Dear Editor and Reviewers,

Please find our responses to reviewer comments below. Reviewer comments are shown in bold, and our responses to each comment are interspersed throughout this document.

Thank you.

**Associate Editor Comments to the Author:  
The review focus on compiling the biological data regarding phenotypic and genetic differentiation between eastern and western monarchs, with the goal of informing researchers and policy-makers who are interested in monarch conservation. As the authors highlight in the cover letter this emblematic species is broad interest because of": (1) the monarch's status as an iconic symbol of conservation; (2) the recent decision by the U.S. Fish and Wildlife Service that a threatened listing is "warranted but precluded"; (3) the looming extirpation of migratory monarchs from western North America."  
  
Please take in consideration both reviewers comments to improve and clarify some issues of the manuscript.  
  
  
Reviewer(s)' Comments to Author:  
  
Reviewer: 1  
  
Comments to the Author  
This is a nice review paper on a species of conservation concern.  Overall, I found it a good summary of available information.**    
  
**Figure 1. Typically an “r” value not R2 is presented for correlations. “only weakly positively correlated across years (R2 = 0.027, p = 0.41” does not seem quite right.  Probably no line (even a dashed line) should be drawn in panel B, and it should simply be referred to as: no correlation was found.Having said this,I would be surprised if cleaning up the data a bit would not reveal a significant correlation.**

As suggested, Figure 1 has been updated so that it reflects *r* (Pearson’s correlation coefficient) rather than the proportion of variation explained. We also removed the trendline and associated confidence intervals.

Reviewer 1 is correct that there are multiple alternative ways to parse the correlation between eastern and western overwintering numbers. Most notably, if the relationship is assessed using a one-year time lag (e.g. eastern overwintering numbers in year *n* compared to western overwintering numbers in year *n + 1*), then the correlation becomes significant. This is currently noted in Appendix 2 and is consistent with a note by Vandenbosch (2007) in the *Journal of the Lepidopterists’ Society*. If you feel that this information is relevant and worth including in the main text, we can do so.  
  
**Line 97: “confined entirely to the east” true, but “east of the Rocky Mountains” would be more clear.**

We have updated the text so that it now reads “east of the Rocky Mountains”  
  
**All the tables: cut them or put in an appendix. They do not add much on their own.**

As suggested, we moved what was Table 1 (studies of host plant adaption) and Table 2 (studies of wing size) to the supplemental material. However, we are inclined to keep what was Table 3 (summary of studies comparing genetic differentiation between eastern and western monarchs) as part of the main text, since the issue of genetic differentiation is central to arguments about the conservation significance of western monarchs. We also feel that this table is important because it highlights how even with different sampling schemes and genetic markers, the general conclusions reached by each study are consistent in finding no detectable genetic differentiation between eastern and western monarchs.  
  
**Reviewer: 2**  
  
**Comments to the Author  
This review focuses on phenotypic and genetic differentiation between eastern and western monarchs, addressing questions about whether western monarchs should be considered a distinct biological entity. While the manuscript provides an interesting summary of research addressing this question, I found very little new here. Thus, while I don’t have major criticisms of the study, I did not find it a compelling synthesis.   
  
Here are comments that could be used to improve the manuscript.**  
  
**The authors state on line 43 that it is self-evident that the presence of populations outside of North America provide redundancy and thus demographic resilience. However, these populations are all orders of magnitude smaller than the eastern migratory population, and in the case of the western population, arguably near extinction. And, as the authors note at the end of the paper, populations outside of North America have reduced genetic diversity.**

Our intention here was to highlight the different interpretations of the term “adaptive capacity,” which is widely used in endangered species assessments. A recent paper by Nail et al. (2019) stated that the presence of monarch populations outside of North America provides the species with adaptive capacity, based on the idea that these peripheral populations reduce the risk of stochastic extinction (e.g. through a catastrophic winter storm affecting eastern overwintering populations). This point—that numerous and geographically widespread populations provide redundancy—is what we consider to be self-evident. By contrast, as you note, it is questionable as to whether the peripheral populations provide adaptive capacity in an evolutionary sense.

In an attempt to make this distinction more clear, we updated this sentence to read “Here, we focus on adaptive capacity in an evolutionary rather than a demographic sense, as we consider it self-evident that the presence of monarch populations outside of eastern North America provides redundancy and reduces the risk of stochastic extinction (e.g. from extreme weather events affecting eastern overwintering locations) (Nail et al. 2019).”

**The authors focus on 4 ecological factors that could drive phenotypic variation between eastern and western monarchs.    
  
•       Host plant associations: While I agree that eastern and western milkweed assemblages vary, one could argue that the species to which monarchs within each of these populations utilize vary just as much. Monarchs within each population are exposed to very different plants over the course of a year, and monarchs’ migratory behavior means that local adaptation is not expected. Not surprisingly, it has not been shown in any of several studies, and pursuing this line of research, as the authors suggest should occur, does not seem fruitful.**

We agree that the within-year turnover in host plant assemblages is likely to preclude a pattern of local adaptation to any specific host species. We now state that future research into host plant adaptation between eastern and western monarchs is not a high priority, though a better understanding of how western monarchs use native California milkweed species available in March and April (especially *A. cordifolia*) would be useful.  **•       Thermal regimes: It would have been more relevant to use occurrence records of immature monarchs, since it is quite likely that thermal regimes during development exert stronger selective pressure than those experienced as adults; eggs, larvae and pupae are less able to escape temperature extremes. There have been both mechanistic and niche analyses of thermal tolerances for monarch eggs and larvae that should have been referenced.**

We chose to focus on adult records because they are much more numerous in online databases, e.g. those through GBIF. However, we agree that selection due to thermal regimes should be more pronounced in monarch eggs, larvae, and pupae, because of their relative immobility.

Unfortunately, the GBIF database does not include information on life stage for most observations. Out of a global database with more than 488,000 monarch records, restricting the search to include the terms “larva,” “egg,” or “pupa” yields only 470 results. The Monarch Larva Monitoring Project contains multiple decades of observations on eggs and larvae, although this dataset is not publicly available, and the sampling locations are highly non-random.

Instead, we used monarch observations through iNaturalist and the associated R package iNatTools. Out of the approximately 121,000 currently available research grade observations available through iNaturalist, about 32,000 are annotated to indicate a life stage of larva, pupa, or egg ([link here](https://www.inaturalist.org/observations?taxon_id=48662&term_id=1&term_value_id=4,6,7) has URL specifying these search terms). This includes approximately 13,500 observations of larvae, pupae, and eggs from July and August in areas of the United States and Canada where migrants breed in the summer. This analysis yields a similar result to that presented based on records for adults: immature monarchs in western North America experience higher daytime high temperatures during July and August (median daytime high = 28.91ºC) compared to immature eastern monarchs (median daytime high = 26.80ºC). We report these results in the caption of Figure 2 but retain the original figure, since the results are qualitatively very similar.

We added references to Zalucki and Rochester (1999) and Zalucki et al. (2015). Other modeling efforts (e.g. Batalden et al. 2014, Lamoine 2015) that focus exclusively on eastern North American monarchs are not cited. We also note in Appendix 1 that for both larval and adult collection records, the BIOCLIM variable corresponding to “mean temperature of the warmest quarter” (BIO10) is slightly higher in eastern compared to western North America, owing to higher nighttime low temperatures in eastern North America. However, the daily maximum temperatures during July and August, which comprise the peak of summer breeding, are on average about 2ºC higher for monarchs in western North America. We also note that a substantial proportion of western monarch records come from locations predicted to have unsuitable EI values in CLIMEX models. **•       Natural enemies: While it is interesting to speculate about how natural enemies may be driving phenotypic or genetic differentiation between eastern and western monarchs, it is likely that within population differences in natural enemies are larger than between population differences. I agree, however, that OE could drive selection, although there is not evidence that it has, at least between the eastern and western migratory North American populations. There were no additional lines of research suggested in this section; I would argue that this might be the most fruitful avenue for ongoing research.**

We added a paragraph that suggests follow-up studies comparing OE virulence between eastern and western North America. The original study comparing eastern and western OE genotypes was conducted in 2008, when there was still a relatively robust migratory western population. It would be interesting to test for whether western OE has become more virulent over the past decade, potentially coinciding with an increase in the proportion of year-round breeding monarchs in western North America.

**•       Scale of migration. I would need to be convinced that there is a big difference between flying ~800 km and 1500-3000. A butterfly that needs to fly 800 km should maximize flight efficiency, right?  There should be a citation for distances for flown by eastern monarchs (lines 168-170).**

We updated the metrics cited in an attempt to better highlight the discrepancy in migration distance between eastern and western monarchs. The originally cited estimate of 800 km for western monarchs comes from releases of tagged butterflies in eastern Washington and Oregon that were later recaptured in California. Such mark-recapture efforts are useful for determining the direction and distance of migratory flights but may not be accurate reflections of the western migration as a whole. Yang et al. (2016) used stable isotope data from overwintering sites along the California coast, and although this paper did not provide an average migration distance estimate, a substantial fraction (~30%) of the butterflies measured in that study originated from sites along the California coastline. Thus, a more realistic estimate for the average migration distance of a western monarch is perhaps 500 km, although this is somewhat speculative. For eastern monarchs, the listed range of 1,500-3,000 km is obviously quite broad and is based on tag recoveries from Monarch Watch. The supplementary data from the most recently published stable isotope study for eastern overwintering monarchs (Flockhart et al. 2017) suggest an average flight distance of 2,995 km for the 613 butterflies included. As we now note in the paper, these metrics would correspond to a six-fold difference in average migration distance.

Additionally, we also make reference to one recent study that demonstrated that eastern and western migrants have measurably different flight physiology (Talla et al. 2020). That study found that eastern migrants collected at a stopover site have significantly longer flight duration and distance (e.g. endurance) than western migrants collected from an overwintering site. **While searches for genetic differences between eastern and western monarchs have not shown any divergence, the authors are correct in arguing that this could be due to either panmixia or recent divergence with ongoing low gene flow (the authors and I agree, I think, that the latter is most likely). However, the suggestion that studies that compare overwintering monarchs only should provide the most power for detecting genetic differentiation shows a poor understanding of population genetic theory. Genes that do not promote successful migration would have long been weeded out of the population, and “good migration” genes would not be expected to be more common in the generation that migrates. Perhaps using more butterflies and more markers will uncover some as yet hidden genetic differences between these populations, but the fact that so many people have looked for this and not found any, but have found them between other populations, suggests that eastern and western monarchs are remarkably similar.**

Our intention was not to suggest that comparing eastern and western overwintering butterflies would reveal fixed genetic differences between them. Rather, sampling overwintering butterflies has the potential to capture small allele frequency shifts associated with directional selection caused by season migration. Because the strength of directional selection imposed by migration is expected to differ between eastern and western North America, there is the potential for these allele frequency shifts to be more pronounced in eastern compared to western North America. Other studies have shown that seasonally fluctuating selection pressures can cause repeated and detectable within-population allele frequency shifts that oscillate within years (e.g. Bergland et al. 2014, Paaby et al. 2014, Behrman et al. 2018). We updated the text of the manuscript to hopefully make this distinction clearer. While we agree that natural selection has strongly favored traits and alleles associated with migration in North American monarchs, there is clearly still substantial phenotypic and genetic variation for migration-associated traits (e.g. size, lipid accumulation, flight physiology), and it seems reasonable to suggest that migration-associated traits might be under balancing selection due to changing selection pressures across the migratory cycle.

**Two populations do not need to be behaviorally panmictic to maintain enough gene flow to prevent divergence, so the arguments against demographic panmictia (clusters break up at different times of year, seasonal movement patterns are different, counts are not correlated, there is no observational evidence for large influxes) are not important. As the authors note, there is strong evidence for some gene flow, at least from the west to the east via tagging studies, and not a lot is needed.**

We removed the first paragraph from the section on migration rates between east and west.  **I had hoped that the conclusions section would provide some meat to this paper, but that was not the case. There is an unfocused paragraph on the potential effectiveness and importance of conservation measures in the west, and an unrelated question about whether these will continue if monarchs are not awarded the protection of the Endangered Species Act. There are also research recommendations to promote western monarch conservation, which seem unconnected to the rest of the paper.**

The primary goal of the paper was to provide an up-to-date review of evidence for and against treating western monarchs as a distinct population. We also feel that the discussion of adaptive capacity provided by non-migratory monarch populations is a novel contribution to the discussion of monarch conservation.

In an attempt at having a more cohesive ending to the paper, we removed two paragraphs from the previous Conclusions section and replaced them with a new section entitled “What lessons can be learned from declines in western monarchs?” that includes the following text:

One year after approximately 300,000 monarchs were recorded at overwintering sites in California in 2016, Schultz et al. (2017) proposed a quasi-extinction threshold of approximately 30,000 monarchs and suggested a 72% risk of extinction within the next 20 years. In 2018, western overwintering numbers fell below the quasi-extinction threshold for the first time. By 2020, fewer than 2,000 monarchs were recorded from California overwintering sites. The reasons for this extremely rapid decline are not entirely clear and are hard to disentangle from time series data (see Crone et al. 2019), though climate change—and especially autumnal warming—has been implicated broadly in the decline of western North American butterflies (Forister et al. 2021). The lack of recovery in western monarchs between 2018-2020 is also consistent with positive feedbacks and possible Allee effects. The rapid loss of western monarchs highlights that adoption of mitigation measures to stem declines cannot be reactive; ongoing efforts to restore overwintering sites in California, while well-intentioned, would have been more useful if implemented preemptively.

Despite the looming extirpation of *migratory* monarchs from the western U.S., a potentially growing population of year-round resident monarchs has established in coastal California (Satterfield et al. 2016, Crone and Schultz 2021), particularly in urban areas with cultivated, non-native milkweed (especially *A. curassavica*). While likely several orders of magnitude smaller than historical migratory populations, these year-round breeding populations may prevent a complete extirpation of monarchs from western North America. It is currently unclear whether year-round breeding populations in California as well as the U.S. Gulf Coast and Florida may act as demographic sources or sinks for migratory monarchs. There is some evidence of monarchs interrupting their migration and joining year-round breeding populations in both eastern (Satterfield et al. 2018) and western (James and Kappen 2021) North America. The distinction between outright extinction versus loss of migration is important and has been noted previously: indeed, the 2014 petition to list monarchs under the ESA highlights that while species-level extinction is unlikely, the phenomenon of long-distance migration is at risk (also see Wells et al. 1983, Brower and Malcolm 1991). From an evolutionary perspective, migration is likely a derived condition within the *Danaus* clade containing monarchs (Zhan et al. 2014). Even though migration is not uncommon among other Danaine butterflies (Ackery and Vane-Wright 1984), monarchs are unique in the scale and scope of their migration. Future research into partial migration—whereby animal populations are composed of a mixture of resident and migratory individuals (Chapman et al. 2011)—using monarch populations from the United States and Australia may provide a glimpse into an uncertain future for migratory monarch butterflies.