environments and show some evidence for phenotypic differentiation—particularly for migration-associated traits—though population genetic and genomic studies suggest that they are genetically indistinguishable from one another. We suggest future studies that could improve our understanding of differences between eastern and western monarchs. We also discuss the concept of adaptive capacity in eastern and western monarchs as well as non-migratory populations outside of the monarch's primary North American range.

Keywords: conservation, monarch butterfly, migration, population ecology, population genetics

Introduction

The North American monarch butterfly (*Danaus plexippus plexippus*) is an iconic species known for its distinctive coloration, association with milkweed host plants, and continent-scale seasonal migration (Gustafsson et al. 2015). Over the past two decades, monarchs have become the focus of intense conservation attention, including a petition to the United States Fish and Wildlife Service (USFWS) to have them listed as threatened under the U.S. Endangered Species Act (ESA) (Monarch ESA Petition). Under the ESA, specific populations of vertebrates are eligible for separate listings, as is the case for populations of grizzly bears (USFWS ECOS: Grizzly Bear), gray wolves (USFWS ECOS: Gray Wolf), and particular salmon runs (USFWS ECOS: Chinook Salmon). For invertebrates, however, listing decisions must be made on a species-level basis (National Research Council 1995; Western Association of Fish and Wildlife Agencies 2019).

In the case of monarch butterflies, species-level conservation decisions will require weighing evidence from two geographically and demographically distinct regions that comprise the core of the species' geographical distribution: eastern North America and western North America (Fig. 1A). In addition, monarchs are also established as year-round breeding populations in areas around the world, including many outlying U.S. states and territories (Ackery and Vane-Wright 1984). This manuscript focuses on whether monarch populations outside of eastern North America provide adaptive capacity—broadly defined as the ability to respond to future environmental change—for the species a whole. Here, we focus on adaptive capacity in an evolutionary rather than a demographic sense, as we consider it self-evident that the presence of populations outside of eastern North America provides some degree of redundancy and therefore resilience for monarchs.

Historically, eastern and western monarchs have been regarded as distinct populations (Urquhart 1960). Eastern monarchs overwinter in the Transverse Neovolcanic Range mountains of central Mexico and have a summertime breeding range that covers much of the United States and southern Canada east of the Rocky Mountains. Western monarchs overwinter at hundreds of sites along a stretch of coastline in California and Baja California and have a summertime breeding range that includes parts of California and the interior west. Western monarchs occupy a large geographic area—approximately 30% of the monarch's overall North American range (Fig. 1A)—but comprise a relatively small proportion of the monarch's North American population. Counts of eastern overwintering monarchs are generally two to three orders of magnitude larger than those for western overwintering monarchs (Fig. 1).

Although most conservation attention to date has focused on the larger eastern monarch population, the recent decline of western overwintering populations has been precipitous (Schultz et al. 2017, Pelton et al. 2019, Crone et al. 2019). Declines in western overwintering monarchs have been mirrored by low summer breeding numbers (Espeset et al. 2016), culminating in a >99% reduction in counts of western overwintering monarchs since monitoring began. For two consecutive years, western monarch overwintering numbers have been below their quasi-extinction threshold, raising concerns about their long-term persistence (Pelton et al. 2019; Fig. 1D). How, if at all, should the decline of western monarchs be incorporated into a species-level conservation approach? The answer to this question depends partly on the degree to which eastern and western monarchs constitute ecologically and evolutionary distinct entities. Specifically, if western monarchs are distinct and have the potential to contribute non-redundant adaptive genetic variation to the species, then their decline should be weighed more heavily in a species-level listing decision.

In this review, we evaluate the current state of knowledge regarding ecological, phenotypic, and genetic differentiation between eastern and western North American monarchs. In each section, we suggest future experiments and analyses that could be done to address current gaps in knowledge. We then discuss adaptive capacity in eastern and western monarchs as well as non-migratory monarch populations outside of North America.

Ecological and phenotypic divergence between eastern and western monarchs

Eastern and western North American monarchs are geographically separated by the Rocky Mountains and occupy distinct biotic and abiotic environments. These different environments have the potential to exert divergent selection pressures and drive phenotypic differentiation. Studies have used measurements from both wild-caught and common garden reared monarchs to test for phenotypic differentiation between eastern and western monarchs. We focus on four primary ecological factors—though there may be others—that are strong candidates to drive phenotypic differentiation between eastern and western monarchs: host plant associations, thermal environments, interactions with natural enemies, and migratory behavior.

Host plant associations

Monarchs encounter more than 100 native species of milkweed (Apocynaceae: Asclepiadoideae) host plants throughout their North American range (Woodson 1954) and have been documented using more than 40 of these species as larval hosts (Malcolm and Brower 1986; Borders and Lee-Mäder 2018). Eastern and western milkweed assemblages differ greatly: the eastern species perceived to be of greatest importance to monarchs (*Asclepias syriaca*, *A. viridis*, *A. incarnata*) are confined entirely to the east, and the primary western hosts (*A. speciosa*, *A. fascicularis*) are either partly or entirely restricted to the west. This divergence in larval host plant assemblages has the potential to contribute to adaptive differentiation in eastern and western monarchs. Two studies to date have tested for patterns of local adaptation to host plant assemblages, both using common garden experiments (Table 1). Neither study found strong evidence for local adaptation to host plant assemblages, as measured by host plant by population interactions (Ladner and Altizer 2005) or sympatric/allopatric contrasts (Freedman et al. 2020).

Future studies of host plant adaptation in eastern and western monarchs would benefit from focusing on milkweed species whose phenology makes them important for monarchs, such as spring-emerging species like *A. asperula* and *A. viridis* (eastern) and *A. californica* and *A. cordifolia* (western). Although these species may not be as abundant as *A. syriaca* or *A. speciosa*, they are primary hosts during a critical demographic window (spring return migration) and could therefore still exert strong natural selection and contribute to adaptive divergence between eastern and western monarchs.

Thermal regimes

Eastern and western monarchs occupy generally distinct thermal regimes. Summer-breeding monarchs in western North America are typically found in areas with a broader range of daytime high temperatures, despite having a more compact geographic range (Fig. 2a; see Appendix 1). Western monarchs also occur in areas with limited summer precipitation (Fig. 2b), which may determine milkweed availability and explain why western monarch occurrence records are biased towards areas with surface water (Dingle et al. 2005) and particular land cover patterns (Dilts et al. 2019). Only one study to date has directly compared eastern and western monarchs with respect to rearing temperature (Davis et al. 2005). This study compared eastern and western monarchs under a range of temperature treatments and found that western monarch larvae were lighter in coloration than eastern larvae regardless of temperature treatment, with this result interpreted as evidence for adaptive variation: lighter cuticular color should be favorable for living in high summer temperatures (Davis et al. 2005). In addition to differences in temperature in summer breeding areas, western overwintering sites in California also tend to have slightly higher mean temperatures but lower diurnal fluctuations and lesser temperature extremes than eastern overwintering sites in Mexico (Leong 1990, Brower et al. 2008, Brower et al. 2009).

Future studies would benefit from repeating earlier studies on thermal performance in monarchs using both eastern and western monarchs. For example, the often-cited estimates of developmental degree days for monarch larvae come from Australia (Zalucki 1982) and could be repeated using side-by-side rearing of eastern and western monarchs under conditions featuring natural insolation (Rawlins and Lederhouse 1981). Likewise, it would be useful to identify genes that may be involved in thermal tolerance in monarchs, since these could potentially differ in frequency or level of expression between eastern and western monarchs. Genes involved in thermal tolerance may also be targets of natural selection in a warming climate (e.g. Somero 2010).

Interactions with natural enemies

Eastern and western monarchs likely interact with disparate assemblages of natural enemies. In eastern North America, various studies have reported on the effects of predation and parasitism by Tachinid flies (Oberhauser 2012), fire ants (Calvert 2004), ladybugs (Koch et al. 2003), and other arthropods (Rayor 2004, Prysby 2004, Oberhauser et al. 2015, Hermann et al. 2019). Few studies have focused on natural enemies in western monarchs, and this would be a useful avenue for future research.

The best-studied interaction between monarchs and their natural enemies is with the protozoan parasite *Ophryocystis elektroschirrha* (OE). Common garden cross-infection experiments with OE showed no differences in tolerance or resistance between eastern and western monarchs (de Roode et al. 2008), despite evidence for (1) higher natural prevalence of OE in western compared to eastern North America (Altizer and de Roode 2015); (2) genetic variation among monarch genotypes for tolerance and resistance (de Roode and Altizer 2010, Lefevre et al. 2011); (3) variation among OE strains in virulence (de Roode et al. 2008, de Roode and Altizer 2010); (4) evidence that monarch populations in Hawaii and South Florida do show evidence for divergence in OE tolerance and resistance phenotypes (Sternberg et al. 2013). Together, these results suggest that differences in virulence among OE genotypes are capable of selecting for genetically-based differences in tolerance and resistance in monarch populations, though such differences are not observed in eastern versus western monarchs.

Migration-associated traits and behaviors

The most conspicuous difference between eastern and western monarchs is the scale of their seasonal migration. Mark-recapture studies with eastern monarchs show that they generally fly between 1,500-3,000 km during their fall migration to Mexico, with some individuals covering more than 4,000 km. By contrast, tagging studies with western monarchs have found maximum flight distances of ~1,300 km, with more typical flight distances of <800 km (James et al. 2018). Studies using stable isotope data corroborate these differences in migration distance between eastern and western monarchs (Wassenaar and Hobson 1998, Hobson et al. 1999, Flockhart et al. 2017a, Yang et al. 2016).

Migration acts as a strong selective filter for migratory monarchs, favoring individuals with larger and more elongated forewings (Altizer and Davis 2010, Yang et al. 2016, Flockhart et al. 2017b). Researchers have generally found that western monarchs have slightly smaller and less elongated forewings than eastern monarchs, potentially as a result of divergent selection due to differences in migration distance (Altizer and Davis 2010, Li et al. 2016, Freedman and Dingle 2018). Studies that have directly compared eastern and western monarchs—both wild-caught and common-garden reared—are shown in Table 2. Eastern monarchs have consistently larger forewings than western monarchs across all studies and comparisons (Table 2). However, these differences are relatively modest in wild caught individuals (eastern monarchs are between 1-8% larger), and even less pronounced for common-garden reared monarchs (~1%). Future studies could focus on accounting for the sources of environmental variation (e.g. rearing temperature, host plant identity, photoperiod conditions) in migration-associated traits that could potentially explain phenotypic differences between eastern and western monarchs. Environmental contributions to migration-associated traits may be particularly important for understanding differences observed between wild-caught eastern and western monarchs, especially in light of studies showing that larval host plant species can influence adult monarch size (e.g. Pocius et al. 2017, Decker et al. 2019, Freedman et al. 2020).

Genetic studies of differentiation between eastern and western monarchs

Population genetics

Researchers have been investigating the potential for genetic differentiation between eastern and western monarchs since at least 1991. As early as 1995, researchers cautioned against human-assisted movement of eastern and western monarchs across the continental divide, in part because of the perceived risk of gene flow potentially disrupting patterns of local adaptation (Brower et al. 1995). The current consensus—developed over the last eight years and with the advent of novel sequencing methods—is that there is a lack of genetic differentiation between eastern and western monarchs.

Recent research strongly suggests that eastern and western monarchs form a genetically indistinguishable population that spans most of their North American range. The exception to this pattern is in South Florida, where monarchs are predominantly non-migratory (Brower 1961, Zhan et al. 2014). This result is summarized in Table 3 (also reviewed in Pierce et al. 2015) and is robust to the kind and number of markers analyzed (i.e. microsatellites versus single nucleotide polymorphisms from whole genome sequencing) and consistent across studies. The most comprehensive study on the topic is Talla et al. (2020), which used whole genome resequencing for 14 eastern and 29 western monarchs and found no evidence for any genetic differentiation, including no fixed differences between east and west and no windows of elevated F_{ST} in genome-wide comparisons. While these studies are consistent with genetic panmixia between eastern and western monarchs, an alternative interpretation is recent divergence but with ongoing low levels of gene flow.

Existing studies have included comparisons from a mix of breeding, migrating and overwintering monarchs. Future research could directly compare overwintering eastern and western monarchs only, since this should provide the most power for detecting potential genetic differentiation. Because migration distance is expected to act as a strong selective filter, this approach could potentially identify allele frequency shifts related to the differences in migration distance between east and west. By contrast, butterflies sequenced during summer breeding are the offspring of adults that randomly mate at overwintering sites and during spring return migration (Eanes and Koehn 1978), which could reduce any signal of divergent selection associated with fall migration distance.

While current evidence suggests little genetic differentiation between eastern and western monarchs, studies that include non-migratory monarchs from South Florida, the Caribbean, Central and South America, the Atlantic, and the Pacific do all find clear evidence for genetic differentiation in these peripheral populations (Lyons et al. 2012, Pierce et al. 2014, Zhan et al. 2014, Hemstrom et al., in review). This pattern suggests that existing methods are capable of detecting genetic differentiation among more divergent monarch lineages, including for monarchs in South Florida, which are genetically distinct from eastern monarchs despite a large influx of eastern migrants each year (Knight and Brower 2009, Vander Zanden et al. 2018). The genetic differences between North American and non-North American monarchs are also generally accompanied by more pronounced phenotypic differences than those observed between eastern and western monarchs (Li et al. 2016, Freedman et al., in review). However, it is possible that expanded sampling involving hundreds or thousands of monarchs sampled across a large number of markers could reveal subtle patterns of genetic differentiation between eastern and western monarchs that present studies have not detected.

Migration rates between east and west

Despite showing evidence for recurrent gene flow, eastern and western monarchs are clearly not demographically panmictic. Eastern and western overwintering clusters break up at different times of year, seasonal movement patterns and directions are different, and the timing of reproduction and number of generations per year may also be different. Overwintering counts of eastern and western monarchs show little evidence of correlation with each other (Fig. 1B, but see Appendix 2), and there is no observational evidence to suggest large influxes of eastern monarchs into western North America or vice versa.

Given their divergent overwintering destinations, it may at first be difficult to see how eastern and western monarchs could form a single genetic population. Mark-recapture studies (Morris et al. 2015, Billings 2019) and museum records (Dingle et al. 2005) suggest that at least some western monarchs travel to Mexican overwintering sites in the autumn. Billings (2019) compiled results from three years of mark-recapture studies conducted in Arizona. Of the 3,194 tagged and released monarchs, 32 were recovered at California overwintering sites and 12 were recovered at Mexican overwintering sites. Likewise, there is speculation that Mexican overwintering monarchs might recolonize western North America in the spring (Brower and Pyle 2004). None of the more than 2 million monarchs tagged east of the Rockies between August-November have ever been recovered in the west (O. Taylor, unpublished data); however, this may reflect (1) low general recovery rates (~1%) for tagged monarchs (Taylor et al. 2019); (2) low rates of movement from Mexican overwintering sites to western North America; (3) limited human population density in areas where these monarchs might be recovered (i.e. southern Arizona and New Mexico). It is also important to note that even small numbers of migrants between east and west—the classic rule of thumb suggests one migrant per generation (but see Mills and Allendorf 1996)—would be sufficient to prevent genetic differentiation from developing in a large, outcrossing species like monarchs.

Adaptive capacity in monarchs

Adaptive capacity in North American monarchs

The concept of adaptive capacity refers broadly to the ability of populations or species to adapt to future environmental change. North American monarchs possess high levels of genetic diversity, as indicated by high estimates of effective population size ($N_e \approx 2x10^6$) (Zhan et al. 2014). This high level of standing diversity should be associated with robust evolutionary potential. Eastern and western monarchs appear to harbor comparable levels of genetic diversity, as seen in measures of allelic richness using microsatellites (Pierce et al. 2014), the ratio of heterozygote to homozygote genotypes (Zhan et al. 2014), and various other measures (Talla et al. 2020, Hemstrom et al. in review). The lack of fixed genetic differences between eastern and western North America suggests that there are no strongly selected genetic variants that contribute to adaptation specifically to eastern or western North American environments (Talla et al. 2020). Experiments that reciprocally translocate eastern and western monarchs and assess their ability to exhibit appropriate migration-associated behaviors (e.g. directional orientation) would help to establish whether eastern and western monarchs are actually interchangeable. A number of previous studies have involved transplanting eastern monarchs westward (e.g. Urquhart and Urquhart 1977, Mouritsen et al. 2013), though the inferences that can be drawn from these studies may be limited (see Brower et al. 1995, Oberhauser et al. 2013).

Adaptive capacity in non-migratory monarch populations around the world

Many non-migratory, year-round breeding populations of monarchs have become established in locations around the world over recent evolutionary history (Vane-Wright 1993, Zalucki and Clarke 2004, Pierce et al. 2014, Zhan et al. 2014). These sites include multiple locations that fall under the purview of USFWS: American Samoa, Guam, the Northern Mariana Islands, Hawaii, Puerto Rico, and the U.S. Virgin Islands. Year-round breeding populations are also present in South Florida, coastal Georgia and the Carolinas, the Gulf Coast states, and southern California. Whether these recently-derived non-migratory populations can act as meaningful reservoirs of genetic diversity and adaptive capacity remains an open question (Reppert and de Roode 2018).

A recent review by Nail et al. (2019) suggested that the monarch's global distribution provides the species with adaptive capacity. While this may be true in a demographic sense—having widely distributed populations around the world reduces the risk of a stochastic extinction event for the species as a whole—recent research suggests that non-migratory populations may not provide adaptive capacity in an evolutionary sense. Derived monarch populations have reduced allelic richness (Pierce et al. 2014) and effective population sizes (Zhan et al. 2014, Hemstrom et al. in review), suggesting a loss of standing genetic diversity associated with founding bottlenecks in these populations. The reduction in genetic diversity in these peripheral populations could conceivably compromise their ability to adapt to future environmental change.

A number of recent studies have addressed the question of adaptive capacity in non-migratory monarchs. Freedman et al. (2018) found that non-migratory monarch populations from Queensland retain migration-associated traits such as induction of reproductive diapause, suggesting that the loss of migration may be due to a lack of relevant seasonal cues, rather than an inability to sense and/or integrate those cues. However, two recent studies (Tenger-Trolander et al. 2019; Tenger-Trolander and Kronforst 2020) found that commercially-reared monarchs whose breeding history precludes seasonal migration can lose their ability to consistently directionally orient, a critical part of their ability to complete migration. These studies suggest that some aspects of monarch migration are phenotypically plastic and may be shielded from selection and maintained in non-migratory populations, while other migration-associated traits might be selected against and lost. Freedman et al. (2020) also found that non-migratory monarch populations from Pacific Islands and Puerto Rico developed more slowly and were smaller as adults than their North American ancestors, potentially suggesting a general loss of vigor in these populations. Finally, non-migratory monarch populations tend to have high prevalence and abundance of infection with OE (Altizer et al. 2000, Bartel et al. 2011, Satterfield et al. 2015). Despite having greater tolerance and resistance to OE, non-migratory populations' parasite loads may render them less capable of completing long-distance flights (Bradley and Altizer 2005), thus generating a positive feedback loop of infection and non-migratory status (Faldyn et al. 2018). Together, these results call into question the notion that derived non-migratory monarch populations are adequate stand-ins for their migratory North American ancestors if the goal is to conserve functional genetic diversity.

Conclusions

Eastern and western monarchs are geographically and demographically distinct, though there is only modest evidence for phenotypic differentiation and no current evidence for genetic differentiation between them. In response to declining numbers of overwintering monarchs, many western states have proposed their own conservation measures. For example, The Western Association of Fish and Wildlife Agencies drafted its own Western Monarch Butterfly Conservation Plan in 2019 (Mawdsley et al. 2020), and numerous municipalities and land trusts have monarch conservation plans (see Jepsen and Black 2015). These are welcome and necessary initiatives, and recovery of western monarchs is a worthwhile goal in and of itself. However, it remains to be seen whether the pursuit of statewide conservation measures in the West is contingent upon a federal listing decision.

Research into the basic biology and migratory cycle of western monarchs is ongoing. Recent studies have begun to reveal natal origins of western overwintering butterflies (Yang et al. 2016), habitat correlates of larval and adult occurrence records (Stevens and Frey 2010, Dilts et al. 2019, Waterbury et al. 2019), windows of opportunity for larval development (Yang and Cenzer 2020), molecular mechanisms underlying diapause termination (Green and Kronforst 2019), and potential reasons for population declines (Pelton et al. 2019, Crone et al. 2019). A key to understanding western monarch population dynamics is research into the early spring period when western monarchs leave their overwintering grounds (Nagano et al. 1993, Espeset et al. 2016, Pelton et al. 2019): where do these monarchs go, what host plants do their offspring utilize, and how sensitive is the timing of this process to temperature and precipitation conditions? Furthermore, the role of non-native milkweed plantings, whose phenology differs from those of native milkweed species, should be investigated as a potential contributor to the decline of overwintering western monarchs. This line of research could include comparisons of cardenolide fingerprints (e.g. Malcolm et al. 1989, Knight and Brower 2009, Satterfield et al. 2018) of western monarchs through time to determine whether non-native milkweeds have become more prevalent, as well as community science initiatives to document the prevalence of non-native host use in areas near overwintering sites.

Policy-makers who are considering how to contextualize the decline of western monarchs will need to decide whether to adopt a parsimonious or precautionary approach in their decision-making. A parsimonious approach based on presently available genetic data would suggest that western monarchs do not constitute a distinct population: at present, there are no diagnostic criteria that could reliably be used to distinguish an eastern from a western monarch. A precautionary approach would recognize the potential for western monarchs to provide adaptive capacity and would involve treating the two populations as distinct based on their phenotypic and demographic differences. In the meantime, there is a clear role for scientists to collect additional data to resolve the somewhat mysterious discrepancy between phenotypic and genotypic patterns in North American monarch butterflies.

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Study	Eastern hosts used	Western hosts used	Experimental details	Local adaptation measure	Strength of local adaptation
Ladner and Altizer (2005)	*A. syriaca* *A. incarnata*	*A. speciosa* *A. fascicularis*	Cut stems used for larval performance assays	Oviposition preference	Host*population p = 0.39
				Larval survival	Host*population p = 0.89
				Larval growth rate	Host*population p = 0.84
Freedman et al. (2020)	*A. syriaca* *A. incarnata*	*A. speciosa* *A. fascicularis*	Live intact host plants used for assays	Larval survival	Sympatric/allopatric p = 0.48
				Larval growth rate	Sympatric/allopatric p = 0.15
				Time to eclosion	Sympatric/allopatric p = 0.73
				Eclosion mass	Sympatric/allopatric p = 0.95

Table 1: Summary of common garden experiments that have tested for local adaptation to host plant assemblages in eastern and western monarchs. The column for strength of local adaptation reports the metric used for assessing local adaptation and corresponding p-value.

Study	Wild caught?	Sample	N	Length (mm)	Area (mm^2)	%Diff Length	%Diff Area	Notes
Beall and Williams (1945)	Wild	Eastern	1274	51.82	NA	3.02%	NA	
		Western	296	50.30	NA			
Altizer and Davis (2010)	Wild	Eastern	302	51.33	872.3	2.19%	3.24%	37 eastern and 29 western maternal families
		Western	259	50.23	844.9			
	Common garden	Eastern	584	50.92	869.3	0.43%	1.42%	
		Western	260	50.70	863.5			
Li et al. (2016)	Wild	Eastern	60	51.51	852.2	0.88%	1.42%	
		Western	59	51.06	840.3			
Freedman and Dingle (2018)	Wild	Eastern	844	50.91	849.5	1.58%	2.32%	Includes samples from Li et al. (2016) and Yang et al. (2016)
		Western	809	50.12	830.2			
Talla et al. (2020)	Wild	Eastern	32	52.10	883.0	3.9%	8.21%	
		Western	31	50.16	816.0			
Freedman et al. (in revision)	Common garden	Eastern	171	51.12	898.1	0.63%	1.28%	12 eastern and 16 western maternal families
		Western	187	50.80	886.7			

Table 2—Summary of studies that have directly compared wing morphological data from eastern and western monarchs. The columns for length and area reflect overall averages based on data in papers and do not separate males and females. The %diff columns reflect the degree to which eastern monarchs are larger than western monarchs.

Study	Number and Location of Monarchs Sampled	Type of Sequencing / Number of Loci Analyzed	Brief Summary of Findings
Brower and Boyce (1991)	<u>28 total:</u> • **12 eastern** (Sierra Chincua) • **12 western** (Natural Bridges SP) • **1 Tobago** • **3 Trinidad**	Allozymes / 13 fragments used in analysis	No differentiation between any samples. Based on subsequent studies, lack of differentiation between North American and Caribbean samples likely an artefact of low statistical power.
Shephard et al. (2002)	<u>1194 total:</u> • **152 eastern** (100 Michoacan, 52 Kalamazoo) • **160 western** (50 San Diego, 55 Santa Barbara, 55 San Luis Obispo) • **855 Australia** • **48 Hawaii**	Allozymes / 7 fragments used in analysis	No differentiation between eastern and western samples, with the possible exception of San Diego. Substantial gene flow inferred between Santa Barbara and Kalamazoo samples.
Lyons et al. (2012)	<u>262 total:</u> • **100 eastern** (St. Marks, FL) • **100 western** (Pismo Beach, Santa Barbara) • **46 Hawaii** • **16 New Zealand**	Microsatellites / 17 sequenced, 11 used in analysis	No differentiation between eastern and western samples. Hawaii and New Zealand clearly distinct from North America. Note that Pierce et al. (2014) included the same North American monarchs and found the same results.
Zhan et al. (2014)	<u>92 total:</u> • **25 eastern** (MA, NJ, FL, TX, MX) • **3 western** (CA) • Various other locations in Central America, South America, Pacific, Atlantic	Whole genome resequencing / ~10 million SNPs with average genome-wide coverage >95%	No differentiation between eastern and western samples. Substantial differentiation between North America and all other locations, including South Florida.
Talla et al. (2020)	<u>43 total:</u> • **14 eastern** (MA, NJ, FL, TX, MX) • **29 western** (Big Sur, Oceano, Carpinteria)	Whole genome resequencing / ~20 million SNPs with average genome-wide coverage >95%	No differentiation between eastern and western samples. Overall genome-wide F_{ST} ~0.001, with no fixed differences between samples. *Note that eastern samples are the same as those used in Zhan et al. (2014).
Hemstrom, et al. (in review)	<u>281 total:</u> • **45 eastern** (MX) • **40 western** (5 sites in CA) • Various other Pacific locations	RAD-seq / ~70,000 SNPs	No differentiation between eastern and western samples. Overall genome-wide F_{ST} between east and west ~0.001, with no fixed differences between samples. Substantial differentiation between other samples.

Table 3—Summary of studies that have directly compared eastern and western monarchs to determine genetic differentiation.

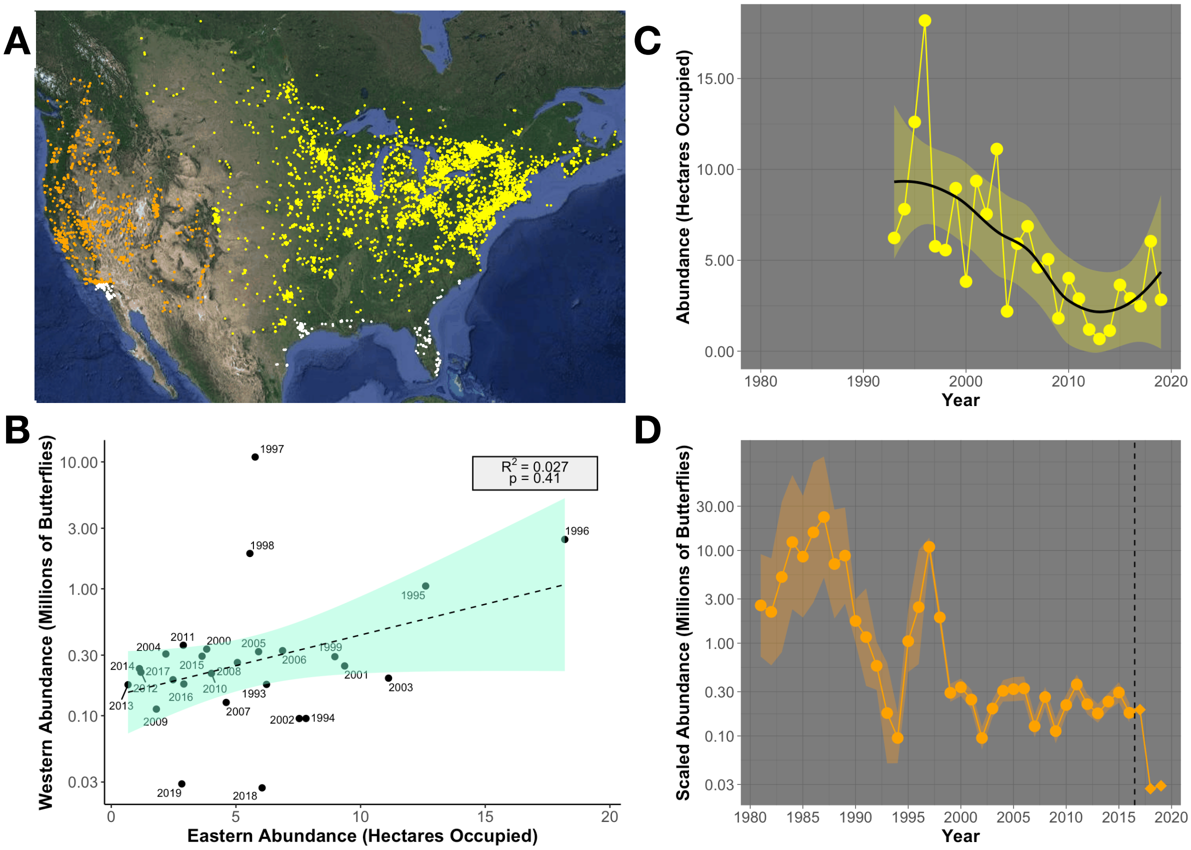

Figure 1 - (A) Distribution of summer-breeding monarch butterflies in the United States and Canada. Points correspond to records from GBIF (n = 20,552) in July and August and are separated into eastern (yellow), western (orange), and year-round breeding (white) locations. Map generated using ggmap (Kahle and Wickham 2013) and the Google Maps API. (B) Overwintering numbers for eastern and western monarchs are only weakly positively correlated across years ($R^2 = 0.027$, $p = 0.41$), suggesting that they are demographically distinct populations. Note that axes are on very different scales. (C) Overwintering abundance for eastern monarchs from Mexico, based on data from WWF Mexico. Abundance is based on aerial estimates of forest area occupied. The trend line shows LOESS-smoothed change through time and 95% confidence intervals around this trend. Note that overwintering abundance estimates are also available for some years between 1976-1994 but are not shown here. Overwintering eastern monarch densities are likely between 21-28 million butterflies/hectare (Thogmartin et al. 2017). (D) Overwintering abundance for western monarchs from sites in California. Abundance estimates from 1981-2016 take into account sampling effort and are the same as the values presented in Schultz et al. (2017); abundance estimates for 2017-2019 are based on non-adjusted counts.

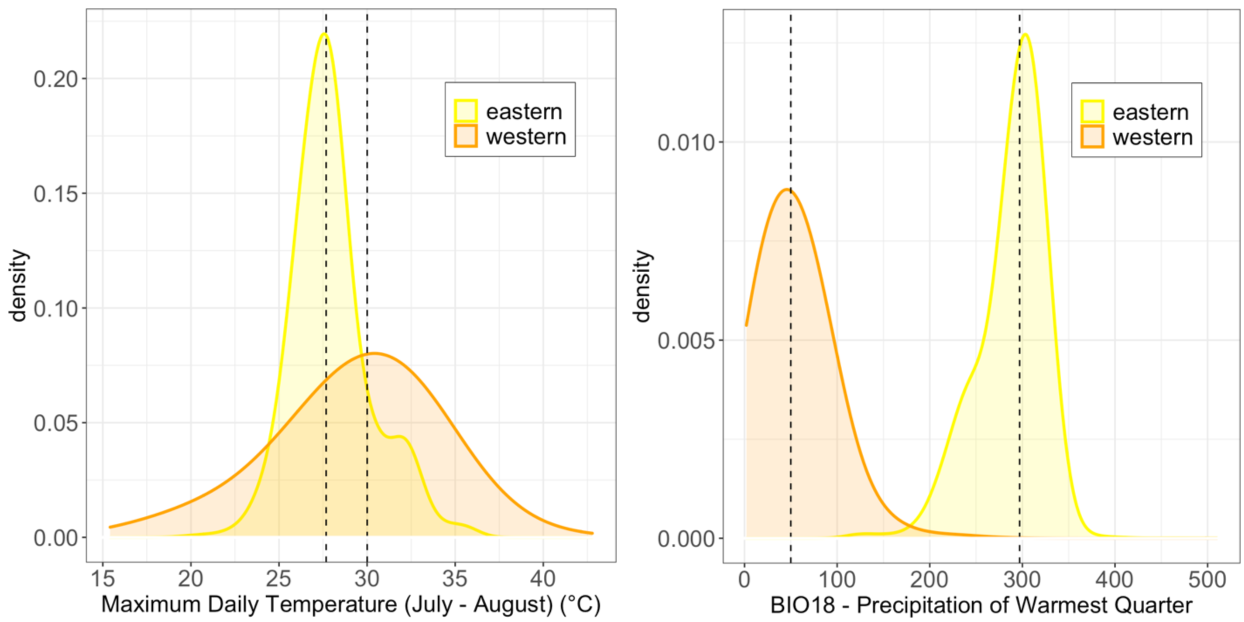

Figure 2 – (Left) Distribution of daily high temperatures during the peak of the monarch's summer breeding season (July and August) in eastern and western North America, corresponding to the occurrence records shown in Figure 1A. Western monarchs were recorded in locations that had median summertime maximum temperatures that were 2.3°C warmer than comparable eastern locations. (Right) Summer records of monarchs in western North America come from areas that receive little summer precipitation.