Comments to the Author

The authors present a population genomics study on the demographic signatures of range expansion in monarch butterflies. This is an interesting system to study these questions, in part because this expansion has also led to events of loss of migration. The authors do a good job at introducing the system and the background on the potential connections between changes in behavior and population genetic signatures of expansion. Although I think this would be a manuscript of wide interest to readers of Molecular Ecology, I raise some minor concerns that I think need to be clarified.   
  
My main concern is that I don’t understand the biological hypothesis behind the models the authors chose. I think it is important to clarify in the introduction what hypothesis are being tested with these models in terms of population genetic differences of range expansion. It was also hard for me to understand what additional information we are learning from this study that was not presented in the Zhan et al 2014 paper that they cite. I understand the model that the authors present is more complex, but it was not clear in my reading how this study changes the knowledge we previously had about this expansion. Is this the first time the direction of the expansion has been described? If so, this needs to be highlighted.

**We apologize for not being clearer about what hypotheses our demographic modelling addresses. The demographic models that we evaluated are part of a set of pre-defined demographic scenarios proposed by other authors and available as part of the dadi analysis pipeline. In our case, we focused on models with two populations, but with widely variable structures and numbers of estimated parameters: for example, some models (e.g. found and grow, Figure 1B) are relatively simple and involve a single population splitting into two, followed by growth in both of the descendent lineages, whereas other models (e.g. the three epoch model, Figure 1A) are more complicated and involve multiple rounds of population expansion or contraction in the ancestral population prior to divergence, followed by additional expansion or contraction in the descendent lineages. Our approach does not favor one scenario *a priori*, and instead is intended to provide information about the likelihood of a particular model generating site frequency spectra similar to those that we observed in our data. So, the hypotheses that we are evaluating are themselves contingent upon the structure of the models that are most favored. As such, it is somewhat difficult to thoroughly describe all of the possible hypotheses in the introduction of the paper. We have added this sentence to the introduction to hopefully clarify what benefits this demographic modeling approach may offer:**

**“Further demographic modeling that accommodates a broad range of potential establishment scenarios—including variable establishment timing, founding population sizes, and past changes in population size—might help to resolve the discrepancy between historical and model-based estimates of expansion timing.”**

**With regards to the Zhan et al. (2014) paper, it is definitely true that our analysis is a direct extension of the approach that they use. However, we feel that our approach has two main advantages. First, the Zhan analysis pools together six disparate Pacific populations (18 individuals) into a single Pacific lineage, which might obscure the stepwise nature of this expansion event. By contrast, we focus only on the Hawaiian population, which is inferred to be the first “stepping stone” in the Pacific expansion and therefore should have the most straightforward demographic history. Second, the Zhan analysis only reports the results of a single demographic scenario (extended data Figure 3a in their paper), which makes it difficult to assess whether this scenario is appropriate relative to other possible scenarios. We instead tested many different demographic scenarios, which provides added context when evaluating specific parameter estimates. We have updated our methods section to reflect this difference.**

**Beyond these methodological differences, the data that we present here also include sampling from six locations in the Pacific not reported in Zhan et al. (2014): Guam, Rota, Saipan, Norfolk Island, New South Wales, and Victoria. This allowed us to uncover a separate expansion event out of Hawaii and also to assess patterns of differentiation within the Mariana Islands and across the Australian continent. This provides a more nuanced picture of the monarch’s Pacific expansion and highlights the role that seasonal migration and its loss can play in structuring spatial genetic variation.**

I think that the patterns the author find for the Hawaiian populations are very interesting. The signatures of greater than expected heterocigocity there, the patterns with rare alleles that the authors mention, and the admixture in these populations could represent multiple events of gene flow from the North American population. I understand that the travel distance is large, but how much does a single butterfly fly in a migratory event? And could climatic events have promoted movement there multiple times?

**We agree that the Hawaiian population is interesting and somewhat enigmatic. Although our demographic models supported very low (in fact, practically non-existent) rates of per-generation migration between North America and Hawaii, we do not entirely dismiss the possibility for ongoing gene flow between North America and Hawaii. Tropical storm systems in the eastern Pacific sometimes originate along the coast of Mexico and track continuously to Hawaii (e.g., Hurricane Lester in 2016, see p. 19 of this report: https://www.nhc.noaa.gov/data/tcr/EP132016\_Lester.pdf), which might aid in monarch movement (also see Clarke and Zalucki 2004). Furthermore, monarchs that appear somewhat regularly in the United Kingdom almost all are associated with Autumn storms passing over the North Atlantic and are recorded at the same time as migratory North American birds, further suggesting wind-aided long-distance movement. This is of course quite speculative, but we did add a brief mention to this possibility in the discussion with the following text:**

**“However, we do note that monarchs appear to be capable of wind-aided dispersal over extremely long distances, as evidenced by occasional records from the United Kingdom that coincide with records of migratory North American birds blown by storms (Asher et al. 2001); thus, ongoing movement from North America to Hawaii is not entirely implausible.”**

**During their North American migration, monarchs regularly cover distances of >4000 km, although this is usually accomplished over the course of multiple weeks and with numerous stopping points along the way, with average daily movements more likely to be around 40-60 km per day (Taylor et al. 2019, Frontiers in Ecology and Evolution).**   
  
Finally, below I include some minor comments that could help streamline and clarify the manuscript:  
  
Line 9: include the number of monarchs for each group.

**The abstract has been updated to include sample sizes for each group. We also note that the total number of sequenced monarchs was actually 275, instead of the previously-reported 281 (some samples that were originally included in the experimental design were lost during DNA extraction / library prep).**  
  
Line 29: I suggest not including this sentence.

**As suggested, we have removed this sentence.**  
  
Lines 55-56: Does this mean phenotypic differentiation or genetic differentiation? If this has been quantified, include citation.

**We are unaware of any studies that have systematically reviewed the degree of within-species variation (phenotypic or genetic) between fully migratory, partially migratory, and fully sedentary species. As such, we have updated this sentence to the following:**

**“Migratory species that show evidence for partial migration, whereby species are comprised of both migratory and non-migratory populations (Chapman et al. 2011), often feature populations that are highly differentiated, both phenotypically (*e.g.*, Dingle 1980, Altizer and Davis 2010) and genetically (*e.g.*, Zhan et al. 2014, Gomez-Bahamon et al. 2020).”**

Line 97: what patterns?

**We have updated this to read “overall patterns of genetic relatedness”**  
  
Line 292: consider estimating Tajima’s D for all the populations that are considered the result of a single expansion together. If the values are positive, this is consistent with the hypothesis that it is a single event, if not, this could reflect more than one event.

**As suggested, we estimated Tajima’s D by pooling all of our expansion populations from the Pacific (i.e., all populations besides North America). This pooled sample had a Tajima’s D of -0.715, consistent with an excess of rare alleles and recent population expansion. As you mention, this could potentially indicate multiple expansion events, as opposed to a single wave of expansion, although this is difficult to determine using this single summary statistic and a sample of pooled populations. To avoid confusion, we removed instances from the manuscript where we refer to a “single expansion event” across the Pacific, since what we really mean to imply is a series of discrete stepwise expansion events.**  
  
Lines 393-395: is there evidence of extremely small effective population sizes? What are the estimates from the models?

**The demographic models that we present in this paper only produced estimates of effective population sizes for the Hawaiian population, as this was the only population considered in our demographic scenarios. However, we did also use the program NeEstimator to estimate effective population sizes for monarchs from North America, Guam, and Rota; unfortunately these were the only populations for which we had large enough sample sizes after filtering to generate model output. The Ne estimates produced by this program were approximately 1.02 million for the North American population, roughly similar to estimates suggested in Zhan et al. (2014) of ~1.8 million. By contrast, Ne estimates in the Mariana Islands were much smaller, at ~2000 for Guam and ~4000 for Rota. These values are also consistent with our other reported summary statistics, and hence we feel comfortable with the statement (now on lines 405-406) that “many nonmigratory Pacific monarch populations likely have extremely small effective population sizes that are susceptible to very strong genetic drift.”**  
  
Line 411: if these come from a single breeder could there be genomic signatures of inbreeding in those monarchs? Is that evident from your data?

**It is difficult to distinguish between a pattern of inbreeding attributable to recent rearing history (akin to that expected from a butterfly rearing operation) versus the more general phenomenon of inbreeding due to small population sizes. With enough sampling, it is conceivable that we might find individuals with very large shared haploytpes, though our sample sizes for Hawaii were relatively small (n = 9 after filtering) and our reduced-representation sequencing method is not well-suited to detect this pattern. However, we do now estimate FIS for the Hawaiian population to more explicitly test for the degree of inbreeding within sequenced Hawaiian individuals. We found an FIS value of 0.055 for the Hawaiian population, which indicates a deficit of heterozygote genotypes, though not to an exceptional degree.**  
Line 417: I look forward to reading this!!

**Stayed tuned!**  
  
Table 1: consider coloring the names of the populations by the regions.

**As suggested, we have updated the text color of the table so that populations are grouped according to their region of origin (consistent with the table caption).**   
  
I think the PCA is a nice way of visualizing some of the patterns described. Consider including it in the main text, perhaps with the admixture panel.

**We agree that PCA is a useful way of visualizing some of the patterns that we describe, especially because in our case it recapitulates the geography of the range expansion that we describe. However, we were asked by previous reviewers to move this figure to the supplementary materials because they found it to be redundant with the other panels in Figure 2. We have elected to keep this figure in the supplement.**