

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.