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Migration genetics take flight: genetic and genomic insights into monarch butterfly migration

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Monarch butterflies have emerged as a model system in migration genetics. Despite inherent challenges associated with studying the integrative phenotypes that characterize migration, recent research has highlighted genes and transcriptional networks underlying aspects of the monarch's migratory syndrome. Circadian clock genes and the vitamin A synthesis pathway regulate reproductive diapause initiation, while diapause termination appears to involve calcium and insulin signaling. Comparative approaches have highlighted genes that distinguish migratory and nonmigratory monarch populations, as well as genes associated with natural variation in propensity to initiate diapause. Population genetic techniques demonstrate that seasonal migration can collapse patterns of spatial structure at continental scales, whereas loss of migration can drive differentiation between even nearby populations. Finally, population genetics can be applied to reconstruct the monarch's evolutionary history and search for contemporary demographic changes, which can provide relevant context for understanding recently observed declines in overwintering North American monarch numbers.

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Monarch butterflies: a model system for studying migration genetics

Migration has evolved across the tree of life as an adaptation to seasonally variable environments and resource availability [9]. Despite its ubiquity and its importance for ecosystem functioning [26], patterns of disease transmission [1], and nutrient fluxes [19], animal migration remains relatively poorly understood from a genetic perspective. The monarch butterfly, *Danaus plexippus*, is a promising model system for advancing the study of migration genetics: unlike many other taxa that migrate long distances, monarchs are short-lived, amenable to large-scale rearing and experimentation, and have an emerging toolkit of functional genomic approaches available.

Monarch butterflies are perhaps the single best-studied migratory insect, known for their spectacular multigenerational movements within North America and their annual return to high-elevation Mexican overwintering sites. Research into monarch migration has traditionally focused on describing their migratory routes (e.g. [44]), characterizing physiological differences between summer-breeding versus autumn-migratory butterflies (e.g. [14,5,7]), and understanding environmental cues associated with migration initiation (e.g. [4,15]). Over the past 15 years, monarchs have emerged as a model system in ecological genomics. Major advances in the study of monarch migration genetics have included the development of an expressed sequence tag for characterizing gene expression differences between summer-breeding and fall-migratory monarchs [51,52]; the publication of the first monarch reference genome [47] and, more recently, a chromosome-level genome assembly [20]; the development of an extensive toolkit for dissecting the neural basis of the monarch's circadian clock and sun compass navigation system [27,29,50]; a genome-wide comparison of migratory and nonmigratory populations from around the world [48]; and recent studies that have identified genes and regulatory networks associated with reproductive diapause initiation [24] and termination [18].

In this review, we briefly highlight recent research that applies tools from genetics and genomics to gain insights into the biology of monarch migration. We note that another review [31] excellently summarized recent research into the neurogenetic basis of monarch migration. As such, we only briefly discuss the neurobiology of the monarch's sun compass navigation system and instead focus our review on two broad areas: (1) studies that use genomic approaches to identify genes and patterns of gene expression that underpin certain aspects of the monarch's migratory syndrome and (2) studies that use population genetic techniques to study connectivity of

migratory and nonmigratory populations and demographic changes in monarch populations through time.

Genomic approaches to studying monarch migration: linking genotype and phenotype

Migration is a behavior whose genetic basis is notoriously difficult to study, in part because it represents a syndrome of interrelated physiological, morphological, behavioral, and metabolic traits. Monarch migration is likely to have a complex genetic architecture [17,32] and to be subject to both genetic and epigenetic control, which has made the search for genes associated with migration challenging. That said, recent studies have made substantial contributions to our understanding of genes and transcriptional networks associated with components of the migratory syndrome in monarchs (Table 1). These studies fall into two general categories: (1) RNA-sequencing-based approaches that contrast monarchs across environmental contexts to identify migration-associated patterns of gene expression and (2) genome-wide association studies that use natural variation in migratory tendency to identify genetic variants that may be associated with migration.

Transcriptional processes associated with monarch migration

Circadian clock genes have long been implicated in monarch migration, beginning with studies that linked the monarch sun compass-based navigation system to expression of clock genes in the antennae (e.g. [13,30]). Recent studies have solidified this connection [50] while also highlighting a broader role for circadian clock gene expression in photoperiodic responsiveness of monarchs. In preparation for their autumn migration, monarchs enter a period of reproductive dormancy (diapause) characterized by reduced production of juvenile hormone (JH) [23]. Iiams et al. [24] used a combination of RNA sequencing and, crucially, clock gene knockouts (*Clk*, *Cyc-like*, and *Cry2*) to demonstrate that circadian clock expression is required for reproductive diapause initiation. Interestingly, this work also found a novel role for the vitamin A synthesis pathway in monarch photoperiodic responses, which acts independently from visual inputs and may be a deeply conserved feature of organismal responses to seasonal changes in photoperiod. Iiams et al. [24] also showed that JH production appears to act downstream of circadian clock genes, suggesting that JH is not itself a master regulator of migration behavior.

Progress has also been made in elucidating the internal signals responsible for diapause termination in North American overwintering butterflies. Using wild-collected overwintering monarchs from California, Green and Kronforst [18] measured gene expression in monarch heads across a three-month time course that spanned the transition from diapause to reproductive activity. A key finding of this research was that a period of cold exposure is likely involved in initiating a diapause termination timer, potentially mediated through calcium signaling pathways. As with Iiams et al. [24], JH synthesis was proximately involved with reproductive development, but JH itself does not seem to be the ultimate regulator of diapause termination. Green and Kronforst

Table 1

List of genes, transcripts, and/or gene ontology (GO) terms shown to be associated with monarch butterfly migration.

Gene/pathway/GO ID	Associated phenotype(s)	Reference
Retinol dehydrogense 13 (*rdh13*)	Photoperiodic sensitivity/diapause initiation	Iiams et al. [24]
Neither inactivation nor afterpotential B (*ninaB1*)	Photoperiodic sensitivity/diapause initiation	Iiams et al. [24]
Scavenger receptor acting in neural tissues 1 and 2 (*santa maria 1, santa maria 2*)	Photoperiodic sensitivity/diapause initiation	Iiams et al. [24]
Clock (Clk)	Photoperiodic sensitivity/diapause initiation	Iiams et al. [24]
Basic helix–loop–helix ARNT-like 1 (*Bmal1*)	Photoperiodic sensitivity/diapause initiation	Iiams et al. [24]
Cryptochrome 2 (*Cry2*)	Photoperiodic sensitivity/diapause initiation; directional orientation during migration	Iiams et al. [24]; Merlin et al. [30]
JH acid methyltransferase (*jhamt*)	Reproductive activity, diapause termination	Zhu et al. [52]; Green and Kronforst [18]
Krüppel homolog 1 (*Kr-h1*)	Diapause termination	Green and Kronforst [18]
Calcium signaling pathway (GO:0051282, GO:0051283, GO:0051208)	Diapause termination	Green and Kronforst [18]
Collagen type-IV α-1	Migratory versus nonmigratory status, wing muscle development	Zhan et al. [48]
FBXO45	Migratory versus nonmigratory status	Zhan et al. [48]
Insulin-like growth factor 2 (*IGF2*)	Migratory versus nonmigratory status, lipid metabolism	Zhan et al. [48]
E3 ubiquitin–protein ligase Udf4	Diapause initiation	Hemstrom et al. (submitted)
period (*per*)	Directional orientation during migration	Merlin et al. [30]
timeless (*tim*)	Directional orientation during migration	Merlin et al. [30]

Note that this list is not comprehensive and is meant to highlight recent research, as well as genes that are especially promising candidates for being associated with migration.

[18] also suggested that insulin signaling pathways may be an important target of the processes involved in diapause termination. Insulin signaling and its connection to lipid metabolism in monarchs is a potentially fruitful area for future research, especially given the role of insulin signaling in seasonal adaptations in other insect systems [39]. Monarch migration is associated with a pronounced uptick in lipid accumulation [7,14], and insulin-like growth factor 2 is among the genes that most strongly differentiate migratory and resident populations of monarchs [48]; see sections below (Table 1).

While diapause initiation and termination are crucial components of the monarch migratory syndrome, much less is known about the epigenetic basis of other migration-associated traits that differ between summer-breeding and autumn-migratory monarchs. Here, it could be helpful to measure gene expression patterns in monarch tissues outside of the adult monarch head that are likely to play a role in migration (e.g. wing muscles and the fat body). Additionally, characterizing patterns of gene expression in developing larvae and/or pupae exposed to divergent environmental conditions could help to identify the developmental trajectory that distinguishes summer-breeding from autumn-migratory monarchs.

Genetic variation associated with differences in monarch migration

While monarchs are best-known from their migratory range in North America, nonmigratory populations are also established in locations around the world, corresponding to at least three independent losses of migratory behavior (Figure 1). Zhan et al. [48] sequenced whole genomes from 80 monarchs across this global range in order to examine the history of monarch expansion and identify genes associated with migration. This approach identified hundreds of candidate loci associated with migration, most notably a 21-kb region containing an F-box protein (FBXO45) and a collagen type-IV α-1 gene that showed strong evidence for convergent positive selection across nonmigratory populations. This collagen type-IV α-1 gene was more highly expressed in nonmigratory populations and has been associated with wing muscle development in *Drosophila* [38], though because of its critical role in basement membrane formation during early phases of development, functional validation using knockout approaches may not be possible.

An alternative approach to studying migration genetics in monarchs involves leveraging naturally occurring variation in responsiveness to environmental cues, such as declining photoperiod, within monarch populations. Hemstrom [21] performed a family-structured genome-wide association study using monarch butterflies from Australia that differ in their propensity to enter reproductive arrest upon exposure to declining photoperiod, following the approach of Freedman et al. [12]. This research pinpointed a locus on chromosome 11 containing a probable E3 ubiquitin ligase (DPOGS208560) (Hemstrom et al., submitted). Interestingly, DPOGS208560 was not among the genes identified by Zhan et al. [48] as differing between migratory and nonmigratory monarch populations, nor the pathways identified by Iiams et al. [24] as regulating photoperiodic sensitivity. However, the general connection between E3 ubiquitin ligases and F-box proteins (including FBXO45 identified by Zhan et al. [48]) is intriguing given the known role of proteasome complexes in interacting with CRY2 [45,46], which has a known role in monarch photoperiodism.

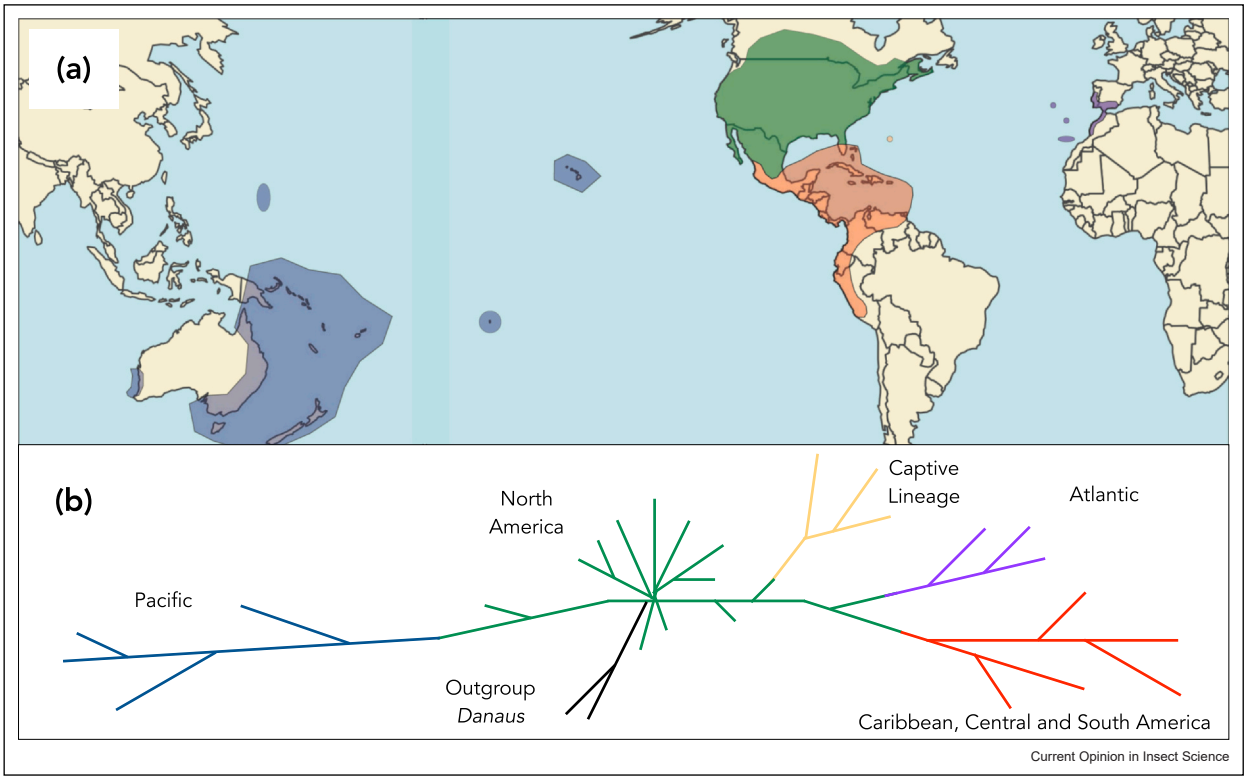
Further research is needed to determine whether traits that differ between migratory and nonmigratory monarchs — including wing morphology [2], resting metabolic rate [48], resistance to the protozoan parasite *Ophryocystis elektroscirrha* [40], and responsiveness to green wavelengths of light [33] — have a clear underlying genetic basis. Here, quantitative trait locus mapping using crosses between migratory and nonmigratory populations with divergent phenotypes could be a promising approach, as previously suggested by Reppert and de Roode [37].

Population genetic approaches to studying monarch migration: global range expansion, population differentiation, and historical changes in population size

Across migratory taxa, population genetic approaches have provided key insights into understanding connectivity and differentiation among populations [28], the importance of migratory divides [8], and the origins of partial migration [16]. Population genetic approaches have also been informative for studying monarchs and placing their migratory behavior into a broader evolutionary context. Recent research has used population genetic approaches to describe the monarch's global range expansion, the role of long-distance migration in mediating spatial population genetic structure, the link between captive breeding and loss of migration, and historical demographic changes in monarch populations over the last 20 000 years.

In contrast to their migratory North American range, where monarchs are genetically panmictic ([41], reviewed in [10]), a number of recent studies have highlighted how range expansion and loss of migration contribute to genetic differentiation in monarch populations around the world. Pierce et al. [36] found that monarchs show evidence for serial stepwise dispersal in each of their three independent out-of-North America expansion events. Hemstrom et al. [22] corroborated the finding of serial

Figure 1



Migratory status across the monarch's global range. **(a)** The global distribution of the monarch butterfly affords opportunities for comparative approaches that contrast the ancestrally migratory North American population (green) with derived nonmigratory populations throughout Central America, South America, and the Caribbean (red), as well as the Pacific (blue) and Atlantic (purple). **(b)** Stylized neighbor-joining tree depicting relationships among global monarch populations, including captive-bred butterflies.
(b) adapted from [42].

stepwise dispersal in Pacific Island monarchs and found that loss of seasonal migration can generate pronounced signatures of isolation by distance even in island populations located less than 40 km apart. By contrast, monarchs sampled across the Australian continent, where they show evidence for long-distance seasonal movement [25], are genetically panmictic, recapitulating the lack of spatial population genetic structure seen in their migratory North American ancestors [22]. Thus, an emerging pattern seems to be that migration in monarchs may collapse any nascent patterns of spatially structured genetic variation, whereas loss of migration can allow for isolation by distance to evolve even over small spatial scales. A similar pattern has been noted in the globally distributed dragonfly *Pantala flavescens* [3].

Loss of migration in monarchs has traditionally been considered in the context of establishment in seasonally stable environments where their milkweed (*Asclepias spp.)* host plants grow year-round [11,48]. However, recent results have highlighted that captive breeding of monarchs, even over relatively short timescales, can have pronounced effects on their ability to respond appropriately to cues associated with initiating autumn migration. Tenger-Trolander et al. [42] showed that a history of captive breeding can disrupt directional orientation abilities and also demonstrated that population genetic divergence between migratory and captive-reared monarchs is remarkably pronounced (i.e. comparable to that seen between North American migrants and nonmigratory populations from other areas of their global range) (Figure 1b). Whether any of the loci that distinguish captive-bred from migratory monarchs have functional connections to directional sun compass orientation system remains to be determined.

Monarchs are the subject of intense conservation attention, in part due to recorded declines in the numbers of overwintering eastern monarchs between 1996 and 2014 [43] and western monarchs between 1996 and 2020 [34]. A potential application of population genomic methods to inform monarch conservation is the use of demographic reconstructions to understand past changes in population size. These approaches are informative because they can

provide broader evolutionary context for understanding recently observed declines. Previous work has shown that monarchs likely underwent a pronounced demographic expansion coinciding with the end of the Last Glacial Maximum [35,48], approximately 10–20 thousand years ago. Other demographic modeling efforts provide modest but inconclusive support for a more recent demographic expansion over the past 250 years for monarchs, coinciding with a clear demographic expansion in the monarch's primary North American host plant, common milkweed (*Asclepias syriaca*), over the same time frame [6].

Forthcoming research has used sequencing of monarch genomes from specimens collected in 1977 and contemporary specimens to compare patterns of genetic diversity (Talla, Mehta and de Roode, in revision). The predominant signal in these data is, as in previous studies, strong evidence for postglacial demographic expansion; however, based on simulations, the absence of a decline in genetic diversity between monarch samples from 1977 and contemporary samples suggests that population declines over that period do not exceed 60% (Talla, Mehta and de Roode, in revision). Further sequencing of historical samples, potentially including ethanol-preserved monarch caterpillars in natural history collections, could help to resolve questions about the magnitude of change in monarch population size over the past century. Likewise, future sampling could help to establish the degree to which mostly non-migratory monarchs in locations such as the U.S. Gulf Coast, coastal California, and Florida are genetically distinct from North American migrants, which has important implications for how these resident populations are considered in conservation decision-making processes.

Conclusions

In spite of the inherent challenges associated with studying migration genetics, substantial progress has been made in describing the genetic basis of certain features of monarch migration. Our understanding of some aspects of the migratory syndrome is relatively thorough; for example, the genomic bases of reproductive diapause initiation and termination have now been fairly well-characterized, as has the neurogenetic basis of sun compass orientation. Future work that illuminates the genomic basis of other aspects of migration, particularly lipid metabolism and wing morphological variation, would represent a substantial advance. Finally, although there are unlikely to be simple proximate controls governing the transition from summer breeding into an autumn-migratory state, research into the monarch epigenome — including the role of histone modification (e.g. [49]) and micro-RNAs in governing transcriptional processes associated with migration — is a promising avenue for future research.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

None.

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