

## 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.