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Integrative Genomics of Migration, Defense, and Host-Plant Chemistry of the Monarch Butterfly

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Abstract The monarch butterfly (*Danaus plexippus*) represents an unparalleled model for studying the genetic, physiological, and ecological bases of complex adaptive traits. Its multigenerational migration, spanning up to 4,000 km across North America, and its specialized larval dependence on toxic milkweeds (*Asclepias* spp.) exemplify coevolutionary and life-history complexity. Recent advances in genomic and molecular biology have transformed monarch research from natural history to a deeply integrative science. Chromosome-scale genome assemblies, long-read sequencing, and transcriptomic profiling now reveal the genetic architecture underlying migration, diapause, chemical defense, and wing patterning. Functional tools such as RNAi, TALENs, and CRISPR/Cas9 enable causal tests linking candidate genes to behavior and physiology. Population-genomic and selection-scan studies identify polygenic bases for migratory versus resident phenotypes, as well as adaptive divergence related to host-plant chemistry and parasite resistance. Complementary metabolomic analyses elucidate how monarchs sequester, detoxify, and biochemically transform milkweed cardenolides, providing a mechanistic bridge between genotype and ecological function. Emerging integrative frameworks-combining genomics, neurobiology, metabolomics, and ecology-are uncovering how genetic and regulatory networks mediate interactions among monarchs, milkweeds, parasites, and environmental stressors. Future research integrating single-cell neurogenomics, pan-genome analyses, and eco-genomic experiments promises to clarify how these traits evolve and persist amid rapid environmental change. By connecting molecular mechanisms to ecological outcomes, monarch genomics now provides not only a foundation for understanding adaptation and coevolution, but also actionable insights for conserving one of the world’s most iconic migratory insects.

Keywords Conservation genomics; *Danaus plexippus*; Defense chemistry; Metabolomics; Plant-insect interactions

1 Introduction

The monarch butterfly (*Danaus plexippus*) has long captured both scientific and public attention as one of the most charismatic insect species. Its extraordinary, continent-scale migration across North America is unique among butterflies, spanning up to 4,000 km and involves multiple successive generations that complete different stages of the annual cycle (Brower, 1995). This migration is tightly coupled to reproductive diapause and seasonal timing, forming one of the most complex life-history strategies known in insects (Agrawal et al., 2014; 2021; 2025). Equally striking is the monarch’s specialized larval dependence on milkweeds (*Asclepias* spp.), which provide not only nutrition but also chemical protection. Monarch caterpillars ingest toxic cardenolides and sequester them through metamorphosis, rendering both larvae and adults unpalatable to vertebrate predators - a classical example of coevolutionary adaptation and aposematism (Agrawal et al., 2012; 2024; 2025).

For much of the twentieth century, research on monarchs was rooted in natural history, ecology, and physiology - from field studies on migratory navigation (Brower, 1995) and overwintering colonies in Mexico (Rendón-Salinas et al., 2023) to biochemical investigations of cardenolide sequestration (Agrawal et al., 2021; 2024). These ecological and behavioral foundations laid the groundwork for monarchs to emerge as a model system in evolutionary biology (Brower, 1995; Oberhauser et al., 2015). The genomic era began with the publication of the first monarch draft genome (Zhan et al., 2011), which provided a platform for identifying genes and regulatory networks underlying migration, chemical defense, wing patterning, and reproductive physiology. Since then, monarch research has become increasingly integrative, bridging genomics, neuroscience, chemical ecology, and conservation biology.

Advances in genomic and molecular tools have transformed monarch research, enabling detailed investigation of the genetic and regulatory bases of complex traits (Li et al., 2025). High-quality reference genomes, long-read sequencing, and chromosome-scale assemblies now provide near-complete maps of coding and non-coding regions, structural variants, and neo-sex chromosomes (Zhan et al., 2011; Mongue et al., 2017; Zhan et al., 2020). Transcriptomic approaches, including tissue-specific and developmental-stage RNA sequencing, reveal dynamic gene expression patterns underlying migration, diapause, and chemical defense. Functional genomics techniques such as RNA interference, TALENs, and CRISPR/Cas9 genome editing have made it possible to experimentally validate candidate genes and regulatory elements, linking genetic variation to phenotypic outcomes (Markert et al., 2016; Zhang and Reed, 2016). Complementary population genomic analyses, leveraging resequencing, Genome Wide Association Studies (GWAS; Uffelmann et al., 2021), and selection scans, are uncovering polygenic architectures for migratory behavior, adaptation to host-plant chemistry, and responses to parasites. Together, these tools provide a comprehensive framework for connecting genotype to phenotype, enabling integrative studies that span molecular mechanisms, ecological interactions, and conservation applications (Li et al., 2025).

In this review, we synthesize current knowledge of monarch ecology, genetics, and metabolomics (Figure 1) across seven major domains: (1) the development of genomic resources and functional tools, (2) the genetic basis of migration and seasonal behavior, (3) molecular evolution of chemical defense and host-plant specialization, (4) sex-chromosome and structural genome evolution, (5) eco-genomic interactions involving parasites, microbiota, and host plants, (6) population genomic signals that inform conservation, and (7) metabolomic perspectives on host-plant chemistry and monarch sequestration. We also highlight methodological frontiers - from long-read pan-genomics to CRISPR-based functional validation, single-cell genomics, and integrated metabolomics - that are poised to resolve outstanding questions about how the monarch's remarkable life history is encoded in its genome and shaped by ecological interactions. Overall, this review aims not only to synthesize recent advances in monarch genomics and chemical ecology, but also to identify conceptual and methodological gaps that must be addressed to link molecular mechanisms to ecological function and conservation outcomes.

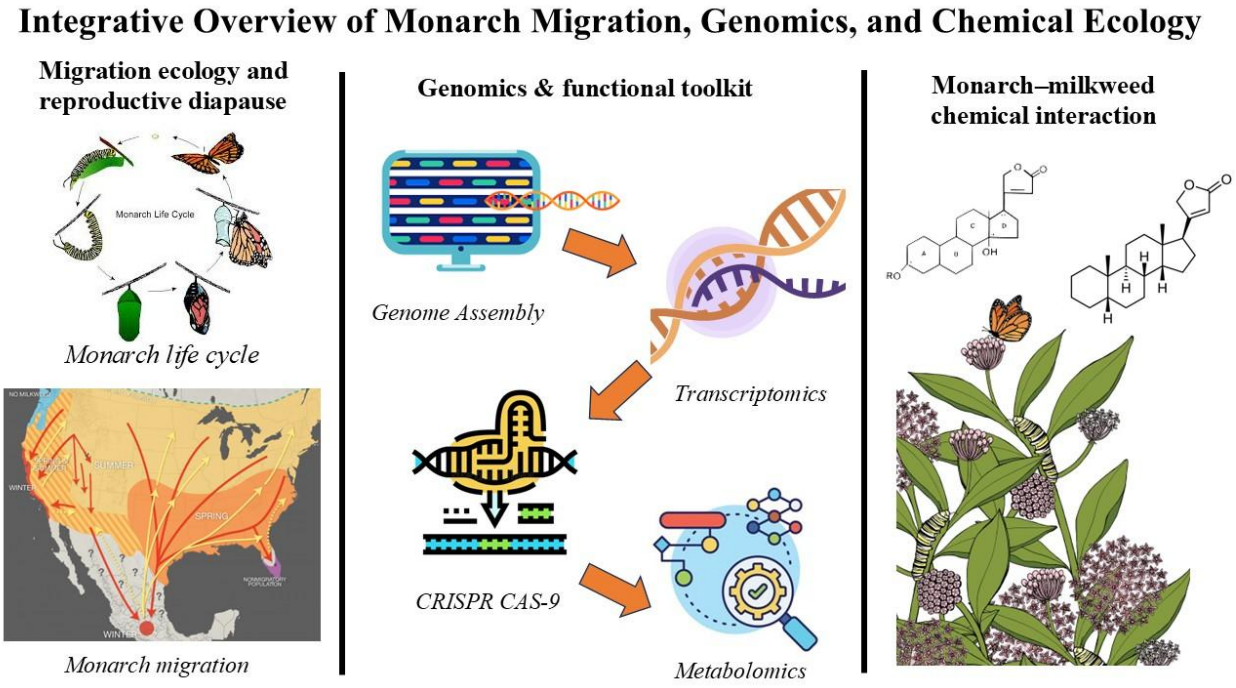

Figure 1 Conceptual model integrating *D. plexippus* migration ecology, genomics, and plant-insect chemical interactions

2 Genomic Resources and Technical Toolkit

2.1 Reference genomes, assemblies, and databases

The initial monarch genome assembly (~273 Mb, ~16 866 predicted protein-coding genes; Table 1) provided a foundational resource for trait mapping and comparative genomics in Lepidoptera (Zhan et al., 2011). Subsequent

chromosome-level assemblies, improved annotations, and curated community databases have substantially expanded the utility of monarch genomics for population, functional, and evolutionary analyses (MonarchBase team, 2012; Mongue et al., 2017). These resources have enabled identification of candidate loci underlying migration, circadian regulation, detoxification, pigmentation, and host-plant interactions, as well as comparative analyses of sex chromosome evolution within *Danaus*.

Recent high-quality assemblies have further resolved structural variants, repetitive regions, and the neo-Z chromosome, providing insight into genomic features that may contribute to adaptation and phenotypic divergence among migratory and non-migratory populations (Table 1). Collectively, these monarch-specific genomic resources now support both hypothesis-driven functional studies and broad-scale evolutionary inference.

Table 1 Genomic and functional resources for *D. plexippus* research

Resource type	Description	Year(s)	Applications	Key references
Genome assemblies (draft → chromosome-scale)	Initial draft genome (~273 Mb) followed by improved, chromosome-scale assemblies using long-read sequencing and Hi-C scaffolding; includes annotation of coding genes, repeats, structural variants, and neo-Z chromosome	2011-2020	Trait mapping, comparative genomics, population genomics, sex-chromosome evolution, structural variant discovery	Zhan et al., 2011; Mongue et al., 2017; Zhan et al., 2020
MonarchBase and genomic databases	Community-curated genome browser and annotation resource integrating gene models, transcriptomes, and functional annotations; linked to NCBI and other repositories	2012-present	Gene discovery, annotation refinement, comparative analyses, education and outreach	MonarchBase Team, 2012; Zhan et al., 2011
RNA-seq atlases (tissues, developmental stages)	Bulk RNA-seq from antennae, brain, fat body, flight muscle, wing discs, larvae, pupae, and adults across migratory and reproductive states	2009-present	Gene expression profiling, circadian biology, diapause regulation, migration physiology, developmental genetics	Merlin et al., 2009; Zhan et al., 2011; de Roode et al., 2011
CRISPR/Cas9 and TALEN applications	Targeted genome editing to disrupt or modify candidate genes; functional validation of regulatory and coding loci in monarchs and related Lepidoptera	2016-present	Causal tests of gene function (navigation, pigmentation, circadian clocks), regulatory element validation	Markert et al., 2016; Zhang and Reed, 2016
Metabolomic datasets (milkweeds and monarchs)	LC-MS/MS and untargeted metabolomics of milkweed secondary metabolites and monarch tissues; quantification of cardenolide diversity, sequestration, and biotransformation	2013-present	Chemical ecology, host-plant adaptation, parasite resistance, eco-genomic integration	Petschenka et al., 2013; Dreisbach et al., 2023; Agrawal et al., 2025
OE parasite genomic resources	Genomic and transcriptomic resources for *Ophryocystis elektroscirrha*, a specialist protozoan parasite of monarchs	2015-present	Host-parasite coevolution, disease ecology, immunity and chemical defense interactions	de Roode et al., 2008; Satterfield et al., 2015

2.2 Functional genomics, genome editing, and multi-omic tools

Functional genomic studies in monarchs have leveraged transcriptomic, proteomic, and metabolomic data to link genetic variation with key migratory, physiological, and defensive traits (Table 1). For example, tissue-specific transcriptomic analyses of antennae revealed circadian clock gene expression patterns essential for time-compensated sun-compass navigation (Merlin et al., 2009), while expression profiling of fat body and flight muscle tissues clarified metabolic shifts associated with long-distance migration and lipid utilization (de Roode et al., 2011).

Monarch research has also been at the forefront of integrating chemical ecology with genomics. Metabolomic analyses of larvae and adults have quantified cardenolide sequestration and detoxification, revealing how

host-plant chemistry interacts with monarch genotype to shape toxin resistance and performance (Agrawal et al., 2012; Petschenka et al., 2013; Dreisbach et al., 2023). Population genomic resequencing across migratory and resident populations has identified loci associated with neural, metabolic, and endocrine function, providing candidates for functional validation (Zhan et al., 2014).

Genome editing approaches, including TALENs and CRISPR/Cas9, have enabled direct tests of gene function in monarchs and related Lepidoptera, allowing researchers to move from association-based inference toward causal understanding of traits such as circadian regulation and pigmentation (Markert et al., 2016; Zhang and Reed, 2016). Together, these monarch-focused functional and multi-omic applications illustrate how established technologies can be combined to address uniquely integrative questions spanning behavior, chemistry, and ecology.

3 Genetic Architecture of Migration and Seasonal Behavior

3.1 Migratory phenotype complexity

Monarch migration is a compound phenotype encompassing sun-compass orientation, reproductive diapause, lipid storage, flight endurance, and seasonal timing (Table 2). Sun-compass orientation depends on antennal circadian clocks and neural integration of solar cues, as demonstrated by experimental disruption of antennal function or clock genes (Merlin et al., 2009; 2020). Reproductive diapause is regulated by photoperiod and hormone signaling (Fleming and Alto, 2006; Green et al., 2019; Freedman et al., 2023), while lipid storage and flight endurance are shaped by metabolic and mitochondrial pathways associated with long-distance flight capacity (de Roode et al., 2011; Zhan et al., 2014). Seasonal timing of migration and breeding integrates environmental cues such as day length and temperature across generations. Collectively, these traits arise from interacting circadian, endocrine, metabolic, neural, and developmental modules, reflecting a modular and polygenic architecture underlying the monarch's migratory phenotype.

3.2 Antennal clocks, sun-compass orientation, and molecular candidates

Peripheral circadian clocks located in the antennae are essential for time-compensated sun-compass navigation, allowing monarchs to adjust orientation as the solar position changes throughout the day (Merlin et al., 2009; Hemstrom et al., 2025). These clocks interact with central brain regions involved in spatial and sensory integration, including the central complex and optic lobes (Merlin et al., 2009; Guerra et al., 2012). Core clock genes (e.g., *period*, *timeless*, *cryptochromes*) coordinate rhythmic gene expression underlying behavioral timing, while transcriptomic analyses implicate broader networks of sensory and metabolic genes contributing to energy allocation and flight performance (Zhan et al., 2011; Zhan and Reppert, 2013). Together, these findings highlight migration as an emergent property of interconnected circadian, sensory, and metabolic pathways rather than a single master regulator.

3.3 Population genomics: migratory vs. resident populations

Comparative genomic analyses of migratory and resident monarch populations reveal modest but consistent differentiation at loci associated with neural signaling, lipid metabolism, and endocrine function, consistent with adaptation to migratory versus sedentary lifestyles (Zhan et al., 2014; de Roode et al., 2013). Divergence at endocrine-related loci involved in juvenile hormone signaling may contribute to differences in reproductive diapause between populations (Hemstrom et al., 2025). Most signals are dispersed across the genome, supporting a largely polygenic architecture for migration, although a small number of loci with larger effect sizes may disproportionately influence key migratory traits (Freedman and Kronforst, 2023).

3.4 Toward causal tests

Advances in genomic mapping, selection experiments, and functional genetic approaches provide promising avenues for testing causal links between genotype and migratory phenotypes. Genome-wide association studies, artificial selection lines, and pedigreed crosses can be combined with CRISPR/Cas9-based validation to interrogate candidate genes involved in circadian regulation, sensory processing, and energy metabolism (Di Cristina et al., 2025). Successful genome editing in monarchs and related Lepidoptera demonstrates the feasibility of moving from genotype-phenotype correlations toward mechanistic understanding of migratory and sensory traits (Markert et al., 2016; Zhang and Reed, 2016).

Table 2 Major genes and molecular pathways leading to diverse *D. plexippus* phenotypes

Category	Resource, gene, or pathway	Trait or biological process	Evidence type	Key references
Genomic resources	Draft and chromosome-scale genome assemblies	Genome organization, migration, chemical defense, sex-chromosome evolution	Comparative genomics, population genomics	Zhan et al., 2011; Mongue et al., 2017; Zhan et al., 2020
Databases	MonarchBase and associated repositories	Gene annotation, transcriptome access	Genome curation, comparative analysis	MonarchBase Team, 2012
Functional genomics	RNA-seq atlases (antennae, brain, fat body, wings)	Circadian rhythms, diapause, flight metabolism	Differential expression	Merlin et al., 2009; de Roode et al., 2011
Genome editing	CRISPR/Cas9 and TALENs	Causal testing of candidate genes	Knockout, allele disruption	Markert et al., 2016; Zhang and Reed, 2016
Migration (circadian clock)	period (per), timeless (tim), cryptochrome 2 (cry2)	Sun-compass orientation, migratory timing	Expression, functional assays	Merlin et al., 2009; Guerra et al., 2012
Migration (sensory integration)	Orco and sensory receptor pathways	Orientation and navigation	Expression, candidate gene inference	Zhan et al., 2011; Zhan et al., 2014
Migration (metabolism and diapause)	Insulin signaling (IGF2), juvenile hormone pathways	Lipid storage, reproductive diapause	GWAS, expression, hormone manipulation	Zhan et al., 2014; Freedman and Kronforst, 2023
Chemical defense	Na^+/K^+-ATPase (ATPα) substitutions	Cardenolide resistance	Biochemical assays, comparative genomics	Petschenka et al., 2013; Agrawal et al., 2012
Detoxification and transport	Cytochrome P450s, ABC transporters	Sequestration and biotransformation of toxins	Expression, metabolomics	Petschenka and Agrawal, 2015; Dreisbach et al., 2023
Metabolomics	Milkweed and monarch metabolite profiles	Host-plant adaptation, parasite resistance	LC-MS/MS, untargeted metabolomics	Dreisbach et al., 2023; Agrawal et al., 2025
Sex chromosomes	Neo-Z chromosome, doublesex and hormone signaling genes	Sex-biased expression, genome evolution	Long-read genomics, expression	Mongue et al., 2017
Eco-genomic interactions	Immunity genes, OE parasite, microbiome pathways	Parasite resistance, fitness trade-offs	Infection assays, RNA-seq, metabolomics	de Roode et al., 2008; Hammer et al., 2014
Conservation genomics	Adaptive alleles and metabolite indicators	Population resilience, migration persistence	Population genomics, metabolomic monitoring	Semmens et al., 2016; Thogmartin et al., 2017

4 Chemical Defense: Na+/K+-ATPase Evolution and Cardenolides

Monarch larvae sequester cardenolides from milkweeds, which bind and inhibit Na^+/K^+-ATPase (ATPα; Mongue et al., 2025). Specific amino-acid substitutions in ATPα, such as *N122H* and *Q111L*, reduce binding affinity for cardenolides and confer resistance (Petschenka et al., 2013; López-Goldar et al., 2024). Convergent evolution has been observed in other specialist herbivores (Agrawal et al., 2024), like *Danaus chrysippus* and *Tetraopes* beetles, which carry similar substitutions conferring toxin resistance. Biochemical assays have demonstrated that these substitutions maintain ion pump function while reducing cardenolide binding, illustrating a clear genotype-phenotype link. Variation in ATPα selectivity among monarch populations correlates with milkweed species in their breeding ranges, highlighting an eco-genomic interaction between host-plant chemistry and monarch defense strategies (Petschenka et al., 2013; Agrawal et al., 2012; 2024).

Metabolomic studies of milkweed species reveal extensive variation in cardenolide composition and other secondary metabolites, which can interact with monarch genotype to influence sequestration efficiency, developmental success, and predator deterrence (Malcolm and Brower, 1989; Petschenka and Agrawal, 2015; Agrawal et al., 2024). Integrating chemical and genomic data allows a detailed understanding of coevolution between monarchs and their host plants.

5 Pigmentation, Mimicry, and Developmental Genetics

Monarch wing coloration exemplifies aposematism, with a consistent orange-black pattern across populations that reinforces predator avoidance in conjunction with cardenolide-based chemical defense. Although pigmentation genetics is not known to contribute directly to migratory behavior, it plays an important indirect role in defense by enhancing the effectiveness of warning coloration and mimicry systems. Functional studies suggest that genes such as *optix*, *cortex*, and *WntA*, known from *Heliconius* butterflies, regulate pigment deposition and patterning in monarchs (Martin et al., 2012; Livraghi et al., 2024; 2025). Regulatory changes in cis-elements of these loci likely modulate spatial expression of pigment genes rather than altering protein sequences directly, a pattern observed broadly across Lepidoptera (Ben Chehida et al., 2025). Transcriptomic analyses of wing discs have identified differential expression of *yellow* and *dopa decarboxylase* during late pupal stages, linking enzymatic pathways to melanin and ommochrome deposition (Shen, 2024). Comparative studies across *Danaus* species may further elucidate the molecular basis of subtle color pattern variation relevant to predator learning and mimicry (De-Kayne et al., 2025).

6 Sex Chromosomes and Structural Genome Evolution

Monarchs possess a neo-sex chromosome (neo-Z) resulting from an autosome-Z fusion approximately 5-10 million years ago (Mongue et al., 2017; Mora et al., 2024). Genes on the neo-Z show accelerated evolution and increased sex-biased expression compared to autosomes. For example, *doublesex* and genes involved in hormone signaling are enriched on the neo-Z (Mora et al., 2024), potentially influencing sexual dimorphism in wing coloration and reproductive timing. Long-read sequencing has identified inversions and structural variants on the neo-Z that may reduce recombination and preserve co-adapted gene complexes related to migration and diapause. Transposable element (TE) accumulation on sex chromosomes also contributes to structural diversification, shaping the evolutionary trajectory of *Danaus* genomes (Davey et al., 2016).

7 Parasites, Microbiome, and Eco-genomic Interactions

The specialist protozoan *Ophryocystis elektroscirrha* (OE) reduces monarch survival, flight performance, and fecundity (Altizer and Oberhauser, 1999; Agrawal et al., 2012). Studies show that monarchs feeding on high-cardenolide milkweeds carry lower parasite loads, linking chemical defense to disease resistance (de Roode et al., 2008; Müller-Theissen et al., 2025). Metabolomic analyses of milkweed secondary compounds, including digitoxin, asclepin, and calotropin, reveal variation in toxicity and sequestration efficiency across populations. Monarch gut microbiomes, dominated by *Enterococcus* and *Lactobacillus* species, influence digestion and detoxification pathways (Sanaei et al., 2024); RNA-seq profiling suggests microbial modulation of host immunity and nutrient assimilation (Hammer et al., 2014; van der Hoeven et al., 2013). Integration of host, parasite, plant chemistry, and microbiome data provides a holistic view of eco-genomic dynamics.

8 Population Genomics, Demography, and Conservation Relevance

Large-scale population genomic analyses indicate that eastern North American monarchs exhibit modest population structure, with subtle differentiation between migratory and non-migratory populations (Zhan et al., 2014; Freedman and Kronforst, 2023). Loci associated with lipid metabolism, circadian regulation, and neural function show signals consistent with local adaptation linked to migratory behavior. Demographic modeling further suggests that overwintering populations in Mexico have declined by more than 80% over the past two decades, underscoring ongoing conservation concern (Semmens et al., 2016; Thogmartin et al., 2017). Genomic diversity metrics are increasingly used to identify conservation-relevant units and track the retention of adaptive variation related to migration and reproduction. Integrating these genomic insights into management strategies-such as habitat restoration and protection of migratory corridors-remains an important but unresolved challenge (U.S. Fish and Wildlife Service, 2020; Erickson et al., 2023).

9 Outstanding Gaps and Priority Directions

Despite remarkable advances in monarch genomics and functional biology, several critical gaps remain that limit our mechanistic understanding of migration, chemical defense, and adaptation to environmental stressors.

9.1 Causal mapping of migratory behavior

Migration in monarchs is increasingly recognized as a polygenic and modular trait shaped by circadian regulation, neural circuitry, endocrine signaling, and metabolic pathways (Table 2). Although population genomic and quantitative genetic studies have identified candidate loci associated with migratory components, direct tests of causality remain limited (Freedman and Kronforst, 2023). Integrating high-resolution mapping with functional validation-such as allele-specific perturbations targeting genes implicated in orientation, diapause, lipid storage, and flight endurance-will be essential for resolving how combinations of alleles produce coordinated migratory phenotypes (Markert et al., 2016).

9.2 Regulatory and cell-type-specific neurogenomics

Migratory orientation and seasonal behavior are mediated by complex neural circuits, particularly in the antennae and central brain. Single-cell RNA sequencing (scRNA-seq), spatial transcriptomics, and ATAC-seq of neural tissues under different photoperiods and temperatures can reveal cell-type-specific regulatory programs. Integration with functional assays will clarify how environmental cues are encoded at the molecular level to influence migration timing and sun-compass navigation (Merlin et al., 2009; Guerra et al., 2012; Agrawal et al,. 2024).

9.3 Pan-genome and structural variation analyses

Recent discoveries of chromosomal rearrangements, including a neo-Z chromosome, highlight the potential importance of structural variation in monarch adaptation (Höök et al., 2024). However, most genomic inferences still rely on a single reference genome. Pan-genome approaches incorporating migratory and non-migratory populations will enable systematic assessment of structural polymorphisms, copy-number variation, and transposable elements that may influence migration, diapause, or chemical defense (Davey et al., 2016; Mongue et al., 2017). A key unresolved question is how such variants contribute to adaptation through regulatory versus coding effects.

9.4 Host-microbe-parasite-plant interactions

Monarch fitness and migration are tightly linked to interactions with milkweed chemistry, gut microbiota, and the protozoan parasite *Ophryocystis elektroscirrha* (OE; Müller-Theissen et al., 2025). Controlled factorial experiments combining milkweed species with differing cardenolide profiles, manipulations of the microbiome, and OE exposure can dissect the genetic and metabolic bases of host tolerance, detoxification, and parasite resistance (de Roode et al., 2008; Satterfield et al., 2015; Müller-Theissen et al., 2025).

9.5 Metabolomics and chemical ecology of host plants and monarchs

A particularly understudied frontier is integrating metabolomics to link plant chemistry**,** monarch metabolism, and stress responses. Milkweeds produce a diverse array of cardenolides, alkaloids, and other secondary metabolites (Hoogshagen et al., 2024) that vary among species, populations, and environmental conditions. Monarch larvae ingest and sequester these compounds (Agrawal et al., 2012; Betz et al., 2025), but the dynamics of sequestration, biotransformation, and excretion remain poorly characterized. Mass spectrometry-based metabolomics, including LC-MS/MS and untargeted metabolite profiling, can quantify both plant and insect chemical landscapes, revealing:

How monarchs metabolically adjust to high vs. low cardenolide diets.

The impact of environmental stressors (temperature, drought, pesticide exposure) on metabolite accumulation and detoxification pathways.

Relationships between metabolite profiles and parasite resistance, flight performance, or survival during overwintering.

Combining metabolomics with transcriptomics and proteomics can map the regulatory networks linking detoxification enzymes (e.g., *cytochrome P450s, ABC transporters)* to physiological and behavioral traits, providing a mechanistic understanding of how monarchs cope with chemically complex host plants.

9.6 Conservation integration

Translating molecular, genomic, and metabolomic insights into actionable conservation strategies remains a critical priority for monarch research (Box 1). Although habitat loss and climate change are widely recognized drivers of monarch declines, increasing evidence suggests that the *quality*, chemical composition, and genetic compatibility of restored habitats may be as important as habitat quantity (Thogmartin et al., 2017; Erickson et al., 2023). Milkweed species and genotypes vary substantially in cardenolide concentration, chemical composition, and inducibility under environmental stress, with downstream effects on monarch detoxification, growth, and resistance to the protozoan parasite *Ophryocystis elektroscirrha* (OE; Agrawal et al., 2012; de Roode et al., 2008; Petschenka and Agrawal, 2015). Metabolomic profiling of candidate milkweeds used in restoration programs could therefore identify plant chemotypes that balance larval performance, chemical defense, and disease suppression, while avoiding unintended consequences such as selecting for excessively toxic plants that impair development or migratory performance (Malcolm and Brower, 1989; Agrawal et al., 2024; 2025).

At the population level, coupling genomic and metabolomic data across landscapes offers a complementary framework for conservation planning. Population genomic analyses reveal consistent signals of selection on loci associated with migration, lipid metabolism, circadian rhythms, and endocrine regulation (Zhan et al., 2014; Freedman and Kronforst, 2023), suggesting that adaptive capacity may erode before demographic declines become evident. Integrating these genomic markers with metabolomic signatures-such as cardenolide sequestration profiles, lipid reserves, and stress-response metabolites-could help identify populations that retain key functional traits required for long-distance migration and overwintering success, while providing early warning indicators of declining resilience (Semmens et al., 2016; Thogmartin et al., 2017). Together, advances in causal genomics, pan-genomics, regulatory neurogenomics, and metabolomics position monarchs as a powerful model for linking genotype, chemistry, behavior, and fitness across ecological scales, informing conservation strategies that preserve both population size and the functional diversity required to sustain migration under ongoing environmental change (U.S. Fish and Wildlife Service, 2020; Erickson et al., 2023).

10 Recommended Approaches and Experimental Roadmap

To advance understanding of monarch migration, chemical defense, and adaptation, research should integrate high-resolution genomic, functional, and ecological approaches. Large, replicated mapping cohorts, including pedigreed crosses and population-resequencing panels, can identify loci underlying complex traits such as migratory orientation, diapause, and lipid storage. Functional validation via CRISPR/Cas9 or TALEN-mediated knockouts and allele swaps will allow causal testing of candidate genes. Complementary single-cell and spatial transcriptomics of antennae and brain tissues can reveal cell-type-specific regulatory programs controlling navigation, circadian timing, and sensory processing.

At the genomic level, long-read sequencing combined with Hi-C scaffolding enables chromosome-scale assemblies and pan-genome analyses, facilitating discovery of structural variants (Livraghi et al., 2024), neo-sex chromosome polymorphisms (Mongue et al., 2017), and transposable element dynamics (De-Kayne et al., 2025). SV-aware association testing can link structural changes to adaptive traits. Integrating these genomic data with metabolomic and proteomic profiling will illuminate the biochemical pathways through which monarchs process milkweed toxins (Agrawal et al., 2012; 2024; 2025) and respond to environmental stressors (Dalla et al., 2014), including parasite exposure (Altizer et al., 2015) and habitat change (Green and Kronforst, 2019).

Finally, experimental designs should bridge molecular and ecological scales. Field and laboratory studies combining genotype, microbiome composition, host-plant chemistry (Dale and Stumpe, 2014), and parasite load can quantify the ecological relevance of genetic and metabolic variation. This integrative roadmap positions future research to resolve mechanistic links from genotype to phenotype to fitness, while providing actionable insights for conservation management, such as selecting milkweed species and populations that optimize monarch survival, detoxification capacity, and migratory performance.

11 Conclusions

Research on the monarch butterfly has progressed from classical natural history and ecological observations to an integrative genomic, functional, and eco-physiological understanding of this iconic species. Chromosome-scale genome assemblies, population resequencing, and functional genomic tools have enabled identification of genes and pathways associated with migratory behavior, circadian rhythms, chemical defense, and sex-chromosome evolution (Satterfield et al., 2015). Studies of host-plant interactions and metabolomics have revealed how cardenolide sequestration and chemical stress responses mediate survival, predator avoidance, and parasite resistance, highlighting the complex interplay between genotype, phenotype, and environment (Agrawal et al., 2012; 2024; 2025). Simultaneously, ecological and population genomic analyses underscore how microbiomes, parasite pressures, and environmental changes shape adaptive variation and influence conservation priorities (de Roode et al., 2008; Dale et al., 2014; Sanaei et al., 2024)

Despite these advances, significant gaps remain in fully elucidating the proximate and ultimate mechanisms underlying monarch adaptation. Key opportunities include causal mapping of migration-related alleles, single-cell and spatial genomics to resolve cell-type specific regulatory networks, pan-genome analyses of structural variants and neo-sex chromosome evolution. Additional metabolomic profiling to link host-plant chemistry with physiological stress responses. Integrative experimental designs combining genotype, metabolome, microbiome, milkweed chemistry, and parasite exposure (Dreisbach et al., 2023; Agrawal et al., 2025) offers a pathway to connect molecular mechanisms with ecological function. By leveraging these multi-dimensional approaches, future research can provide mechanistic insights into migration, chemical defense, and adaptation while directly informing conservation strategies to enhance monarch resilience in the face of habitat loss, climate change, and shifting ecological pressures.

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Conflict of Interest Disclosure

The author declares no conflicts of interest.

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Box 1 Conservation Implications of Monarch Genomics and Metabolomics

Recent advances in monarch genomics and metabolomics provide an opportunity to move beyond abundance-based conservation toward strategies that preserve functional, adaptive capacity across the migratory range.

1. Cheically informed habitat restoration. Milkweed species and populations differ substantially in cardenolide composition, inducibility, and secondary metabolite diversity, with direct consequences for monarch survival, chemical defense, and resistance to the protozoan parasite *Ophryocystis elektroscirrha* (OE) (de Roode et al., 2008; Agrawal et al., 2012; Petschenka and Agrawal, 2015). Metabolomic screening of milkweeds used in restoration projects could identify plant chemotypes that optimize larval performance while enhancing parasite resistance and predator deterrence, avoiding one-size-fits-all planting strategies.

2. Preserving adaptive genetic variation for migration. Population genomic studies indicate that migration, diapause, lipid storage, and navigation are polygenic traits shaped by subtle allele-frequency shifts across many loci (Zhan et al., 2014; Freedman and Kronforst, 2023). Conservation actions that maintain connectivity among breeding, migratory, and overwintering regions are therefore essential to preserve adaptive alleles associated with circadian timing, endocrine regulation, and metabolic endurance.

3. Metaolites as early-warning indicators. Metabolomic profiles-such as cardenolide sequestration patterns, lipid reserves, and stress-response metabolites-may provide sensitive indicators of physiological condition and migratory readiness before population declines are detectable through census data alone (Semmens et al., 2016; Thogmartin et al., 2017). Integrating metabolomic monitoring into long-term surveys could improve detection of sublethal stress caused by climate extremes, pesticide exposure, or host-plant mismatch.

4. Integrating host–parasite–microbiome dynamics. Chemical defense, parasite resistance, and gut microbiome composition interact to shape monarch fitness. Restoration strategies that consider host-plant chemistry alongside disease pressure and microbial interactions may reduce parasite prevalence and improve survival during migration and overwintering (de Roode et al., 2008; Hammer et al., 2014).

5. From molecular insight to management practice. By integrating causal genomics, pan-genomics, regulatory neurogenomics, and metabolomics, conservation efforts can prioritize not only habitat quantity but also genetic, chemical, and physiological quality. Such mechanistically informed strategies are likely to be more robust to environmental change, supporting long-term persistence of migratory monarch populations in an increasingly variable landscape (U.S. Fish and Wildlife Service, 2020; Erickson et al., 2023).