

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 <sup>+</sup> /K <sup>+</sup> -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<sup>+</sup>/K<sup>+</sup>-ATPase Evolution and Cardenolides

Monarch larvae sequester cardenolides from milkweeds, which bind and inhibit Na<sup>+</sup>/K<sup>+</sup>-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).