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Evolution of Odonata: genomic insights

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Odonata is an order of insects that comprises ~6500 species. They are among the earliest flying insects, and one of the first diverging lineages in the Pterygota. Odonate evolution has been a topic of research for over 100 years, with studies focusing primarily on their flight behavior, color, vision, and aquatic juvenile lifestyles. Recent genomics studies have provided new interpretations about the evolution of these traits. In this paper, we look at how high-throughput sequence data (i.e. subgenomic and genomic data) have been used to answer long-standing questions in Odonata ranging from evolutionary relationships to vision evolution to flight behavior. Additionally, we evaluate these data at multiple taxonomic levels (i.e. ordinal, familial, generic, and population) and provide comparative analysis of genomes across Odonata, identifying features of these new data. Last, we discuss the next two years of Odonata genomic study, with context about what questions are currently being tackled.

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Odonata systematics using genomics, transcriptomes, and targeted enrichment

Odonata comprises three extant suborders: the damselflies (Zygoptera), the dragonflies (Anisoptera), and a monogeneric Anisozygoptera. Odonate relationships have been studied for over one hundred and fifty years, initially based largely on morphological datasets. The development of new morphological data tools (e.g. scanning electron microscopy images, micro-computed tomography scans), early molecular Sanger-based methods, and subsequent next-generation sequencing techniques has inspired the re-evaluation of Odonate relationships. Most studies have tended to focus on particular families or superfamilies with fewer ordinal-level studies. Several recent studies have reconstructed ordinal-level phylogenies, however, to assess systematics in this group [3,10,21]. These studies sequenced thousands of genes using reduced representation sequencing methods, based on data from transcriptomes [10,21] or anchored hybrid enrichment methods in [3]; see Table 1 for sequence details. While the foci of these three studies were congruent, they were each unique, with Kohli et al. [10] focusing on the evolution of egg-laying behavior and the relative position of Petaluridae and Gomphidae (based on 105 species), Suvorov et al. [21] focusing on introgression between Zygoptera, Anisoptera, and Anisozygoptera (based on 83 species), and Bybee et al. [3] focusing on resolving taxonomy within the order (based on 136 species). All studies recovered monophyletic suborders, with Anisozygoptera supported as sister to Anisoptera forming a group, Epiprocta. Slight differences between the studies include the relative positions of historically difficult nodes (see Figure 1), including Calopterygoidea (recovered as monophyletic in Kohli et al. [10] and Suvorov et al. [21] but as paraphyletic in Bybee et al. [3]), the relationship of Petaluridae + Gomphidae (recovered as monophyletic or paraphyletic with respect to Cavilabiata depending on phylogenetic reconstruction method), and Libelluloidea (recovered as monophyletic in all studies but with varying support of relationships within the superfamily).

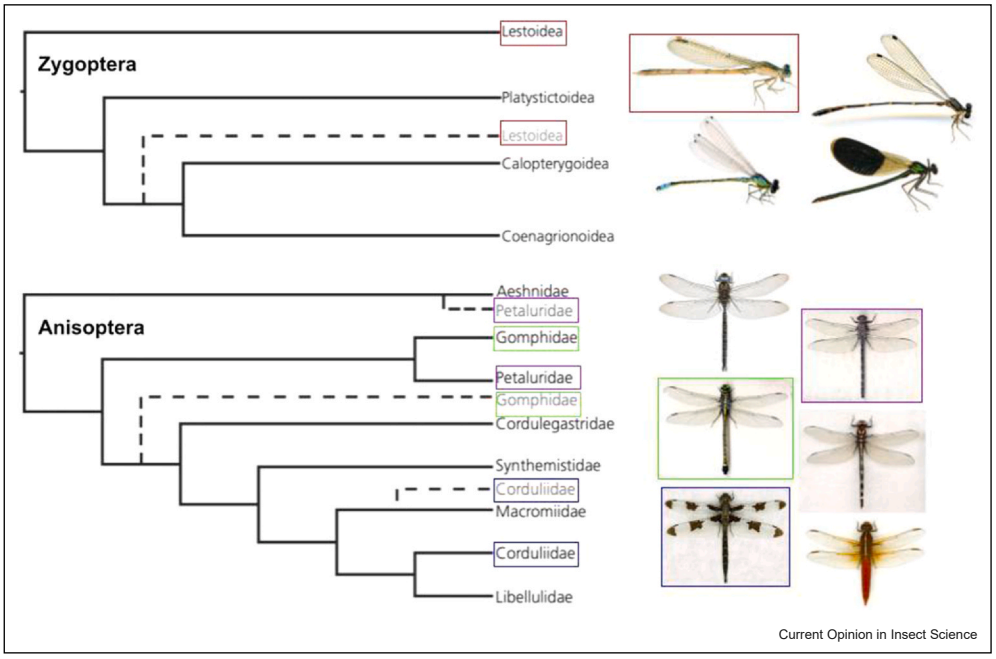
What are the difficult nodes to resolve in the dragonfly and damselfly tree of life?

Although there has been congruence among studies in the relationships of many dragonfly and damselfly taxa, there are a few nodes that consistently lack resolution. In general, the relationships between the clubtail and petaltail families of dragonflies, the relationships among four taxon groups in the superfamily Libelluloidea (Synthemistdae, Macromiidae, Corduliidae, and Libellulidae), and the relationships within the superfamily Calopterygoidea (Chlorogomphidae, Cordulegastridae, and Neopetaliidae) have been challenging (Figure 1).

Petaluridae + Gomphidae + Cavilabiata

Previous phylogenetic hypotheses, based primarily on morphological and/or small targeted locus data, have

Figure 1



Summary trees reflecting difficult nodes to resolve.
Modified from Kohli and Ware (2022).

struggled to resolve the relationship between Petaluridae, Gomphidae, and Cavilabiata (Cavilabiata=Libelluloidea+Cordulegastroidea) (e.g. [2,12]). Despite having many orders-of-magnitude more data, the three recent studies mentioned above with subgenomic data recovered varying relationships depending on the dataset. Petaluridae + Gomphidae were recovered as sister to Cavilabiata with strong support when using concatenation reconstruction approaches; however, when implementing gene tree-based approaches [10,21], this relationship was no longer recovered and instead Gomphidae was recovered as sister to Petaluridae Cavilabiata, with varying support. Kohli et al. [10] showed that the relationships between these groups differed depending on the speed of gene evolution, with faster-evolving genes recovering Petaluridae + Cavilabiata and slowly evolving genes recovering Petaluridae + Gomphidae, and that the branch support was varying from weak to moderate support depending on the percentage of genes used. Suvorov et al. [21] noted that incomplete lineage sorting (ILS) and ancestral introgression are potential processes influencing these conflicting relationships.

Libelluloidea/Libellulidae relationships

Libelluloidea comprises at least 4 taxonomic groups, including the 'River Cruisers' (Macromiidae), 'Emeralds' (Corduliidae), the diverse Synthemistidae ('GSI complex' sensu [25]), and the most abundant and familiar dragonflies, 'Skimmers/Chasers' (Libellulidae). Libellulidae are readily recognizable, often with colored or patterned wings and a boot-shaped series arrangement of veins (the anal loop) in the hindwing. The relationships within the Libelluloidea have historically been difficult to resolve with either morphological and/or molecular data. Several studies have focused on this taxonomic group. Ware et al. [25], Pilgrim and Von Dohlen [16], Letsch [26], Bybee et al. [2], and Letsch et al. [27], with varying taxon samples, failed to resolve the backbone of Libellulidae, and the relationships among the four putative families remained uncertain. In comparison with other parts of Anisoptera Bybee et al. [3] (anchored hybrid evolutions), Suvorov et al. [21] (Transcriptomes) and Kohli et al. [10] (Transcriptomes) similarly recovered lower branch support and lower quartet concordance values for these same nodes. Uncertainty of relationships within this superfamily may be attributed to interfamilial introgression and/or ILS [21], and thus will require extensive taxon sampling of this highly speciose group and/or whole-genomic sequencing data to compile enough phylogenetic signal to potentially resolve these relationships.

Calopterygoidea relationships

Calopterygoidea is a superfamily comprising the often colorful 'banner wing' damselflies, including the charismatic rubyspot damselflies *(Hetaerina)* and the ebony jewel wings *(Calopteryx)*. In past studies, the superfamily

Calopterygoidea was recovered as either monophyletic or paraphyletic depending on the taxon sampling, data type, and reconstruction method. Regarding reconstruction method, when using a concatenation approach, Calopterygoidea was monophyletic for two of the three studies and paraphyletic for the remaining study. In this case, the biggest difference between these phylogenies was the taxon sampling, with paraphyly occurring with increased taxon sampling (47 species representatives) compared with instances of monophyly (19 representatives for both). However, Suvorov et al. [21] did recover a paraphyletic Calopterygoidea when phylogenetic reconstructions were gene tree-based, which they attributed to the likely occurrence of several interfamilial introgression events. Interestingly, Kohli et al. [10] also generated a species tree but still recovered a monophyletic Calopterygoidea, which might have been influenced by differences in data matrix gene composition.

There remain several unresolved evolutionary relationships across the Odonate tree of life, despite the increasing amount of data generated through the advent of technological advancements. However, additional taxon sampling, genome-scale data, and more sophisticated methods explicitly accounting for ILS and introgression may uncover the 'true' (i.e. most robust as possible) relationships within Odonata.

How has genomic data impacted the study of Odonate evolution?

Most studies on the evolution of Odonata have focused on solving systematics-related questions, despite the fact that there are many questions still unanswered related to their functional, population, and conservation genomics. Few studies have undertaken comparative work, and there is a great potential for future work in this area.

Comparative genomics

In 2021 and 2022, the number of publicly available Odonata genome assemblies more than doubled from three to eight (Table 1). Overall, the eight genomes only represent 6 families (Zygoptera: Calopterygidae, Coenagrionidae, Chlorocyphidae, and Platycnemidae; Anisoptera: Libellulidae, Petaluridae). Four of the five recent assemblies are chromosome-length, while the remaining assembly has a contig N50 > 80 mb, a marked improvement in contiguity over the first three Odonata genome assemblies released. All five assemblies have a BUSCO score greater than the original three assemblies, indicating a trend toward greater completeness. However, the assembly of the Chlorocyphidae species *Rhinocypha anisoptera* is of a poor quality, so its comparative uses are likely limited.

Little downstream analysis has been performed on these assemblies. In fact, the assemblies of *Platycnemis pennipes*, *Hetaerina americana*, and *Rhinocypha anisoptera* have not been annotated at the time of submission. We know almost nothing about the genomic evolution of Odonata, but we expect the already-published genomes will yield a wealth of knowledge about chromosomal evolution, duplication events (or the lack thereof), repetitive elements, and gene family evolution. Although limited in sample size, the haploid chromosome number in the available chromosome-level assemblies all differ (*Tanypteryx hageni*=9 [23], *Pantala flavescens*=12 [13], *Ischnura elegans*=14 [17], and *P. pennipes*=13) encompassing a wide range of karyotypes found in Odonata, including the ancestral 2n= 25 for *Platycnemis pennipes* [11]. This dataset could be used to determine the duplication, fusion, and fission events leading to each karyotype. The evolution of gene families related to flight and vision is likely to be of special interest, as Anisoptera in particular are among the strongest fliers and most efficient predators in all Eumetazoa.

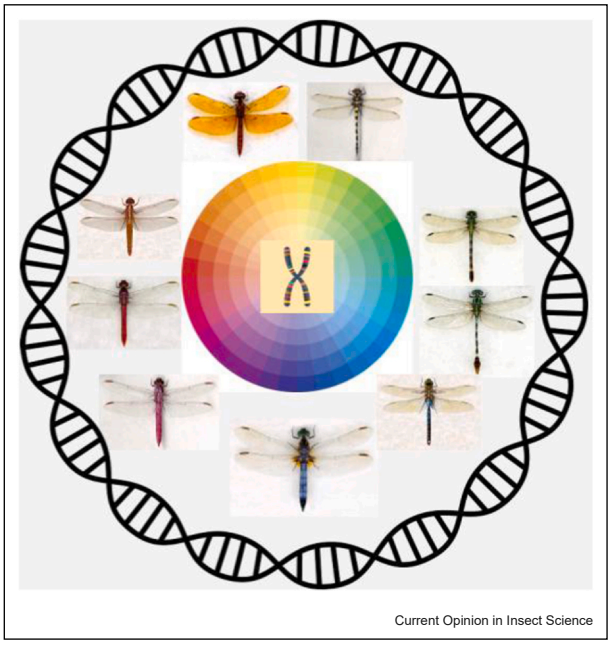
Conservation genomics

Although dragonflies and damselflies are taxa of concern for conservation, few conservation genomics studies exist. Liu et al. (2021) used the genome of *Pantala flavescens* to infer changes in effective population size using SMC++ [22] and linked human activities to a severe decline in population size, but this is the only Odonata genome assembly to be used to generate information pertinent to species conservation. Currently available genomes should be used to investigate a link between species decline and population size, and we advise authors to keep such analyses in mind as they share genomic-level data. Indeed, we call upon the research community to prioritize genomics projects with conservation interests, given the current state of insect decline [4,7,14,18,24].

Population genomics

As highly mobile predators, dragonflies and damselflies could be useful organisms for studying population dynamics. However, population genetic data are scant for Odonata, even when considering available Sanger sequencing data. However, high-throughput sequencing techniques have recently been used to better understand populations of Odonata with much greater efficacy than studies that have relied on traditional Sanger techniques. Johansson et al. [9], for example, sampled *Leucorrhinia dubia* widely across its range, including Europe, Russia, and Japan, generating double digest restriction-site associated DNA (ddRAD) sequences; they found several distinct genetic clusters across Europe and Asia, which reflect the recolonization history of *L. dubia* after the last glaciation. In another recent study [1], ddRAD sequence data are used to corroborate

Figure 2



Color wheel demonstrating the breadth of color across Odonate taxa.

population predictions generated through species distribution models; Biddy focused on *Hetaerina*, the Canyon Rubyspot, and found the species' populations are separated and likely undergoing adaptive changes. These two studies reflect the limited breadth of the current published population genomics data. Author Tolman (current publication) has population genomics data on Petaluridae, which is not yet published, and the authors know of other studies underway; to date, the majority of dragonfly and damselfly population studies published have used Sanger sequencing for data collection, but as sequencing costs decrease, we expect there to be a rise in the number of studies using population genomic data. These results, though limited, highlight the need for conservation for aquatic insects in boreal habitats, affected by climate change.

Functional genomics

Few studies have looked at the functional genomics of Odonates. Those that have been done have focused on vision, color, and the nymph-to-adult transition. Futahashi et al. [5] used transcriptomic data to evaluate wax-based color changes in Odonates, and relatively few other studies have been published yet on the functional genomics of dragonfly wing or body color. Dragonfly color varies dramatically across taxa (e.g. Figure 2). Vision, which is largely impacted by opsin pigments, has been assessed by Futahashi et al. [6] and Suvorov et al. [20]; these studies suggest that there have been duplications and losses of opsins, and in general, the opsins that are expressed in nymphs and adults vary. As Odonates are nonholometabolous, few have studied the genetic mechanisms for their development, but Okude et al. [15] used transcriptomes to assess the transcription factors Krüppel homolog 1 (Kr-h1) and E93, which, among others, are involved in dragonfly metamorphosis. Little other work has been done on these transcription factors in Odonata, but future studies should expand taxon sampling to assess variation among suborders. Simon et al. [19] looked at genes related to embryogenesis and development in *Ischnura*, but this study focused largely on variation among stadia. There is much yet to be done in the field of functional genomics for Odonata. Future studies could use such techniques to assess migration strategies, wing, and body color variation within populations and individuals, as well as to assess reproductive strategies and Odonate immunology. This is an exciting field that will likely continue to expansively grow over the next decade.

Declaration of Competing Interest

None.

Data Availability

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.cois.2023.101073.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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This study, having the most inclusive taxon sampling to date for any ordinal level phylogeny, used a targeted enrichment approach to reconstruct the Odonata tree of life. This study provided support along the backbone, included an updated classification scheme within Zygoptera, and highlighted areas of future research (i.e. more taxon sampling to potentially resolve difficult nodes).

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This study used transcriptomes for a little over 100 dragonflies and damselflies to reconstruct a phylogeny. Fossil calibrations were applied, and divergence time estimation analyses were done to assess the impact of particular fossils on node ages, and to estimate the node ages of clades that are important to the understanding of reproductive biology.

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Glossary

Anchored hybrid enrichment: A targeted enrichment approach to collect genetic information from many loci based on user-defined probe regions.

Comparative genomics: Area of genomics where researchers compare whole-genome sequences of different species to identify genomic similarities and differences.

Conservation genomics: Area of genomics where researchers apply genome sequences to further advance conservation efforts.

Functional genomics: Area of genomics, using either whole-genome or transcriptome data, where researchers identify the link between genes and their functions.

Genome: The complete set of genetic information in an organism.

High-throughput sequencing: Sequencing large quantities of DNA using massively parallel sequencing of DNA libraries separated by synthesis and not based on chain-termination chemistry.

Phylogeny: A graphical representation, in the form of a branching 'tree', that reflects the evolutionary history and relationships among taxa.

Sanger sequencing: A sequencing method developed by Frederick Sanger and colleagues, which uses capillary electrophoresis and chain-termination methodology.

Transcriptome: The genes expressed by an organism at a given time (i.e. the messenger RNA present).