Title: Population genetics of a recent pacific range expansion in monarch butterflies

Running Title: Pacific monarch butterfly genetics

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**Abstract**

Monarch butterflies are best-known from North America but have greatly expanded their geographic range over recent evolutionary history, including an expansion across the Pacific Ocean. Here, we present reduced-representation sequencing data for approximately 280 monarchs from North America and 15 locations across the Pacific. We find support for a stepwise pattern of dispersal across the Pacific, including a previously uncharacterized westward expansion from Hawaii into the Mariana Islands. Monarchs within the Mariana Islands show strong patterns of differentiation, despite being in extremely close proximity; by contrast, migratory North American samples form a single genetically panmictic population across the entire continent. Estimates for the timing of the monarch’s establishment in the Pacific are concordant with a very recent expansion, but with high uncertainty around the precise timing of this event. Estimates of contemporary gene flow suggest extremely low rates of movement between North America and Hawaii. Together, our data argue in favor of deferring to historical records to infer the general timing of the monarch’s range expansion and also provide a striking example of how migratory status influences patterns of genetic differentiation across geographic scales in a widespread insect species.

**Key words:** Range expansion, serial dispersal, monarch butterfly, population genomics

**Introduction**

Species that undergo range expansions often have distinctive patterns of population genetic structure, with decreasing relatedness and increasing contributions of genetic drift in populations further from the original source population [[1](https://paperpile.com/c/tNxuHC/yH35),[2]](https://paperpile.com/c/tNxuHC/jQxu). One commonly encountered form of range expansion is serial stepwise dispersal, in which populations are founded in a stepping-stone fashion [[3](https://paperpile.com/c/tNxuHC/9Zhp),[4]](https://paperpile.com/c/tNxuHC/0Cse). Serial dispersal is characteristic of many post-glacial range expansions into temperate regions and has been shown for species including eider ducks (*Somateria mollissima*) [[5]](https://paperpile.com/c/tNxuHC/S7wa), ragwort (*Senecio halleri*) [[6]](https://paperpile.com/c/tNxuHC/4FF0), and rough-skinned newts (*Taricha granulosa*) [[7]](https://paperpile.com/c/tNxuHC/NrmF). The out-of-Africa expansion of *Homo sapiens* is also characterized by serial stepwise dispersal [[8]](https://paperpile.com/c/tNxuHC/dg3r).

One species that has undergone a dramatic range expansion over its recent evolutionary history is the monarch butterfly (*Danaus plexippus*). Evidence suggests that monarchs historically occupied Central America and the southern United States before undergoing a large demographic expansion approximately 20,000 years ago [[9](https://paperpile.com/c/tNxuHC/wGjC),[10]](https://paperpile.com/c/tNxuHC/79SP). This demographic expansion likely coincided with the end of the last ice age and glacial retreat in North America, which enabled colonization of temperate areas by the monarch’s *Asclepias* host plants and likely set the stage for the onset of continent-scale long-distance migration. More recently, monarchs have become established around the globe in a number of independent out-of-North America expansion events [[11](https://paperpile.com/c/tNxuHC/b7Th),[9]](https://paperpile.com/c/tNxuHC/wGjC). This includes a southern expansion that involved establishment in South America and the Caribbean [[9]](https://paperpile.com/c/tNxuHC/wGjC), an eastward expansion across the Atlantic and into the Iberian Peninsula [[12]](https://paperpile.com/c/tNxuHC/UJ0f), and a westward expansion across the Pacific [[13]](https://paperpile.com/c/tNxuHC/g2dz). Here, we focus exclusively on the monarch’s range expansion across the Pacific.

Historical records suggest that monarchs crossed the Pacific quite recently, with the earliest positive records of monarch occurrences coming from the 1840s in Hawaii [[14](https://paperpile.com/c/tNxuHC/lvj1),[13]](https://paperpile.com/c/tNxuHC/g2dz). By 1871, monarchs had reached Australia [[13]](https://paperpile.com/c/tNxuHC/g2dz) and were established on nearly every major Pacific island group by 1900. Some authors have attributed the recency of the monarch’s appearance in these locations to the “Columbus hypothesis,” which posits that the clearing of forests in the eastern and midwestern United States during the eighteenth and nineteenth century prompted a massive increase in the North American monarch’s population size and scope of migration [[14]](https://paperpile.com/c/tNxuHC/lvj1). However, demographic reconstructions using whole genome sequence data indicate that the monarch’s out-of-North America expansion events happened much more distantly, perhaps as long as 2,000-3,000 years ago [[9]](https://paperpile.com/c/tNxuHC/wGjC). Thus, there is disagreement between demographic models and historical records about the timing of the monarch’s Pacific expansion.

Currently available population genetic data suggest that the monarch’s recent global range expansion happened in a serial stepwise fashion [[15](https://paperpile.com/c/tNxuHC/upvO),[9]](https://paperpile.com/c/tNxuHC/wGjC). Serial expansion is indicative of a natural wave of expansion, rather than a series of independent and deliberate or assisted human introductions [[13]](https://paperpile.com/c/tNxuHC/g2dz). Although the evidence for serial dispersal in monarchs is quite strong, there are a number of unsampled populations in the Pacific that might improve our understanding of range expansion timing and direction.

Finally, in contrast to their migratory North American ancestors, nearly all Pacific island populations have become fully non-migratory, year-round breeding populations. Little is known about how this contemporary loss of migration has affected fine-scale patterns of population differentiation in monarchs. Two studies have addressed this question: Hughes and Zalucki (1984) used four allozyme markers and found relatively high FST between milkweed patches over small spatial scales in Queensland (tens to hundreds of kilometers) [[16]](https://paperpile.com/c/tNxuHC/lH1o). Pierce et al. (2017b) used microsatellite markers and showed that monarchs from the Hawaiian archipelago show little differentiation among islands [[17]](https://paperpile.com/c/tNxuHC/XTdq). However, the conclusions of these studies are limited by the spatial scale of sampling and the number of loci studied. Thus, the degree to which loss of migration shapes fine-scale patterns of population differentiation remains unresolved.

In this study, we sequenced approximately 280 monarch butterflies at >70,000 highly variable genomic sites from the ancestral North American population and many Pacific Island populations, including a number of previously unsampled locations: the Mariana Islands (Guam, Rota, and Saipan) and Norfolk Island. The goals of this study were to understand (1) patterns of relatedness among Pacific and North American populations, (2) genetic differentiation within expansion populations, and (3) expansion timing and amount of ongoing gene flow from North America.

**Methods**

*Sample preparation and sequencing*

Monarchs were collected as either larvae or adult butterflies from locations around the world between 1990 – 2017 (Figure 1a and Table S1).DNA was extracted from samples using a magnetic bead-based protocol [[18]](https://paperpile.com/c/tNxuHC/BRJR) and quantified using Quant-iT PicoGreen dsDNA Reagent (Thermo Fisher Scientific) on a FLx800 Fluorescence Reader (BioTek Instruments). Restriction Associated Digest (RAD) DNA libraries were then created using the PstI restriction enzyme according to ref. [[18]](https://paperpile.com/c/tNxuHC/BRJR) and sequenced using 150bp paired-end sequencing on an Illumina Hi-Seq 4000.

*Sequence alignment, filtering, and genotype calling*

We aligned raw sequence data to version 3 of the monarch butterfly genome assembly [[19]](https://paperpile.com/c/tNxuHC/psiG) using the mem algorithm implemented in Burrows-Wheeler Aligner [[20]](https://paperpile.com/c/tNxuHC/7NQQ). Sequence data was sorted and filtered for PCR duplicates and improper pairs using SAMtools [[21]](https://paperpile.com/c/tNxuHC/EXik). For use in demographic reconstruction, genotypes were then called using the SAMtools genotype likelihood model [[21]](https://paperpile.com/c/tNxuHC/EXik) as implemented in the ANGSD software package with a minimum mapping and base call quality score of 20, a SNP *p*-value of 1e-8, a uniform genotype prior, and a posterior genotype probability cutoff of 0.95 [[22]](https://paperpile.com/c/tNxuHC/hYyB). For demographic reconstruction specifically, we randomly selected SNPs such that no SNP was within 10kb of another using a custom R script to reduce the likelihood that SNPs were in substantial physical linkage prior to demographic reconstruction. For analyses that relied on called genotypes, we removed potential paralogous sites using the ngsParalog tool by removing all sites within a kilobase of any SNP with a log ratio test statistic of > 10 in any population [[23]](https://paperpile.com/c/tNxuHC/OQZa). Individuals with less than 75% of called SNPs were removed. Since strong bottlenecks are likely to remove rare SNPs from the population, we did not use a minor allele frequency filter when calling genotypes for the calculation of the basic diversity statistics (π, HO, and Het/Hom ratio).

*Patterns of relatedness among monarch populations*

We calculated the average number of pairwise differences (µ), observed heterozygosity (Ho), heterozygote/homozygote ratio per individual (Het/Hom), Tajima’s D, and fixation index (FST) between each pair of populations for each SNP using the snpR package (Hemstrom et al. *in prep*). We calculated FST using the R implementation of the GENEPOP software package [[24]](https://paperpile.com/c/tNxuHC/UGgy) with a minor allele frequency cutoff of 0.05. To calculate Tajima’s D [[25]](https://paperpile.com/c/tNxuHC/LJI7), we used all sequenced sites that passed the quality and paralog filters without removing non-paralogous sites or those with low minor allele frequencies. For each of these statistics, the Eastern and Western North American samples were pooled together.

In order to describe basic population structure, we created a neighbor-joining tree [[26]](https://paperpile.com/c/tNxuHC/29Jm) using the ape R package v.5.0 [[27]](https://paperpile.com/c/tNxuHC/jHGn). In order to maximize the amount of genetic data contributing to this tree, the input distance matrix was created using the Identity-by-State approach in ANGSD with the same parameters as above, save for a minor allele frequency cutoff of 0.05 [[22]](https://paperpile.com/c/tNxuHC/hYyB). A Principal Component Analysis (PCA) was also conducted using this dataset. For comparison, NGSadmix was used to generate individual ancestry coefficients for each individual for between 1 and 9 putative population clusters (k) [[28]](https://paperpile.com/c/tNxuHC/cT57). Each value of k was run 10 times, and the results were collapsed into consensus plots using CLUMPP [[29]](https://paperpile.com/c/tNxuHC/ytKX). The pophelper [[30]](https://paperpile.com/c/tNxuHC/nkwt) and snpR (Hemstrom et al. *in prep*) R packages were used to simplify these analyses. No individuals were removed for this analysis.

To quantify the direction and strength of population spread across the Pacific, we calculated the directionality index (***ψ***) [[31]](https://paperpile.com/c/tNxuHC/k8vx) for each pairwise combination of North America, Hawaii, Queensland, Guam, Rota, Norfolk Island populations using the snpR package (Hemstrom et al *in prep*). We created the polarized site-frequency spectra used in these calculations using the δaδi (dadi) [[32]](https://paperpile.com/c/tNxuHC/8M6p) dataset described below by projecting populations down to ten gene copies each using the methods described by ref. [[32]](https://paperpile.com/c/tNxuHC/8M6p) as implemented in snpR (Hemstrom et al *in prep*). Using 10 gene copies tended to produce the highest number of maintained SNPs in the resulting spectra. The SNPs were polarized via reference to whole genome sequence data of the best-sequenced individual of the monarch sister taxon *Danaus erippus* [[9]](https://paperpile.com/c/tNxuHC/wGjC) by alignment to the monarch genome as described above.

In order to determine the effect of migration on population connectivity, we looked for evidence of isolation by distance (IBD) between samples from the Mariana Islands, North America, Australia, and Hawaii. To do so, we first calculated Edwards’ [X] angular genetic distance between each pair of samples from the given populations. We then compared these distances to the geographic distances between samples using a Mantel Test [X].

*Demographic history of the monarch’s expansion*

To describe the patterns of establishment and migration between North America and the Pacific, the demographic reconstruction program dadi [[32]](https://paperpile.com/c/tNxuHC/8M6p) was used to estimate the demographic history of the North American and Hawaiian samples. In order to reduce potential bias due to linkage, filtered SNPs were randomly subsampled such that no SNP was within 10,000bp of any other SNP. The resulting 11,384 SNPs were then projected down to a sample size of 100 gene copies from North America and 10 from Hawaii, resulting in 9370 total SNPs. These projection numbers were picked to maximize the remaining number of SNPs in the dataset.

We fit a range of possible models to the observed data: (1) each of the “Island Model” set described in the dadi\_pipeline [34], which contains some models originally published in ref.(2017) [[33]](https://paperpile.com/c/tNxuHC/QaAW); (2) variations on these models with logistic rather than exponential growth functions; (3) the model described by ref. [[9]](https://paperpile.com/c/tNxuHC/wGjC) for the same comparison; (4) a similar model that allowed for an additional period of growth prior to the establishment of the Hawaiian population and another following establishment. The latter model is shown in greater detail in Figure S1. Each of the dadi\_pipeline models and their logistic growth versions were run three times: once with growth allowed in the founding population post-split, once with growth allowed in the founded population post-split, and once with growth allowed in both populations post-split. Note that in each of these (dadi\_pipeline) models, a source population splits to form two descendant populations, with an optimized parameter (s) controlling the portion of the population that forms each descendant population. When s is optimized to be very small (as it typically was), the founded population represents only a very small proportion of the ancestral population, as is likely realistic for the founding of the Hawaiian population from the North American population. Graphical depictions of the dadi\_pipeline models are available in ref [34] and [33]. The logistic versions of these models are functionally identical, albeit with population growth modeled using a standard logistic growth curve rather than an exponential growth curve.

To optimize the models we fit during the analysis, we used a variation on dadi\_pipeline, the sequential step-down parameter permutation approach described by Portik et al. (2017) [33]. Unlike this method however, we set the starting parameters for each sequential run via weighting the parameters from each run in the previous iteration by the relative AIC score of that iteration, such that all but the worst runs contribute in some degree to the starting parameters for the next step. The number of runs and iterations per step are listed in Table SX. Individual optimization runs were killed if they took longer than 48hrs to complete, since these runs tended to take far longer to finish and often included integration errors due to extremely small population sizes. Most runs completed in under 48hrs and are included in the results.

To extract meaningful parameter units from the results, we assumed 0.3 years per generation and used the per-base mutation rate of 8.4x10-9­ reported from Drosophila melanogaster [2]. These values match those used by Zhan et al. (2014) [3]. Using a potentially more realistic generation time of 7 generations per year resulted in more recent divergence times, and using the slower mutation rate reported for the more closely related Heliconius melpomene of 2.9x10-9 [4] resulted in larger effective size estimates and more distant divergence times, for a net result of slightly more distant divergence times and larger effective sizes. Overall, the results did not differ qualitatively to any substantial degree. In order to determine the length of the considered genomic region, we multiplied the total number of bases sequenced after quality filtering (but not SNP p-value filtering so as to count non-polymorphic sites) by the ratio of SNPs in the final allele frequency spectrum to the total number of called SNPs.

Among the large set of possible demographic models, the simple *found and grow* scenario (Figure S1a), which had a constant ancestral population size in North America, Hawaii colonization, and then population growth in both sites, produced the lowest AIC scores on the final pass of the pipeline. However, the new *three epoch found and grow* model (hereafter *three epoch*), which involved multiple rounds of demographic expansion in the ancestral North American population, followed by colonization and growth in Hawaii, had the lowest possible AIC score across all passes of the pipeline (Figure S2). The *three epoch* model is a more complex version of the model specified in ref. [[9]](https://paperpile.com/c/tNxuHC/wGjC). We report in detail the results of both the *found and grow* and *three epoch* models and, where relevant, highlight discrepancies in the inferences that they produce.

**Results**

*Sequencing results*

After paralog filtering, we were able to genotype 2,159,978 sites in at least 50% of individuals. 541,899 of these sites were polymorphic, and 71,157 had a minor allele frequency above 0.05. After removing individuals sequenced at less than 75% of called genotypes, 413,271 and 70,878 sites remained in each category, respectively. The number of samples from each population after filtering can be found in Table 1.

*Question 1: Overall patterns of relatedness*

Principal component analysis separated North American, Hawaiian, Mariana Islands, and South West Pacific samples along two axis of expansion (Figure 1a). We found decreasing relatedness with the ancestral North American population with increasing distance, as indicated by directionality index (***ψ***) scores (Figure S3). Genetic diversity (π, HO, and Het/Hom), was highest in the ancestral North American populations, followed by Hawaii, Australia, and then the remaining Pacific Island populations (Table 1, Figure S4). Likewise, Tajima’s D is positive in all sites besides North America and Hawaii (Table 1). Fst and NGS results both reflect the patterns we observed in the PCA (Table S2, Figure 1b). IBD patterns were strongest between the Mariana island samples (p = 0.001), were present but not significant in Hawaii and Australia (p = 0.086 and 0.0766, respectively), and were absent in North America (p = 0.489).

*Question 2: Patterns of differentiation within expansion populations*

Samples from the Mariana Islands (especially the well-sampled Guam and Rota populations) appear to form highly distinct populations, despite their close physical proximity (Figure 1a-c). By contrast, populations within Hawaii (Maui and Oahu) and Australia (Queensland, New South Wales, and Victoria) do not show strong patterns of differentiation (Figure 1a-c). Norfolk Island, the other previously unsampled population in our dataset, groups closely with samples from Australia and New Zealand (Figure 1a-c).

*Question 3: Timing of establishment and patterns of ongoing gene flow*

Our two best-performing demographic models (f*ound and grow, three epoch*) gave highly variable estimates of establishment timing and founding populations size, with the *three epoch* model generally producing much broader estimates for these parameters. For example, the simpler *found and grow* models were consistent in indicating a founding time of approximately 105 years ago, while the *three epoch* models suggested establishment times that ranged between approximately 102 to 105 years ago (Fig. 4a, 4b). Similarly, the found and grow models were more consistent in predicting a large founding population of >105 individuals, while the *three epoch* models suggested a broader founding population size of between 10 and 105 individuals (Figure 2a, 2b). These models also differed in their estimates of the *Ne* for the Hawaiian population, with the found and grow model suggesting a large *Ne* of around 106 and the *three epoch* models generally producing estimates of Hawaiian *Ne* between 102 and 105 (Fig. 4c, 4d).

For other parameters, the *found and grow* and *three epoch and grow* models generated similar estimates. Both models suggest very low levels of contemporary migration between North America and Hawaii, with the *found and grow* models converging near 0 for both directions and the *three epoch and grow* models generally suggesting migration rates of < 2.5 x 10-5 individuals per generation (Fig. 2e, 2f). This finding accords with our intuition that trans-oceanic dispersal events in monarchs should be rare events.

**Discussion**

Our analysis suggests a recent natural range expansion characterized by serial stepwise dispersal across the Pacific in monarchs. We find evidence for two independent expansions upon establishment in Hawaii, with a previously uncharacterized westward expansion from Hawaii into the Mariana Islands. Our overall results provide a higher resolution picture of the monarch’s pattern of establishment in the Pacific and show that historical estimates of introduction timing overlap with demographic reconstructions.

Summary statistics are consistent with a scenario of directional dispersal from North America to Hawaii, from Hawaii to Guam, and from Hawaii to Australia. This pattern is reflected in both the positive directionality index measures (0.07, 0.08, and 0.05, respectively) and other summary statistics [[31]](https://paperpile.com/c/tNxuHC/k8vx). Interestingly, monarch populations in Hawaii and Australia seem to maintain relatively high levels of genetic diversity, despite the apparent bottlenecks associated with establishment. This is especially striking in the Australian population, which was itself likely founded by individuals from a much smaller population in New Caledonia [[35]](https://paperpile.com/c/tNxuHC/uX4j). The retention of genetic diversity in Hawaii and Australia may reflect rapid population growth upon establishment, which could temper the loss of allelic diversity that might be predicted with a bottleneck event.

Monarchs in the Mariana Islands represent a distinct expansion event within the Pacific. Within the Mariana Islands, there was a strong pattern of differentiation between islands, especially between the nearby islands of Guam and Rota. This pattern is striking because of their close geographic proximity: these islands are separated by only 40 km of open ocean. By contrast, our samples from North America, despite coming from overwintering sites nearly 2,000 km apart, formed a single panmictic population. This result is consistent with other population genetic analyses of eastern and western North American monarchs [[9,36](https://paperpile.com/c/tNxuHC/F2co)-[39](https://paperpile.com/c/tNxuHC/1s8F)], although it provides the strongest evidence to date that North American monarchs form a single genetically panmictic population.

The strong population genetic differentiation within the Mariana Islands but not at the scale of the entire North American continent highlights both (1) the pervasive role that long-distance migration in North America plays in collapsing any patterns of population structure that might otherwise develop, and (2) the fact that many Pacific monarch populations likely have extremely small effective population sizes that are susceptible to very strong genetic drift. Panmixia over large spatial scales is common in other long-distance migratory taxa including bats [[40]](https://paperpile.com/c/tNxuHC/n711), birds [[41]](https://paperpile.com/c/tNxuHC/mSQA), and eels [[42]](https://paperpile.com/c/tNxuHC/wKr3), though monarchs provide a unique opportunity to compare patterns of population structure across both migratory and non-migratory populations.

In contrast to populations within the Mariana Islands, samples from Hawaiian and Australian monarchs do not provide indications of fine-scale population genetic differentiation that might be expected in non-migratory populations. Within Hawaii, our samples from Maui and Oahu formed a single genetic cluster, consistent with the results of ref. [[17]](https://paperpile.com/c/tNxuHC/XTdq). Likewise, Australian samples from New South Wales and Victoria grouped with samples from Queensland. This result differs somewhat from the results of ref. [[16]](https://paperpile.com/c/tNxuHC/lH1o), who reported considerable among-site genetic variation within Queensland, but is consistent with similar later work [[43]](https://paperpile.com/c/tNxuHC/dse6).

For Hawaiian monarchs, it is not immediately clear why the islands of Maui and Oahu do not form separate populations. One possibility is that prevailing winds promote gene flow between islands and that the orientation axis of the Hawaiian islands relative to prevailing wind direction differs from that seen in the Mariana Islands. Pacific monarchs are likely moved by wind patterns, and it has been suggested that tropical cyclones may have led to the monarch’s establish in Australia [[35]](https://paperpile.com/c/tNxuHC/uX4j). In the case of Australian monarchs, one possibility for the lack of strong differentiation across the continent is that Australian monarchs may exhibit seasonal migration patterns akin to those seen in North American monarchs [[44](https://paperpile.com/c/tNxuHC/Ykeg),[45]](https://paperpile.com/c/tNxuHC/G8Rn). Australian monarchs retain migration-associated behaviors that further support the notion that they may undergo large-scale seasonal movements [[44](https://paperpile.com/c/tNxuHC/Ykeg),[46](https://paperpile.com/c/tNxuHC/wYOk), Hemstrom et al. *in prep*]. Our population genetic data suggest that the lack of continent-wide population structure seen in migratory North American monarchs may be recapitulated in Australia.

Interpreting the results of our demographic models is somewhat more complicated than interpreting basic patterns of relatedness among populations. This is due to the conflicting inferences provided by the two best-performing model structures and the wide range of parameter estimates in the *three epoch* models. Although we present the results of both the simpler *found and grow* and the more complicated *three epoch* models, we are inclined to place more confidence in the estimates produced by the *three epoch* models for two reasons: (1) the demographic scenario that it specifies—recent demographic expansion in the ancestral North American population prior to geographic expansion—has empirical support from other studies [[9](https://paperpile.com/c/tNxuHC/wGjC),[10]](https://paperpile.com/c/tNxuHC/79SP) and accords with our understanding of past changes in climate, and (2) this model structure produces parameter estimates that match our prior understanding for how and when monarch range expansion may have occurred. The latter point is related to the former: because a North American population expansion is not allowed until after the founding of Hawaii in the *found and grow* model, this model forces an ancient founding of the Hawaiian population in order to allow for the ancient growth of the North American population. As such, we focus our discussion on the estimates produced by the *three epoch* model.

In general, our demographic results do not exclude a recent founding of the Hawaiian population by North American monarchs (Fig 4d). While our model optimizations span several orders of magnitude for this parameter, many of the iterations settled on introduction estimates of less than 200 years ago. Since the earliest historical records of monarchs on Hawaii date to roughly 200 years ago (1841), we are inclined to accept the results of these iterations over those with longer estimated divergence times. Other lines of evidence supporting recent (<200 years) Hawaiian establishment include: (1) the lack of noticeable phenotypic differentiation between North American and Pacific Island monarchs, especially relative to the pronounced phenotypic differences in non-migratory populations from the Caribbean and South America, which have historically been treated as separate subspecies [[11]](https://paperpile.com/c/tNxuHC/b7Th), (2) the likely need for human-mediated transport of the monarch’s host plants (some of which are native to subtropical Africa) as a pre-condition of monarch establishment in the Pacific, and (3) recent genomic evidence showing that as little as 20 years of captive breeding (A. Tenger Trolander, *pers. comm.*) is sufficient to generate patterns of genetic divergence comparable to those observed between North American and Pacific populations [[47]](https://paperpile.com/c/tNxuHC/94mb). Interestingly, our re-implementation of the model used by ref. [[9]](https://paperpile.com/c/tNxuHC/wGjC) produced results that were similar to theirs, with the vast majority of model iterations supporting an introduction time of roughly 1000+ years ago (Figure S5). This highlights the need to run a range of possible demographic models when attempting to infer demographic history, since failing to account for underlying complexity in population histories can result in very divergent parameter estimates.

Demographic model results were also variable in their estimates of founding population sizes in Hawaii. Some models produced estimates as high as 10,000 founding individuals, which seems implausible given the incredibly long distance (>3500 km) between North America and Hawaii. The extremely wide range of parameter estimates for founding population size and timing may reflect that, in practice, it is difficult to distinguish between a very recent strong bottleneck versus a more distant but less severe bottleneck. Our model results do provide some support for this, since the model iterations with recent establishments also tended to have smaller establishment population sizes (Figure 2d).

In contrast to variable estimates of establishment timing and founding population size, demographic models were consistent in suggesting very low contemporary migration rates (on the order of 0.0001 individuals per generation from North America to Hawaii and vice versa). Our results thus contrast with those of ref. [[15]](https://paperpile.com/c/tNxuHC/upvO), whose methods suggested much higher migration rates (nearly 10 individuals/generations) for both North America to Hawaii and vice versa. We are more confident in our results due to (1) the much larger number of sampled loci, (2) the more realistic demographic model that we used in our analysis, and (3) the absence of modern records of regular North America to Hawaii establishment events.

In conclusion, we have shown that monarchs colonized the Pacific as part of a single, recent out-of-North America expansion event, with at least two subsequent expansions out of Hawaii. Furthermore, we show that the loss of migration coupled with strong genetic drift can generate strong patterns of differentiation between monarch populations at the scale of islands within an archipelago, as seen in comparisons between the islands of Guam and Rota. This is in stark contrast to the continent-wide panmixia that is maintained by seasonal migration within North America, and suggests that future studies must sample at both fine and coarse spatial scales if they hope to recover accurate population structures. Finally, understanding the magnitude of genomic, phenotypic, and ecological differentiation between migratory North American monarchs and populations in outlying U.S. states/territories (including Hawaii, American Samoa, and the Mariana Islands) has important conservation implications as the U.S. Fish and Wildlife Service considers the concept of adaptive capacity in a petition to list the monarch under the Endangered Species Act [[48]](https://paperpile.com/c/tNxuHC/GENO).

**Data Accessibility**

All scripts used for analysis are available at <https://github.com/hemstrow/F-H_2018>. Sequence data will be made available through NCBI upon publication.

**Author Contributions**

WBH, MGF, and MRM designed the research. MGF and MPZ provided samples used for sequencing. WBH and MGF performed data analysis. All authors contributed to writing and editing the manuscript.

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**Competing Interests**

The authors declare no competing interests.

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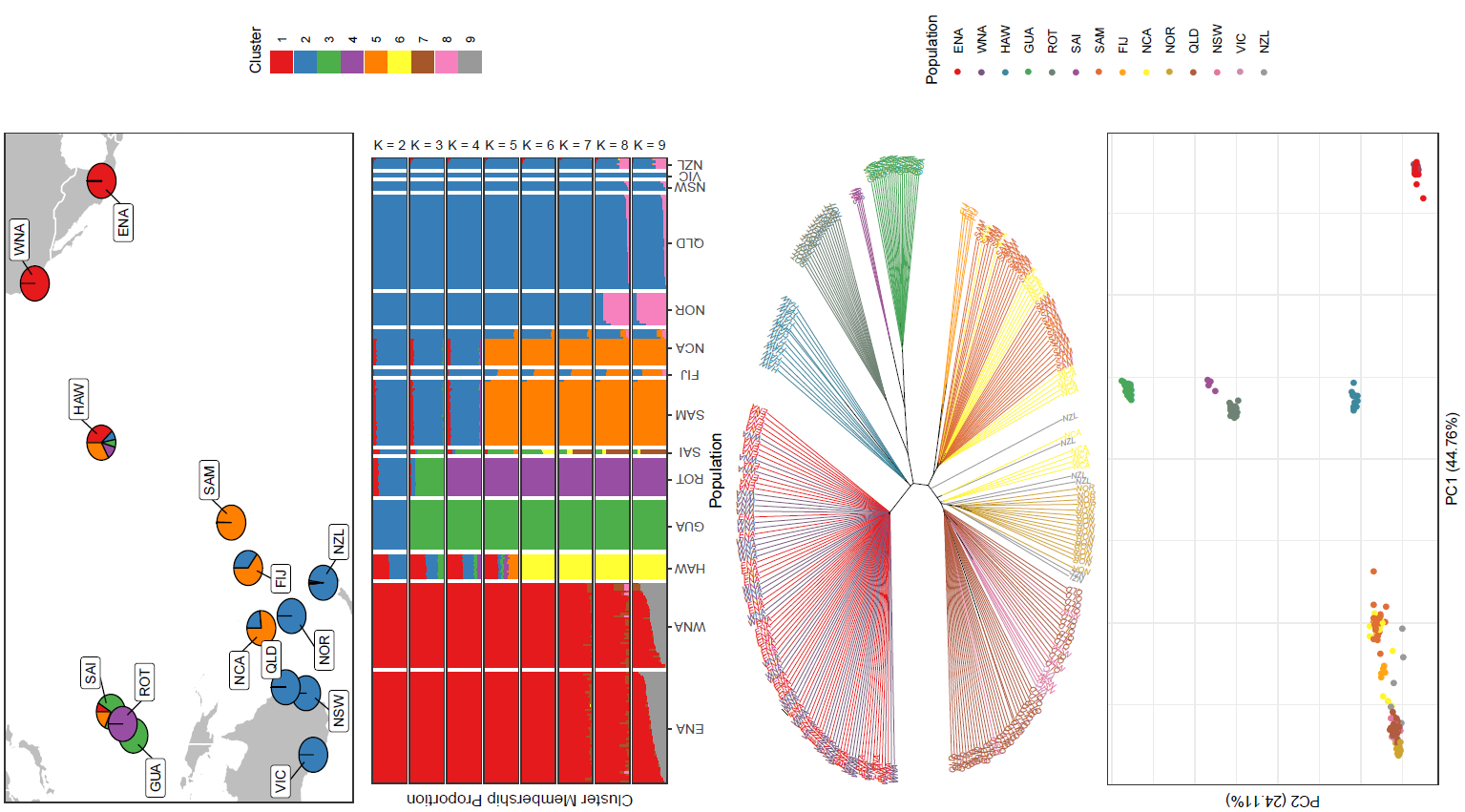
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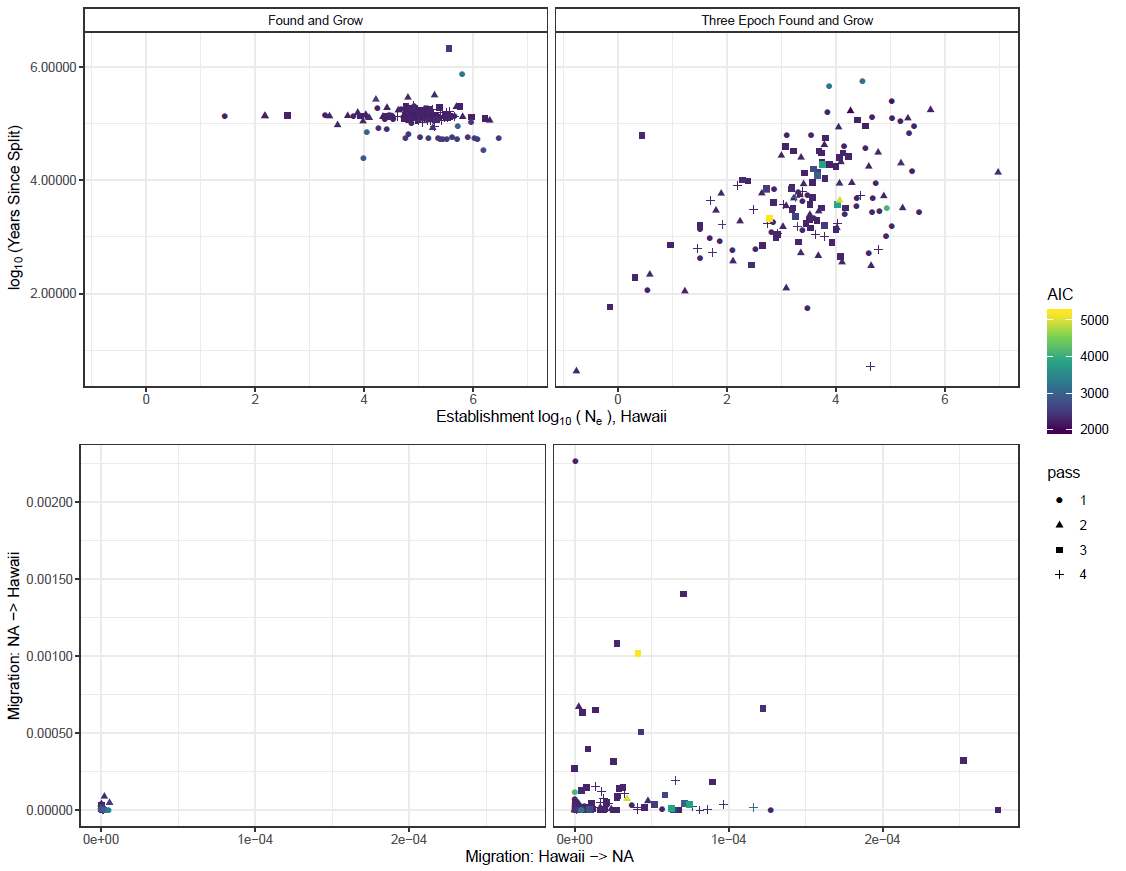
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|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Population** | **# Samples** | **Tajima's D** | **H­O** | **π** | **Het/Hom** |
| **NAM** | 83 | -1.92 | 0.055 | 0.064 | 0.059 |
| **HAW** | 9 | -0.211 | 0.048 | 0.055 | 0.051 |
| **GUA** | 19 | 0.091 | 0.031 | 0.032 | 0.032 |
| **ROT** | 16 | 0.388 | 0.035 | 0.038 | 0.037 |
| **SAI** | 4 | 0.326 | 0.022 | 0.025 | 0.023 |
| **QLD** | 15 | 0.349 | 0.042 | 0.044 | 0.043 |
| **NSW** | 5 | 0.433 | 0.038 | 0.042 | 0.039 |
| **VIC** | 2 | 0.899 | 0.041 | 0.043 | 0.042 |

**Table 1 -** Number of samples remaining after filtering, Tajima’s D, Observed Heterozygosity (HO), nucleotide diversity (π), and the average ratio of Heterozygous to Homozygous sites across all individuals in each population.

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**Figure 1 –** Relatedness among sampled populations. Top to bottom: (a) Map of sampled populations, with pie charts reflecting results from NGSadmix. (b) NGSadmix plots showing the proportion of ancestry across clustering values between k = 2 and k = 9. At k = 5, Hawaii reflects a mixture of ancestry comprising North American, Mariana Islands, and southwestern Pacific samples. At k = 6, Hawaii becomes its own cluster. At values beyond k = 6, populations are subdivided. (c) Neighbor joining tree. (d) Principal component analysis largely recapitulates the geographical distribution of samples, with PC1 explaining 44.7% of variation and corresponding to the east-west axis of differentiation. Abbreviations in labels are as follows: ENA = Eastern North America, WNA = Western North America, HAW = Hawaii, GUA = Guam, ROT = Rota, SAI = Saipan, SAM = Samoa, FIJ = Fiji, NCA = New Caledonia, NOR = Norfolk Island, QLD = Queensland, NSW = New South Wales, VIC = Victoria, NZL = New Zealand.



**Figure 2 -** Results of the dadi optimization runs for the “Found and Grow” (Left) and “three epoch” (Right) demographic models. (a) Hawaii establishment effective size and years since establishment. (b) Current effective size estimates in North America and Hawaii. (c) Migration rates from North America to Hawaii and vice versa.