CHAPTER 3

# Transcriptomic insights into Odonata ecology and evolution

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**Overview**

Chapter 3 attempts to define transcriptomics, a powerful tool for scientific research that has advanced our knowledge of Odonata evolution in many meaningful ways. It then goes on to outline its strengths and weakness for ecological and evolutionary research. The chapter frames the current state of transcriptomics research in Odonata. It focuses specifically on color, color vision, embryogenesis, and phylo-transcriptomics, as these are currently the deepest areas for transcriptomics among Odonata to date. The chapter also describes the authors' advocacy for future research using transcriptomics and it presents clearly and concisely their arguments to convince the reader that there are exceptional opportunities among Odonata. This is particularly so when transcriptomics is combined with genomics.

## 3.1 Introduction

Transcriptomes can be powerful tools for scientific research and have advanced our knowledge of Odonata evolution in many meaningful ways. Where genomes (DNA) are generally used to capture a snapshot of current and past genetic information, transcriptomes (RNA) can offer a more dynamic look at genetic patterns (e.g. post-translational modifications, expression) within a given organism. DNA is the foundation of chromosomes that, when combined to include the complete set of genetic DNA of an organism, are called the genome. RNA is the basis of the exome, the portion of the genome that is transcribed into messenger RNA (mRNA), translated into protein, and usually expressed in the cell. The exome represents all possible transcribable portions of the genome, while the transcriptome is the mRNA present in an individual, tissue, or cell.

Transcriptomes can provide a report of the overall mRNAs and their expression levels in the whole organism (i.e. full body mRNA extraction), a portion of an individual (e.g. head, thorax, abdomen), a tissue type (e.g. antennae, epidermis, eyes, fat bodies, muscle), or even single cells. The transcriptome can also be isolated from different life stages (e.g. embryo, nymphs (larva), teneral, and adults), different environments (e.g. predatory vs. non-predatory environments), under different physiological demands (e.g. flying vs. resting), etc. By extracting mRNA from different life stages, different tissues, in different environments at different times, and/or while conducting different behaviors, a unique view into gene expression can be obtained. This is the value of transcriptomes and something genome sequencing cannot provide (Figure 3.1).

Multiple other implications of transcriptomics include gene annotation and model prediction (Saha et al., 2002), quantification of differential gene expression (Vijay et al., 2013) as well as deciphering the evolutionary patterns of intra- and inter-specific gene expression (Brawand et al., 2011; McManus et al., 2010; Suvorov et al., 2013). Within Odonata research several studies utilized RNA-seq to address such questions as

Seth M. Bybee et al., *Transcriptomic insights into Odonata ecology and evolution*. In: *Dragonflies and Damselflies*. Second Edition.
Edited by Alex Córdoba-Aguilar, Christopher D. Beatty and Jason T. Bried, Oxford University Press. © Oxford University Press (2023).
DOI: 10.1093/oso/9780192898623.003.0003

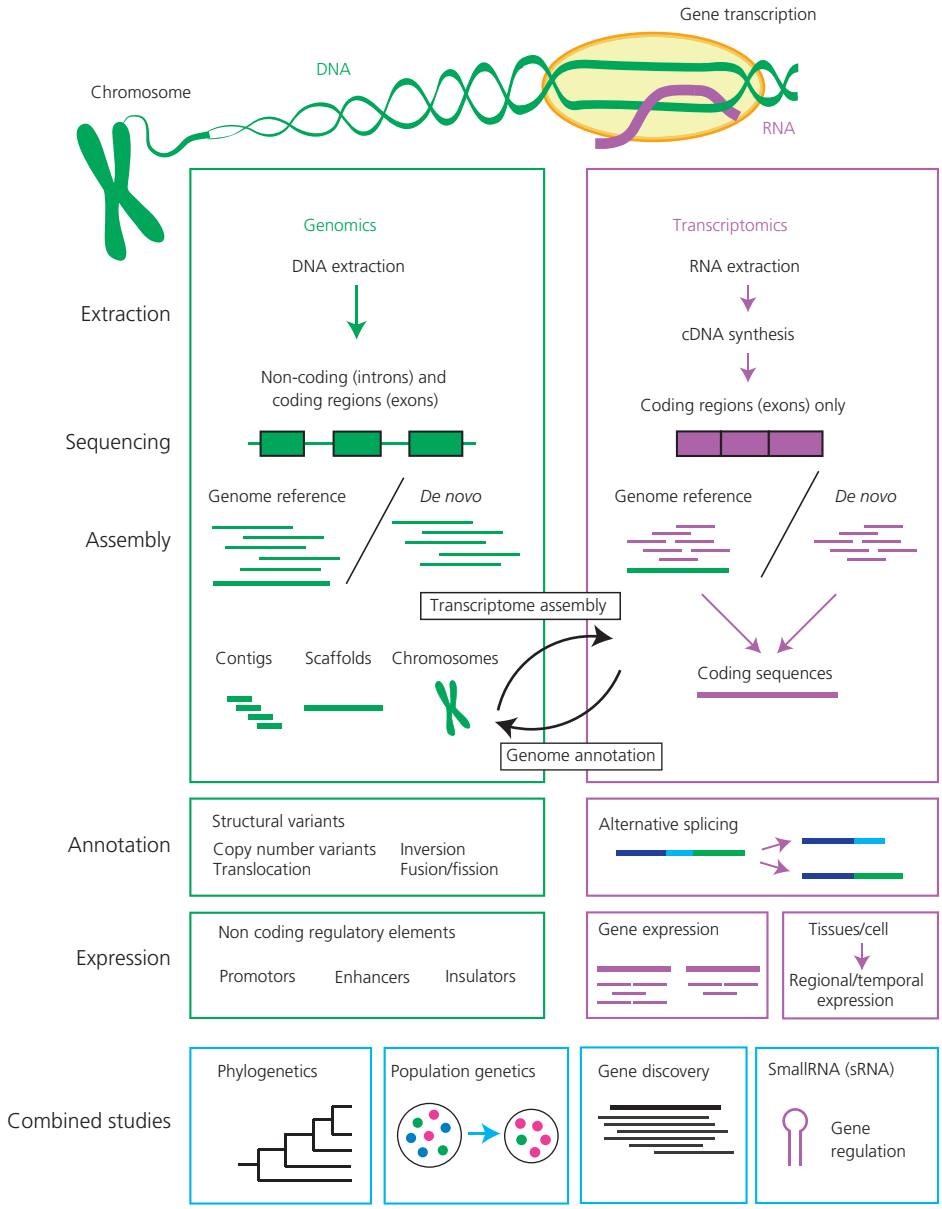


**Figure 3.1 A comparison of genomics and transcriptomics methods in odonate biology and evolution.**
Gene expression begins with the unfolding of coiled DNA. This allows the enzyme, RNA polymerase, to access and transcribe DNA into RNA. Genes can be studied at the level of the DNA (genomics) or RNA (transcriptomics), depending on the study design. Sequencing is carried out on DNA, therefore for transcriptomics studies, an additional step must be taken to reverse transcribe RNA to complementary DNA (cDNA). Genomic sequencing has the capability of capturing all genetic information encoded by the genome, including regions that are not transcribed during gene expression (introns). In contrast, transcriptomic sequencing (RNA-seq) only captures genetic material that has been expressed (exons) in the cells or tissues used for RNA extraction. Sequencing data from either "-omics" strategy can be assembled into longer transcripts using a template from previous genomics studies, or more typically for non-model species, using assembly algorithms when a template is not available (*de novo assembly*). Both assembled genomes and transcriptome sequence data can be used to inform the other, the genome providing a spatial map for the alignment of RNA-seq data and RNA-seq data providing additional genetic coverage for genome assembly. Since transcriptomes capture only

(*Continued*)

transcriptome assembly and annotation of *Ischnura elegans* (Chauhan et al., 2014) and *Megalopreus caerulatus* (Feindt et al., 2018), identification of sex-biased gene expression (Chauhan, Wellenreuther, and Hansson 2016), detection of viral sequences (Johnston, Mikolajewski, and Rolff 2015), phylogeny reconstruction and divergence time estimation (Kohli et al., 2021; Suvorov et al., 2021), tempo and mode of opsin gene family evolution (Futahashi et al., 2015; Suvorov et al., 2017) and inference of introgression through evolutionary history of Odonata (Suvorov et al., 2021). The first step in transcriptomic analyses for taxa without available reference genomes typically begins with *de novo* transcriptome assembly from RNA-seq libraries (Grabherr et al., 2011). Additional steps may include orthology annotation of transcriptomic contigs to identify the presence or absence of certain genes in the assembly. The annotated transcriptomes can be further used to extract orthologous loci, for example, for downstream phylogenetic analyses (Waterhouse et al., 2018). Furthermore, the read coverage resulting from short reads mapped back to the assembled contigs will serve as a proxy for transcript/gene expression intensity.

Despite this great potential of high throughput transcriptomics, it has important caveats. Due to the highly plastic nature of gene expression (Hodgins-Davis and Townsend 2009), some genes will be missed from transcriptome annotations. Furthermore, this effect may lead to erroneous inference of differential gene expression, since the expression profile includes a distribution of expression intensities determined by the environment. Variation in gene expression between specimens can be large and may lead to variable results, as individuals often express different genes and gene expression levels—even among individuals of the same sex from the same locality and environment performing the same behaviors (e.g. Schilder and Marden 2007; Marden 2008). Nonetheless, sequencing additional biological replicates may partially alleviate these problems (Schurch et al., 2016). Finally, the presence of mRNA does not mean it is translated or expressed from what is transcribable from the DNA (e.g. post-translational modifications and alternative splicing resulting in isoforms). This must be taken into consideration when interpreting results from transcriptomics-based studies.

We have tried to identify both the strengths and the weaknesses of transcriptomes, but if they are used in the proper context, transcriptomes can be an exceptional tool for research. This chapter attempts to outline a broad range of applications for transcriptomics within odonate research (color vision, color, embryogenesis, and phylotranscriptomics) with an eye toward the future.

## 3.2 Color vision

Anyone that has stared deeply into the dragonfly eye is drawn in by what appears to be an entire universe of visual action. Both at the morphological and molecular levels, dragonfly eyes are among the most complex of all arthropods. In some dragonfly species the eyes make up nearly 75% of the entire head and can contain more than 30,000 facets (Sherk 1978). Odonates have the capacity for color discrimination (Huang et al., 2014) and Yang and Osorio (1996) explored their underlying chromatic processing; however, there is a great amount to still be learned. Within the eye there are genes (opsins) that, when coupled with chromophore pigment, form the photopigment that support color vision. Physiological measurements from the eyes of dragonflies (Laughlin 1976; Yang and Osorio 1991) and damselflies (Shultz et al., 2008; Huang et al., 2014) have estimated between three and five photopigments sensitive across the visible light spectrum from ultraviolet (UV) well into the long wavelength (LW). From a physiological perspective, Odonata do not need more than five opsin copies to form photopigments that can discriminate colors across the entirety of the visible light spectrum. However, when dragonfly eyes were examined at the molecular level using transcriptomics, upwards of 30 copies of opsins

expressed genes, full assembly yields individual gene transcripts. In contrast, genomic data can be assembled up to the level of the chromosome, with sufficient sequencing data. Once genes have been identified and annotated, the genome provides information that informs on how genes have not only diverged from related species but also how they have changed positionally within the genome (e.g. inversion, translocation, fusion). RNA-seq data provides additional information on post-transcriptional modifications (e.g. alternative splicing). Gene expression is largely under the control of non-coding regulatory elements, such as promotors, enhancers, and insulators. The level of gene expression can be studied using transcriptomics to examine expression in particular tissue types (regional expression) or during different developmental stages/under particular environmental conditions (temporal expression). While researchers may decide to undertake genomic or transcriptomic studies to explore a particular research question, both sequencing strategies are complementary and together can inform on a wide range of research topics, including phylogenetics, population genetics, gene discovery, or more focused studies of gene regulation (e.g. non-coding small RNAs).

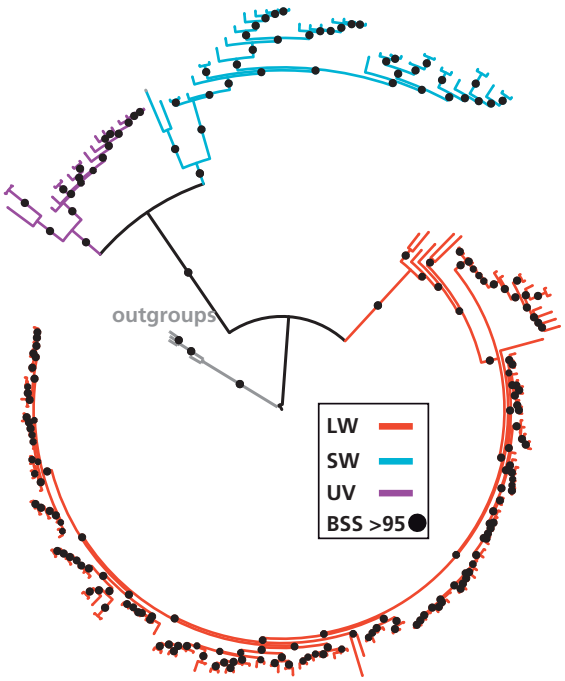


**Figure 3.2 Phylogeny of opsin classes within Odonata.**
The phylogeny represents 13 species of Odonata and demonstrates the lack of UV opsin duplications and the extent of duplications in the SW and LW sensitive opsins.

mRNAs were found (Futahashi et al., 2015; Suvorov et al., 2017). Among the three opsin classes (UV, short wavelength (SW) and LW) LW opsins show by far the most diversity (Figure 3.2; see also Futahashi et al., 2015; Suvorov et al., 2017). SW-sensitive opsins are also relatively diverse, but no UV duplication has been detected across the major families of Odonata. Ancestral state reconstruction estimates the common ancestor of Odonata had an opsin repertoire of one UV, two SW, and six (Futahashi et al., 2015) to ten LW (Suvorov et al., 2017) copies. UV duplications are relatively rare across insects in general (e.g. Carulli et al., 1994; Briscoe et al., 2010; Perry et al., 2017; Sharkey et al., 2017), but with UV color being common among adults (Futahashi 2020) and color being a common (if not the ultimate) signal for species recognition in odonates, it is interesting that there has not yet been a duplication identified among the UV opsins. This is also interesting as it appears that duplications within the SW- and LW-sensitive odonate opsins are commonplace. It could be that SW duplicates are spectrally shifted toward the UV to provide deeper color discrimination in that portion of the visible light spectrum. However, in other insect groups, such as *Heliconius* butterflies, UV duplications have provided deeper discrimination among UV pigments (Briscoe et al., 2010; Bybee, Yuan et al., 2010).

The patterns of selection also vary between the opsin classes. Again, UV exhibits the strongest selection, with SW and LW opsins having more "relaxed" purifying selection pressures. LW exhibits the most relaxed selective pressures, which perhaps explains the larger diversity of LW opsins. Even more interesting is that positive selection is strongest before gene duplication and diffuses toward the terminals of the topology, which suggests that odonate opsins are evolving under the permanent heterozygote model (Suvorov et al., 2017). It is worth noting that duplications within both the SW and LW opsin classes can have very few amino acids and even nucleotide differences, and therefore may share similar function as photopigments within the eye.

The permanent heterozygote model has been proposed for odonate opsin evolution (Suvorov et al., 2017), but the reason for the explosive opsin diversity is still unclear. In other animal groups with large opsin repertoires (e.g. stomatopods) it has been demonstrated physiologically that these opsin repertoires provide extensive sensitivities among the photoreceptors. One of the reasons for the diversity of opsin genes in Odonata may be that both dragonflies and damselflies use opsins in a stage- and region-specific manner (Sharkey 2015; Futahashi et al., 2015; Lancer, Evans, and Wiederman 2020). For example, both SW and LW opsins are the primary visual genes in the eye of nymphs, a likely reflection of the LW shifted light environment of the aquatic nymph compared to terrestrial adults (Sharkey 2015). The adult eye is more complex and is divided into dorsal and ventral regions. The expression of opsins is similarly more complex (Futahashi et al., 2015). For example, UV and SW opsins dominate the dorsal region to maximize the detection of small dark targets against the blue sky (Labhart and Nilsson 1995). There is also evidence from flies (e.g. the aquatic culicids) where opsins are used in motion regulation and chemosensation and play a role in the function of the visual system (Feuda et al., 2021). It may be that physiological evidence is lacking and that Odonata do have a complex color vision system yet to be described. However, the data thus far do not appear to support that odonates use the diversity of opsins to provide extensive photoreceptor sensitivities.

## 3.3 Transcriptomic insight into the eco-evolutionary role of color variation

### 3.3.1 Ecological significance of color variation within and between species

Insect colors are not only beautiful, but also they are extremely diverse, both within and among species. These color patterns can vary in their configuration, luminance, and polarization, and with this variety convey multiple channels of information (Endler and Mappes 2017). For example, many insects (including dragonflies) use colors for physiological adaptations (Cooper 2010), communicating species identification, and distinguishing individual quality (Cuthill et al., 2017). Color diversity and variation is particularly pronounced in odonates, where many species display large inter- and intraspecific color diversity (Bybee et al., unpublished data) that is paralleled by some of the most advanced visual systems among insects (Samways 2008; Bybee et al., 2012; see also Section 2.2). For example, for species-rich odonate families that live in open areas (e.g. ponds, marshes, and streams), body color patterns function both in species recognition and intraspecific communication (Sánchez-Guillén et al., 2020; Wellenreuther et al., 2014). Sexually dimorphic color differences are particularly common within odonate species, and intra- and interspecific interactions in this group are commonly based on both body and wing color phenotypes. It is thus no surprise that colors in Odonata are often implicated in sexual selection via female mate choice (i.e. intersexual selection) and/or male–male competition (Grether 1996, 2015; Sánchez-Guillén et al., 2020). In some species, exuberant color differences segregate even within the same sex of a species, in the form of color polymorphisms as described for the damselflies genera *Argia*, *Enallagma*, and *Ischnura* (Andrés, Sánchez-Guillén, and Rivera 2000; Bybee et al., 2012; Fincke et al., 2005). Perhaps, the best described polymorphism is that of the female-limited and genetically inherited colors of the blue-tailed damselfly *Ischnura elegans*, the males of which are monomorphic in color. However, females of this species fall into one of three phenotypically visible and distinct color morphs: the male-mimicking androchrome morph, and the more cryptic and dull colored infuscans and infuscans-obsolete morph (Sánchez-Guillén et al., 2005). This species belongs to Coenagrionidae, the largest damselfly family, which includes 95 genera and 1,082 species worldwide (Paulson 2009), and of these, over 100 species are color polymorphic (Fincke et al., 2005; Sánchez-Guillén et al., 2020). This polymorphism has been well studied and it is known to affect mate choice and sexual conflict (Svensson, Abbott, and Hardling 2005). Evidence from crossing experiments in several species suggests, including its sister species *I. graellsii*, a genetic basis to color (Cordero 1990; Sánchez-Guillén et al., 2005). These studies were able to demonstrate that color inheritance in *I. elegans* follows a classic Mendelian pattern, involving a few alleles at a single locus (Sánchez-Guillén et al., 2005).

### 3.3.2 Evolution of color phenotypes

The existence of several female color morphs is a conspicuous characteristic of *I. elegans* and other *Ischnura* damselfly species with a female limited color polymorphism. These polymorphisms are typically characterized by one male-like (androchrome) and several non-male-like (gynochrome) morphs. Andromorph females look and often behave like males, whereas gynomorph females exhibit a more cryptic and female-like coloration and behavior. The prevalence of female color indicates the presence of alternative reproductive strategies and is thought to result from sexual conflict over optimal mating rates, where females generally benefit from lower mating rates than males (Van Gossum et al., 1999; Van Gossum, Stoks, and De Bruyn 2001; Sánchez-Guillén et al., 2017). The intense sexual conflict in this group leads to extensive mating harassment and negative frequency-dependent selection, because males form search images for the common morphs, similar to the apostatic selection on common prey by predators, and then this morph is disproportionately targeted. Transcriptome studies have shown that female immune responses are upregulated, indicating that intense male mating harassment may lead to a heightened immune response in females due to injuries, toxins, and related impacts on female health (Chauhan et al., 2016). Androchrome females are often the minority morph, and commonly experience lower levels of male harassment, and studies have shown that they also have a lower fecundity (Sánchez-Guillén et al., 2017). Gynochrome females, in contrast, tend to have higher fecundity and can persist as the majority morph in the population (Sánchez-Guillén et al., 2017), even when extensive mating leads to a fitness and fecundity reduction. The occurrence of alternative strategies in females over time and space in *Ischnura* damselflies allows for a rapid response of the population to the prevalent ecological conditions and consequently, morph frequencies commonly vary across environmental gradients. For example, a latitudinal cline in andromorph morph

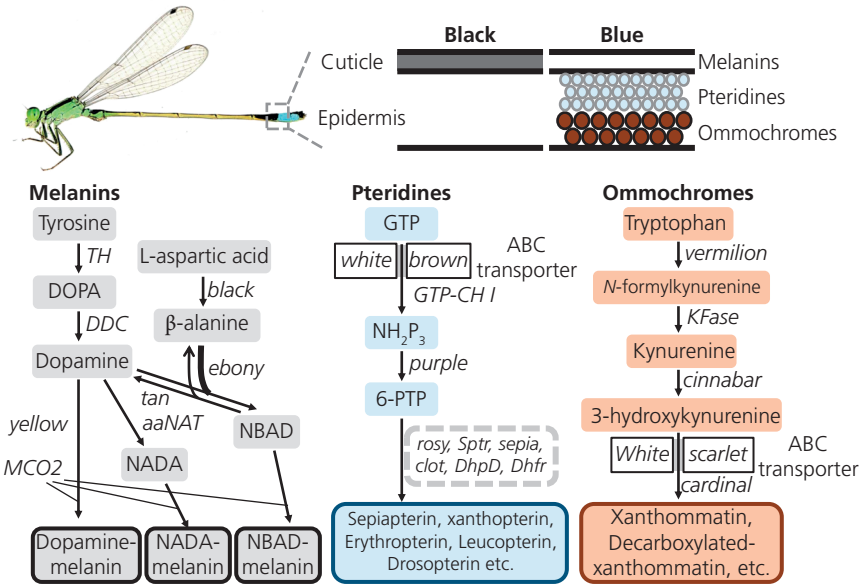


**Figure 3.3 Schematic diagram of black and blue coloration in Odonata.**
Summary of major pigment synthesis pathways in insects. Figure modified from Okude and Futahashi, 2021.

frequency in *Ischnura senegalensis* is a classic signature of natural selection acting on color, with the fitness of andromorphs increasing with latitude implying a gene-by-environment interaction (Takahashi et al., 2011). Transcriptome profiling of *I. elegans* validated the presence of dozens of genes in three pigmentation pathways: the pteridine, melanin, and ommochrome (Figure 3.3), thereby providing a good transcriptomic resource for future work on color polymorphisms (see Figure 5 from Chauhan et al., 2014 for more detail).

### 3.3.3 Pigments

Like other organisms, the colors of Odonata can be classified into pigments and structural colors. The pigments of Odonata are mostly derived from the three major insect pigments: melanins, ommochromes, and pteridines (Figure 3.3) (Okude and Futahashi 2021). Melanins are present on the cuticular surface and, in insects, are derived mostly from dopamine (Arakane et al., 2016; Futahashi and Osanai-Futahashi 2021). Black and brown coloration of the wings and body is mostly attributed to melanins, and orange color in some species (e.g. wings of *Mnais* species and female thorax of *Ischnura* species) is also composed of melanin-related pigments (Hooper Tsubaki, and Siva-Jothy 1999; Okude and Futahashi 2021). Ommochromes are the major red pigments in libelullid dragonflies (e.g. *Crocothemis*, *Sympetrum*), and the yellow-to-red color change is controlled by the redox reaction of ommochromes in a sex-specific manner (Futahashi et al., 2012). Ommochromes sometimes play roles in the darkening at lower temperatures by migrating of pigment granules within cells in a temperature-dependent manner (Veron, O'Farrell, and Dixon 1974; Umbers et al., 2014; Okude and Futahashi 2021). Pteridines are present in yellow, green, and light blue colors, and often emit strong fluorescence under UV light (Henze et al., 2019; Futahashi 2020; Okude and Futahashi 2021).

### 3.3.4 Structural colors

Some Odonata species exhibit iridescent structural colors in their wings and/or bodies, which is primarily attributed to the multilayer structure (Stavenga et al., 2012; Nixon, Orr, and Vukusic 2013, 2015; Guillermo-Ferreira et al., 2015, 2019). Pigments also contribute to structural colors, and the accumulation of melanins is required to develop the metallic blue colors in mature males of *Calopteryx* damselflies (Stavenga et al., 2012). Light-blue colors are found in many odonate species, which are structural colors caused by coherent light scattering (Prum, Cole, and Torres 2004). Both ommochromes and pteridines are important for the

light blue coloration (Figure 3.3; Veron et al., 1974; Prum et al., 2004; Henze et al., 2019; Okude and Futahashi 2021). From the simulations of the reflection spectrum, light blue coloration is predicted by coherent scattering of pteridine-containing nanospheres, and color saturation and brightness increase by absorption of ommochrome-containing granules (Henze et al., 2019). Some odonate species secrete whitish or bluish wax on their surface. These waxes strongly reflect ultraviolet light (Silberglied 1979; Robertson 1984; Futahashi et al., 2019; Futahashi 2020). For example, waxes of libelullid dragonflies are mixtures of long-chain methyl ketones and long-chain aldehydes that form self-assembled light-scattering fine structures (Futahashi et al., 2019).

### 3.3.5 Genes involved in body color formation

The synthetic pathways for melanins, ommochromes, and pteridines are conserved across various insects (Figure 3.3; Futahashi and Osanai-Futahashi 2021). RNA sequencing and genome analysis have identified many of the pigment synthesis genes in Odonata (Chauhan et al., 2014, 2016; Ioannidis et al., 2017; Okude et al., 2017; Feindt et al., 2018). One homologous gene has been identified in Odonata for the majority of pigment synthesis genes (Okude and Futahashi 2021). In the genus *Ischnura*, some studies (Chauhan et al., 2016; Takahashi et al., 2019; Willink et al., 2020) have analyzed genes with differential expression between sexes and/or female morphs. To date, three melanin synthesis genes (*black*, *ebony*, and *yellow*) and one ommochrome synthesis gene (*cinnabar*) were identified as differentially expressed genes. In addition, specific elongation of very long-chain fatty acids (ELOVL) family genes (e.g. there are 17 paralogs in libelullid dragonfly species) was specifically expressed at the wax-producing regions, suggesting its involvement in wax synthesis (Futahashi et al., 2019). Recently, RNAi experiments have revealed that the *multicopper oxidase 2* (*MCO2*) gene, which is essential for melanin synthesis, is necessary for black and orange color formation (Okude et al., 2017; Okude et al., 2021; Okude and Futahashi 2021). It should be noted that the *MCO2* gene RNAi does not affect light blue or green coloration (Okude et al., 2021; Okude and Futahashi 2021).

## 3.4 Embryogenesis

Embryogenesis, the central life cycle, is a continuum of morphological changes, starting with blastoderm formation, germ-band formation, followed by elongation, segmentation, and appendage formation. Insect embryonic patterning can be distinguished into long, intermediate, and short germ-band types according to the time segments are specified during embryogenesis (Davis and Patel 2002; Liu and Kaufman 2005). Most insects develop as short and intermediate germ-band embryos—which is also considered to be the ancestral type of embryogenesis in insects, where only anterior segments, typically the head and thoracic regions, are initially specified during the blastoderm stage. Posterior segments sequentially develop from anterior to posterior as the germ band elongates (Tautz, Friedrich, and Schröder 1994; Nakagaki, Sakuma, Machida 2015). In contrast, long germ-band segmentation, where all segments develop simultaneously at the blastoderm stage before the embryo elongates, occurs only in holometabolous insects.

Odonata display an intermediate germ-band type (Sander 1976) where an anterior stretch of the germ anlage subdivides rapidly to yield the anterior segments. The newly formed embryo starts to elongate and differentiates into the anterior broad protocephalon and posterior protocorm (rest of the embryo). The remaining segments are added successively (Sander 1996) from a cellularized region at the posterior of the embryo, the "growth zone" (Tautz et al., 1994). The following describes briefly major odonate embryonic stages based on morphological characteristics of the embryo according to Ando (1962) and Suzuki, Watanabe, and Tojo (2020).

1. Egg cleavage: Fertilized eggs undergo the typical superficial cleavage. After several divisions, nuclei approach the periphery of the egg and differentiated yolk nuclei migrate from the periphery to position in the central yolk.
2. Blastoderm formation: Cleavage nuclei at the egg periphery undergo repeated mitotic divisions to increase their number and finally cover the whole egg surface to form the blastoderm.
3. Germ disc formation: The number of posterior cells increase remarkably and differentiate into the germ disc. Anterior cells of the blastoderm form the extraembryonic areas and the future serosa.
4. Embryo elongation and invagination (anatrepsis): The newly formed embryo elongates and invaginates into the yolk with its posterior end (anatrepsis). At this stage the amnion production also continues, and the amnio-serosal fold develops. At the end of anatrepsis the rudiments of labrum, antenna, and the three thoracic segments have been formed.
5. Segmentation of the embryo: Segmentation of the embryo continues, and the embryonic labrum develops into the "labial mask." The abdomen

becomes entirely segmented and the appendages of the embryo develop remarkably. The embryo has reached its maximum length.
6. Katatrepsis (revolution): In all Odonata species this process occurs midway in embryonic development. The previously separated amnion and serosa fuse over the embryo's head and rupture. The embryo moves along the ventral surface of the egg toward the anterior pole, and the antero-posterior axis of the embryo reverses.
7. Development in the post-revolution stage: Dorsal closure continues, and the labium and the thoracic appendages become segmented. As the embryo proceeds to develop, the yolk sac shrinks and is absorbed into the embryo. The head becomes sclerotized and compound eyes have fully developed. Shortly before hatching, the embryo starts to actively move, and the tracheal system differentiates.

The duration of the embryonic development is species-specific and also depends on external factors such as water temperature (Koch 2015; Mendonca et al., 2018). In addition, embryonic diapause in hibernating species can occur at different developmental stages, the embryo hibernates before katatrepsis, or the full-grown embryo hibernates in the egg (Ando 1962).

### 3.4.1 Gene expression during embryogenesis

In contrast to the extensive work describing morphologically embryonic stages of several odonate species (e.g. by Ando 1962 and references therein and the recent work of Suzuki et al., 2020), almost no gene expression data from odonate development is available. In general, only for a handful of odonate species have "omic" studies been performed, such as for *Enallagma hageni* (Shanku, McPeek, and Kern 2013), *Ischnura elegans* (Chauhan et al., 2014; Chauhan et al., 2016, Lancaster et al., 2016; Simon et al., 2017), *Coenagrion puella* (Johnston and Rolff 2013), *Megaloprepus caerulatus* (Feindt et al., 2018), *Calopteryx splendens* (Ioannidis et al., 2017), and *Ladona fulva* (unpublished). Indeed, only one study provides transcriptional information across odonate embryogenesis to highlight gene sets involved in morphogenesis (Simon et al., 2017); it analyzed transcriptomic data across the whole embryonic development of the blue-tailed damselfly *Ischnura elegans*. Based on the nine stage-specific embryonic profiles, they identified distinct transcriptional clusters according to early-, mid-, and late embryogenesis, and corresponding characteristic developmental genes. During early embryogenesis several homeobox genes such as *homothorax* and *Ultrabithorax* or genes involved in segmentation such as *hunchback* and *Delta* could be identified. For these developmental genes also more detailed expression patterns—temporal and spatial—are also known for the cricket *Gryllus bimaculatus* (Donoughe and Extavour 2016). In contrast, during the late embryogenesis of *Ischnura elegans* and shortly before the hatching of the embryo, transcriptional active genes are mainly involved in muscle structure and function such as Tropomyosin, Myosin, and Actin.

Undoubtedly, there is an urgent need to further study in depth the temporal and spatial expression patterns of developmental genes in Odonata to understand the transcriptional basis of morphogenetic events that occurred in one of the earliest lineages of winged insects. The blue-tailed damselfly *Ischnura elegans* might represent here a promising new model system. The damselfly can be reared in the laboratory (Van Gossum et al., 2003; Piersanti et al., 2015), belongs to one of the most common groups of damselflies in Europe, and is particularly abundant at eutrophic sites (Svensson et al., 2005; Wellenreuther et al., 2011; Boudot and Kalkman 2015). The life cycle is highly variable and temperature dependent, with coastal populations and the occurrence of a second generation (bivoltinism) having a life cycle of 8–12 weeks (10–22 days of embryonic development) (Van Gossum et al., 2003; Simon et al., 2017). However, life history plasticity is well known in Odonata (Stoks, Johansson, and De Block 2008; Koch 2015).

## 3.5 Phylo-transcriptomics

High-throughput transcriptomics has invigorated evolutionary research of non-model organisms (Ungaro et al., 2017) by producing relatively inexpensive "omic"-sized datasets that can be used for tackling various biological questions (Todd, Black, and Gemmell 2016). Specifically, in the past decade RNA-seq technologies have had a sizable impact on phylogenetics by allowing researchers to produce multi-locus data (Cheon, Zhang, and Park 2020), which are critical for more accurate reconstruction of evolutionary histories. The recent studies estimated large-scale phylogenetic resources (in terms of sequence and taxon sampling) for different groups of organisms using transcriptomic approaches (Misof et al., 2014; One Thousand Plant Transcriptomes Initiative 2019).

Two complimentary phylogenetic efforts using transcriptomic data are currently available (Kohli et al., 2021). Kohli and colleagues (2021) focus on the evolution of oviposition and the relationship of Petaluridae and Gomphidae (see also Chapter 21) and Suvorov and colleagues (2021) produced a phylogenetic estimate of Odonata. The resulting phylogeny allowed for the detection of multiple patterns of deep ancestral introgression within Odonata. Perhaps the most compelling evidence that has been uncovered is for deep ancestral introgression between the Zygoptera and Anisozygoptera, which, based on the fossil record, became genetically isolated after the Lower Jurassic. Species of Anisozygoptera exhibit anatomical characteristics of both Anisoptera and Zygoptera suborders. Some general features of Anisozygoptera that relate them to Zygoptera include dorsal folding of wings during perching in adults, characteristic anatomy of proventriculus (a part of digestive system that is responsible for grinding of food particles), and absence of specific muscle groups in the larval rectal gills, whereas abdominal tergite shape, rear wing geometry and larval structures are similar to Anisoptera. More recent studies also revealed that Anisozygoptera ovipositor morphology shares similarity with Zygoptera; muscle composition of the head resemble characteristics of both Anisoptera and Zygoptera; and thoracic musculature of Anisozygoptera nymphs exhibit similarity between Anisoptera and Zygoptera (Büsse, Helmker, and Hörnschemeyer 2015. Thus, Anisozygoptera represent a morphological and behavioral "intermediate," which is supported by Suvorov and colleagues (2021), who observed consistent recovery of introgression between Zygoptera and Anisozygoptera. Moreover, this study suggests unidirectional introgression where early Anisozygoptera was a recipient taxon from a zygopteran donor, though a lesser degree of gene flow in the opposite direction cannot be ruled out either. Further, the analyses of introgression patterns showed that a sizable fraction (~27–33%) of the Anisozygoptera genome descends from a zygopteran lineage, most likely from members of the subfamily Lestoidea. Taken together, these observations strongly suggest a xenoplasious origin of Anisozygopteran traits (i.e. traits introduced to a recipient taxon via introgression) that are shared with Zygoptera. However, the possibility that some trait hemiplasy may have resulted from Incomplete lineage sorting (ILS) is not fully rejected. Based on the gathered evidence for introgression, Suvorov and colleagues (2021) suggest that ancestral lineages that gave rise to modern-day Anisozygoptera and Zygoptera experienced introgression in their past evolutionary history.

According to the hybrid swarm hypothesis (Seehausen 2004), introgression can trigger a rapid cladogenesis resulting in the establishment of ecologically different species with niche-specific adaptive phenotypes. Some of the notable examples can include adaptive radiations caused by ancestral introgression in cichlid fishes, and intricate inter-specific introgression patterns in big cats that could be linked to rapid diversification of modern-day species in that lineage (Meier et al., 2017; Figueiró et al., 2017). Interestingly, the large fraction of introgressed material estimated within the Anisozygopteran lineage does not appear to have facilitated any adaptive radiation. This proposition is supported by an extremely low species diversification within the suborder (just three extant species) and also by the lack of fossil record for Anisozygoptera.

## 3.6 Future directions

Another major strength of transcriptomes is their ability to be coupled with physiological approaches to establish the function of genes. Establishing the function of genes has always been a focus of genetic research and doing so in wild organisms, such as Odonata, provides deep insight into the evolutionary origin, ecological use, and maintenance of genes in the genome. Coupling physiological data with transcriptomic data in the areas above will undoubtedly lead to findings among odonates that have yet to be unlocked and allow us to fully appreciate the uniqueness of the group. We end by providing some future directions for odonate research that involve transcriptomics. Some involve the research presented above and some ideas are novel for dragonflies, but these ideas are by no means exhaustive. The possibilities within Odonata are endless.

### 3.6.1 Color vision

What is tantalizing, yet completely understudied and untested, is that opsin duplications appear to line up with the diversity of color found across Odonata (Figure 3.4). This is only an observation and there are patterns within the group (e.g. some damselfly visual systems are less complex but display great color diversity) that may argue against the overall pattern of opsin and color diversity seen across Odonata. Coupling additional physiological data gathered from the visual system (e.g. spectral sensitivities, spectral responses, and chromatic processing) in light of current and additional transcriptomic data would likely set up a complex evolutionary history between color vision and color evolution.

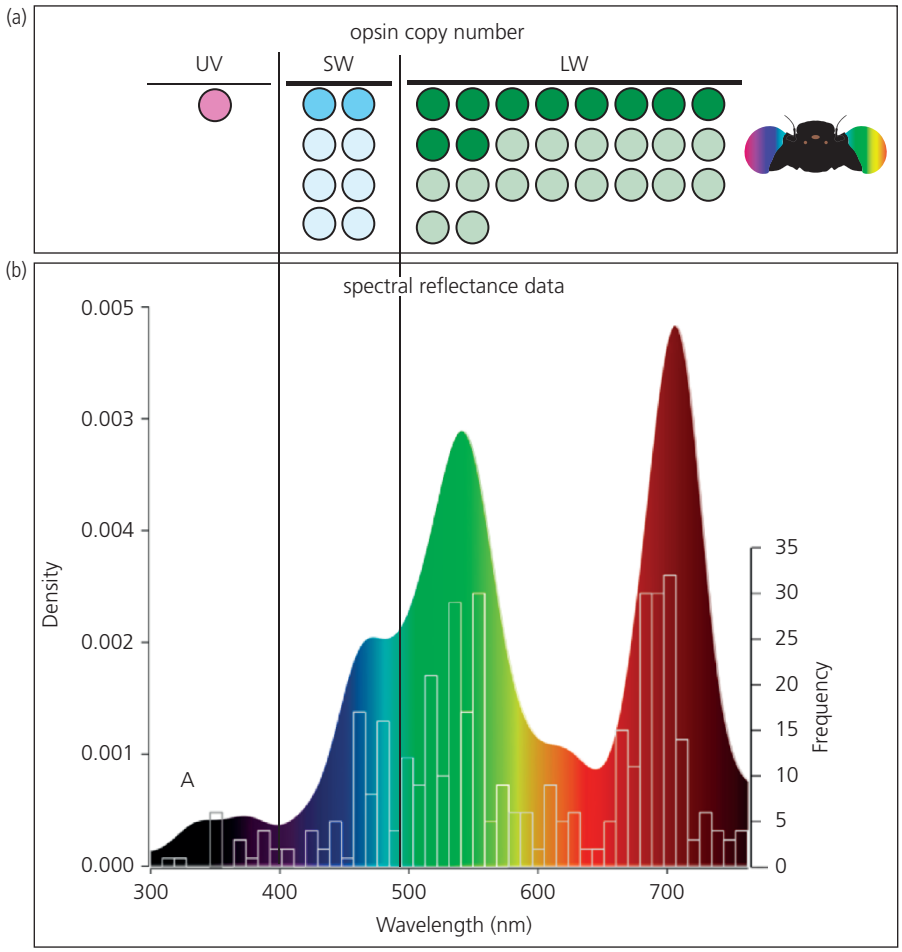


**Figure 3.4 Opsins and odonate color.**
A: The ancestral estimates for each opsin class (full color) along with the estimated maximum number of opsin duplicates per class currently known (transparent color). UV is ultraviolet, SW is short wavelength or blue, and LW is long wavelength. B: Represents the diversity, density, and frequency of colors across the visible light spectrum for 258 odonates from Asia (Vietnam), Africa (Rwanda), and South America (French Guiana) representing the most commonly encountered families. We note only that the relationship between opsin diversity and color diversity are similar. No formal analysis has been performed.

### 3.6.2 Color

An examination of how color has evolved through the identification of not only the genes but also the pathways responsible for the diversity and maintenance of color will have broad ramifications for our understanding of color signaling (i.e. how color may impact both the behavior and overall ecology) among insects, and across the animal kingdom as well. Functional analysis of genes involved in the synthesis of dragonfly ommochromes and pteridines will hopefully uncover genes important for various color formation in Odonata and thus provide a deeper understanding of odonate behavior.

### 3.6.3 Embryogenesis

Comparative transcriptome studies of Odonata embryogenesis and integrating developmental data into evolutionary research is further necessary to elucidate ancient winged insect developmental mechanisms. Current research supports a dual evolutionary origin of insect wings, including dorsal limb branch induction and margin outgrowth. However, in-depth

transcriptional studies—temporal or spatial—in one of the oldest winged insect orders, the Odonata, are almost completely lacking. Comparative transcriptome studies would therefore represent a rich and promising area of research and would allow us to polarize ancestral differences in gene expression and the origin of insect wings. In addition, Odonata are a promising indicator species to monitor freshwater ecosystems, one of the most endangered ecosystems in the world as a result of anthropogenic interference like pollution. Exploring transcriptional plasticity across developmental stages also in relation to anthropogenic factors could further help to gain a better understanding of how and when species can respond to environmental change (Anastasiadi et al., 2021).

### 3.6.4 Phylogenomics

Whole genome data have the advantage that next to primary sequence information, phylogenetic information from meta-characters such as intron positions, near intron pairs, domain arrangements, molecule structure, methylation patterns, and repetitive elements can be collected. However, there is still a lack of odonate genomic resources (Bybee et al., 2016; Hotaling, Kelley, and Frandsen 2020; Hotaling et al., 2021) and the (at least) tenfold difference in genome size between Odonate species further challenges high-quality assembled and annotated genomes. Therefore, transcriptome level data, particularly that accompanied by genomes, will further allow for exceptional insights not only into phylogeny but also in the evolutionary mechanisms (i.e. introgression, ILS) that have shaped the group. Furthermore, relationships that have been historically difficult to resolve may become resolved, or at least transcriptomic data will provide insight into their evolutionary history and why certain portions of the odonate tree of life may never be resolved (i.e. a phylogenetic representation of relatedness is not an accurate representation of evolution).

## 3.7 Conclusion

This chapter outlined a few research areas where odonates have gained traction as a result of transcriptome data. There are more that could be included and certainly there are many, many more to be developed. Odonates have a unique phylogenetic position among insects and contain subordinal groups that make for exceptional comparative research. Transcriptomes should play a central role in such studies and offer unique vantage points that no other data type, including genomes, can offer.

## Acknowledgments

We thank Alex Córdoba-Aguilar, Christopher Beatty, Jason Bried, and Catalina Suárez-Tovar for the invitation to write this chapter. We thank two reviewers, Dr Rosa Ana Sánchez Guillen and Dr. Jessica Ware, who provided thoughtful reviews and helpful suggestions to improve this chapter. We are grateful for members of the odonate community throughout the world for years of spirited discussion as we have developed the research addressed in this chapter. We are also grateful to members of our labs that offered hard work, insight, and data generation. This chapter was supported by a grant from the National Science Foundation to SMB (DEB-2002432).

## References

Anastasiadi, D., Venney, C., Bernatchez, L., and Wellenreuther, M. (2021). Generational signatures of epigenomic variation in plant and animals. *Trends in Ecology & Evolution* **36**(12): 1124–40.

Andō, H. (1962). *Comparative embryology of Odonata with special reference to a relic dragonfly* Epiophlebia superstes Selys. Japan Society for the Promotion of Science, Tokyo.

Andrés, J. A., Sánchez-Guillén, R. A., and Rivera, A. C. (2000). Molecular evidence for selection on female color polymorphism in the damselfly *Ischnura graellsii*. *Evolution* **54**(6): 2156–61.

Arakane, Y., Noh, M. Y., Asano, T., and Kramer, K. J. (2016). Tyrosine metabolism for insect cuticle pigmentation and sclerotization. In: *Extracellular composite matrices in arthropods*. Cohen, E. and Moussian, B. (eds), pp. 165–220. Springer, Cham.

Boudot, J. P. and Kalkman, V. J. (2015). *Atlas of the European dragonflies and damselflies*. KNNV Publishing, Zeist.

Brawand, D., Soumillon, M., Necsulea, A., Julien, P., Csárdi, G., Harrigan, P., ... and Van Gossum, H. (2011). Thermal plasticity in life-history traits in the polymorphic blue-tailed damselfly, *Ischnura elegans*: no differences between female morphs. *Journal of Insect Science* **11**(1): 112. https://doi.org/10.1673/031.011.11201

Briscoe, A. D., Bybee, S. M., Bernard, G. D., Yuan, F., Sison-Mangus, M. P., Reed, R. D., ... and Chiao, C. C. (2010). Positive selection of a duplicated UV-sensitive visual pigment coincides with wing pigment evolution in Heliconius butterflies. *Proceedings of the National Academy of Sciences of the United States of America* **107**(8): 3628–33.

Büsse, S., Helmker, B., and Hörnschemeyer, T. (2015). The thorax morphology of Epiophlebia (Insecta: Odonata) nymphs–including remarks on ontogenesis and evolution. *Scientific Reports* **5**(1): 1–14.

Bybee, S., Córdoba-Aguilar, A., Duryea, M. C., Futahashi, R., Hansson, B., Lorenzo-Carballa, M. O., Schilder, R., Stoks, R., Suvorov, A., Svensson, E. I., Swaegers, J., Takahashi, Y., Watts, P. C., and Wellenreuther, M. (2016). Odonata

(dragonflies and damselflies) as a bridge between ecology and evolutionary genomics. *Frontiers in Zoology* **13**(1): 1–20.

Bybee, S. M., Johnson, K. K., Gering, E. J., Whiting, M. F., and Crandall, K. A. (2012). All the better to see you with: a review of odonate color vision with transcriptomic insight into the odonate eye. *Organisms Diversity & Evolution* **12**(3): 241–50.

Bybee, Seth M., et al. "UV photoreceptors and UV-yellow wing pigments in Heliconius butterflies allow a color signal to serve both mimicry and intraspecific communication." *The American Naturalist* 179.1 (2012): 38–51.

Carulli, J. P., Chen, D.-M., Stark, W. S., and Hartl, D. L. (1994). Phylogeny and physiology of Drosophila opsins. *Journal of Molecular Evolution* **38**: 250–62.

Chauhan, P., Hansson, B., Kraaijeveld, K., de Knijff, P., Svensson, E. I., and Wellenreuther, M. (2014). De novo transcriptome of Ischnura elegans provides insights into sensory biology, colour and vision genes. *BMC Genomics* **15**(1): 1–14.

Chauhan, P., Wellenreuther, M., Hansson, B. (2016). Transcriptome profiling in the damselfly Ischnura elegans identifies genes with sex-biased expression. *BMC Genomics* **17**(1): 1–12.

Cheon S., Zhang J., and Park C. (2020). Is phylotranscriptomics as reliable as phylogenomics? *Molecular Biology and Evolution* **37**: 3672–83.

Cooper, I. A. (2010). Ecology of sexual dimorphism and clinal variation of coloration in a damselfly. *The American Naturalist* **176**(5): 566–72.

Cordero, A. (1990). The inheritance of female polymorphism in the damselfly *Ischnura graellsii* (Rambur) (Odonata: Coenagrionidae). *Heredity* **64**: 341–6.

Cuthill, I. C., Allen, W. L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M. E., Hill, G. E., Jablonski, N. G., Jiggins, C. D., Kelber, A., Mappes, J., Marshall, J., Merrill, R., Osorio, D., Prum, R., Roberts, N. W., Roulin, A., Rowland, H. M., Sherratt, T. N., Skelhorn, J., Speed, M. P., Stevens, M. P., Caswell Stoddard, M., Stuart-Fox, D., Talas, L., Tibbetts, E., and Caro, T. (2017). The biology of color. *Science* **357**(6350): eaan0221.

Davis, G. K. and Patel, N. H. (2002). Short, long, and beyond: molecular and embryological approaches to insect segmentation. *Annual Review of Entomology* **47**(1): 669–99.

Donoughe, S. and Extavour, C. G. (2016). Embryonic development of the cricket Gryllus bimaculatus. *Developmental Biology* **411**(1): 140–56.

Endler, J. A. and Mappes, J. (2017). The current and future state of animal coloration research. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372**(1724): 20160352.

Feindt, W., Oppenheim, S. J., DeSalle, R., Goldstein, P. Z., and Hadrys, H. (2018). Transcriptome profiling with focus on potential key genes for wing development and evolution in Megaloprepus caerulatus, the damselfly species with the world's largest wings. *PloS One* **13**(1): e0189898.

Feuda, R., Goulty, M., Zadra, N., Gasparetti, T., Rosato, E., Pisani, D., Rizzoli, A., Segata, N., Ometto, L., and Rota-Stabelli, O. (2021). Phylogenomics of opsin genes in Diptera reveals lineage-specific events and contrasting evolutionary dynamics in Anopheles and Drosophila. *Genome Biology and Evolution* **13**(8): evab170. doi:10.1093/gbe/evab170

Figueiró, H. V., Li, G., Trindade, F. J., Assis, J., Pais, F., Fernandes, G., Santos, S. H. D., Hughes, G. M., Komissarov, A., Antunes, A., Trinca, C. S., Rodrigues, M. R., Linderoth, T., Bi, K., Silveira, L., Azevado, F. C. C., Kantek, D., Ramalho, E., Brassaloti, R. A., Villela, P. M. S., Nunes, A. L. V., Teixeira, R. H. F., Morato, R. G., Loska, D., Saragüeta, P., Gabaldón, T., Teeling, E. C., O'Brien, S. J., Nielsen, R., Coutinho, L. L., Oliveira, G., Murphy, W. J., and Eizirik, E. (2017). Genome-wide signatures of complex introgression and adaptive evolution in the big cats. *Science Advances* **3**(7): e1700299.

Fincke, O. M., Jödicke, R., Paulson, D. R., and Schultz, T. D. (2005). The evolution and frequency of female color morphs in Holarctic Odonata: why are male-like females typically the minority? *International Journal of Odonatology* **8**(2): 183–212.

Futahashi, R. (2020). Diversity of UV reflection patterns in Odonata. *Frontiers in Ecology and Evolution* **8**: 201.

Futahashi R., Kawahara-Miki R., Kinoshita M., Yoshitake K., Yajima S., Arikawa K., and Fukatsu T. (2015). Extraordinary diversity of visual opsin genes in dragonflies. *Proceedings of the National Academy of Sciences of the United States of America* **112**: E1247–56.

Futahashi R., Kurita R., Mano H., and Fukatsu T. (2012). Redox alters yellow dragonflies into red. *Proceedings of the National Academy of Sciences of the United States of America* **109**(31): 12626–31.

Futahashi, R, Osanai-Futahashi, M. (2021). Pigments in insects. In: *Pigments, pigment cells, pigment patterns*. Hashimoto, H., Goda, M., Futahashi, R., Kelsh, R., and Akiyama, T. (eds), pp. 3–43. Springer, Singapore.

Futahashi R., Yamahama Y., Kawaguchi M., Mori N., Ishii D., Okude G., Hirai Y., Kawahara-Miki R., Yoshitake K., Yajima S., Hariyama T., and Fukatsu T. (2019). Molecular basis of wax-based color change and UV reflection in dragonflies. *eLife* **8**: e43045.

Grabherr M. G., Haas B. J., Yassour M., Levin J. Z., Thompson D. A., Amit I., Adiconis X., Fan L., Raychowdhury R., Zeng Q., Chen Z., Mauceli E., Hacohen N., Gnirke A., Rhind N., di Palma F., Birren B. W., Nusbaum C., Lindblad-Toh K., Friedman N., and Regev A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nature Biotechnology* **29**: 644–52.

Grether, G. F. (1996). Intrasexual competition alone favors a sexually dimorphic ornament in the rubyspot damselfly *Hetaerina americana*. *Evolution* **50**(5): 1949–57.

Grether, G. F., Drury, J. P., Berlin, E., and Anderson, C. N. (2015). The role of wing coloration in sex recognition and

competitor recognition in rubyspot damselflies (*Hetaerina spp*.). *Ethology* **121**(7): 674–85.

Guillermo-Ferreira, R., Bispo, P. C., Appel, E., Kovalev, A., and Gorb, S. N. (2015). Mechanism of the wing colouration in the dragonfly *Zenithoptera lanei* (Odonata: Libellulidae) and its role in intraspecific communication. *Journal of Insect Physiology* **81**: 129–36.

Guillermo-Ferreira, R., Bispo, P. C., Appel, E., Kovalev, A., and Gorb, S. N. (2019). Structural coloration predicts the outcome of male contests in the Amazonian damselfly *Chalcopteryx scintillans* (Odonata: Polythoridae). *Arthropod Structure and Development* **53**: 100884.

Henze, M. J., Lind, O., Wilts, B. D., and Kelber, A. (2019). Pterin-pigmented nanospheres create the colours of the polymorphic damselfly Ischnura elegans. *Journal of the Royal Society Interface* **16**(153): 20180785

Hodgins-Davis, A. and Townsend, J. P. (2009). Evolving gene expression: from G to E to G×E. *Trends in Ecology & Evolution* **24**: 649–58.

Hooper, R. E., Tsubaki, Y., and Siva-Jothy, M. T. (1999). Expression of a costly, plastic secondary sexual trait is correlated with age and condition in a damselfly with two male morphs. *Physiology and Entomology* **24**: 364–9.

Hotaling, S., Kelley, J. L., and Frandsen, P. B. (2020). Aquatic insects are dramatically underrepresented in genomic research. *Insects* **11**(9): 601.

Hotaling, S., Sproul, J. S., Heckenhauer, J., Powell, A., Larracuente, A. M., Pauls, S. U., Kelley, J. L., and Frandsen, P. B. (2021). Long-reads are revolutionizing 20 years of insect genome sequencing. *Genome Biology and Evolution* **13**(8): evab138. doi:10.1093/gbe/evab138

Huang, S., Chiou, T., Marshall, J., and Reinhard, J. (2014). Spectral sensitivities and color signals in a polymorphic damselfly. *PLoS One* **9**: e87972.

Ioannidis, P., Simao, F. A., Waterhouse, R. M., Manni, M., Seppey, M., Robertson, H. M., Misof, B., Niehuis, O., and Zdobnov, E. M. (2017). Genomic features of the damselfly *Calopteryx splendens* representing a sister clade to most insect orders. *Genome Biology and Evolution* **9**(2): 415–30.

Johnston, P. R., Mikolajewski, D. J., and Rolff, J. (2015). Identification of viruses associated with larvae of the dragonfly *Leucorrhinia dubia*, and damselfly *Coenagrion puella* from RNA sequencing data. *International Journal of Odonatology* **18**: 81–8.

Johnston, P. R. and Rolff, J. (2013). Immune- and wound-dependent differential gene expression in an ancient insect. *Developmental and Comparative Immunology* **40**(3–4): 320–4.

Koch, K. (2015). Influence of temperature and photoperiod on embryonic development in the dragonfly *Sympetrum striolatum* (Odonata: Libellulidae). *Physiological Entomology* **40**(1): 90–101.

Kohli, M., Letsch, H., Greve, C., Béthoux, O., Deregnacourt, I., Liu, S., Zhou, X., Donath, A., Mayer, C., Podsiadlowski, L., Gunkel, S., Machida, R., Niehuis, O., Rust, J., Wappler, T., Yu, X., Misof, B., and Ware, J. (2021). Evolutionary history and divergence times of Odonata (dragonflies and damselflies) revealed through transcriptomics. *iScience* **24**: 103324.

Labhart, T. and Nilsson, D.-E. (1995). The dorsal eye of the dragonfly Sympetrum: specializations for prey detection against the blue sky. *Journal of Comparative Physiology A* **176**: 437–53. doi:10.1007/BF00196410

Lancaster, L. T., Dudaniec, R. Y., Chauhan, P., Wellenreuther, M., Svensson, E. I., and Hansson, B. (2016). Gene expression under thermal stress varies across a geographical range expansion front. *Molecular Ecology* **25**(5): 1141–56.

Lancer, B. H., Evans, B. J. E., and Wiederman, S. D. (2020). The visual neuroecology of Anisoptera. *Current Opinion in Insect Science* **42**: 14–22.

Laughlin, S. B. (1976). The sensitivities of dragonfly photoreceptors and the voltage gain of transduction. *Journal of Comparative Physiology A* **111**: 221–47. doi:10.1007/BF00606466

Liu, P. Z. and Kaufman, T. C. (2005). Short and long germ segmentation: unanswered questions in the evolution of a developmental mode. *Evolution & Development* **7**(6): 629–46.

Marden, J. H. (2008). Dragonfly flight performance: a model system for biomechanics, physiological genetics, and animal competitive behaviour. In: *Dragonflies and damselflies. Model organisms for ecological and evolutionary research*. Córdoba-Aguilar, A. (ed), pp. 249–61. Oxford University Press, Oxford.

McManus, C. J., Coolon, J. D., Duff, M. O., Eipper-Mains, J., Graveley, B. R., and Wittkopp, P. J. (2010). Regulatory divergence in Drosophila revealed by mRNA-seq. *Genome Research* **20**(6): 816–25.

Meier, J. I., Marques, D. A., Mwaiko, S., Wagner, C. E., Excoffier, L., and Seehausen, O. (2017). Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nature Communications* **8**(1): 1–11.

Mendonca, F. Z., Bernardy, J. V., Oliveira, C. E. K., Oliveira, P. B. G., and De Marco, P. (2018). Temperature effect on the development of tropical dragonfly eggs. *Neotropic Entomology* **47**(4): 484–91.

Misof, B., Liu, S., Meusemann, K., Peters, R. S., Donath, A., Mayer, C., Frandsen, P. B., Ware, J., Flouri, T., Beutel, R. G., Niehuis, O., Petersen, M., Izquierdo-Carrasco, F., Wappler, T., Rust, J., Aberer, A. J., Aspöck, U., Aspöck, H., Bartel, D., Blanke, A., Berger, S., Böhm, A., Buckley, T. R., Calcott, B., Chen, J., Friedrich, F., Fukui, M., Fujita, M., Greve, C., Grobe, P., Gu, S., Huang, Y., Jermiin, L. S., Kawahara, A. Y., Krogmann, L., Kubiak, M., Lanfear, R., Letsch, H., Li, Y., Li, Z., Li, J., Lu, H., Machida, R., Mashimo, Y., Kapli, P., McKenna, D. D., Meng, G., Nakagaki, Y., Navarrete-Heredia, J. L., Ott, M., Ou, Y., Pass, G., Podsiadlowski, L., Pohl, H., von Reumont, B. M., Schütte, K., Sekiya, K., Shimizu, S., Slipinski, A., Stamatakis, A., Song, W., Su, X., Szucsich, N. U., Tan, M., Tan, X., Tang, M., Tang, J., Timelthaler, G., Tomizuka, S., Trautwein, M., Tong, X., Uchifune, T., Walzl, M. G., Wiegmann, B. M.,

Wilbrandt, J., Wipfler, B., Wong, T. K., Wu, Q., Wu, G., Xie, Y., Yang, S., Yang, Q., Yeates, D. K., Yoshizawa, K., Zhang, Q., Zhang, R., Zhang, W., Zhang, Y., Zhao, J., Zhou, C., Zhou, L., Ziesmann, T., Zou, S., Li, Y., Xu, X., Zhang, Y., Yang, H., Wang, J., Wang, J., Kjer, K. M., and Zhou, X. (2014). Phylogenomics resolves the timing and pattern of insect evolution. *Science* **346**(6210): 763–7.

Nakagaki, Y., Sakuma, M., and Machida, R. (2015). Expression of engrailed-family genes in the jumping bristletail and discussion on the primitive pattern of insect segmentation. *Development Genes and Evolution* **225**(5): 313–18.

Nixon, M. R., Orr, A. G., and Vukusic, P. (2013). Subtle design changes control the difference in colour reflection from the dorsal and ventral wing-membrane surfaces of the damselfly *Matronoides cyaneipennis*. *Optics Express* **21**: 1479–88.

Nixon, M. R., Orr, A. G., and Vukusic, P. (2015). Wrinkles enhance the diffuse reflection from the dragonfly *Rhyothemis resplendens*. *Journal of the Royal Society Interface* **12**: 20140749.

Okude G., Fukatsu, T., and Futahashi, R. (2021). Electroporation-mediated RNA interference method in Odonata. *Journal of Visualized Experiments* **168**: e61952.

Okude, G. and Futahashi, R. (2021). Pigmentation and color pattern diversity in Odonata. *Current Opinion in Genetics and Development* **69**: 14–20.

Okude, G., Futahashi, R., Kawahara-Miki, R., Yoshitake, K., Yajima, S., and Fukatsu, T. (2017). Electroporation-mediated RNA interference reveals a role of multicopper oxidase 2 gene in dragonfly's cuticular pigmentation. *Applied Entomology and Zoology* **53**(3): 379–87.

One Thousand Plant Transcriptomes Initiative. (2019). One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* **574**: 679–85.

Paulson, D. (2009). *Dragonflies and damselflies of the west*. Princeton University Press, Princeton.

Perry, M., Konstantinides, N., Pinto-Teixeira, F., and Desplan, C. (2017). Generation and evolution of neural cell types and circuits: insights from the Drosophila visual system. *Annual Review of Genetics* **51**: 501–27.

Piersanti, S., Rebora, M., Salerno, G., Cordero-Rivera, A., and Frati, F. (2015). A method for rearing a large number of damselflies (*Ischnura elegans*, Coenagrionide) in the laboratory. *International Journal of Odonatology* **18**(2): 125–36.

Prum, R. O., Cole, J. A., and Torres, R. H. (2004). Blue integumentary structural colours in dragonflies (Odonata) are not produced by incoherent Tyndall scattering. *Journal of Experimental Biology* **207**: 3999–4009.

Robertson, H. M. (1984). Pruinosity in odonates reflects UV. *Notulae Odonatologicae* **2**: 68–9.

Saha, S., Sparks, A. B., Rago, C., Akmaev, V., Wang, C. J., Vogelstein, B., Kinzler, K. W., and Velculescu, V. E. (2002). Using the transcriptome to annotate the genome. *Nature Biotechnology* **20**: 508–12.

Samways, M. J. (2008). Dragonflies as focal organisms in contemporary conservation biology. In: *Dragonflies and damselflies.* Córdoba-Aguilar, A. (ed), pp. 97–108. Oxford University Press, Oxford.

Sánchez-Guillén, R. A., Fadia-Ceccarelli, S., Villalobos, F., Neupane, S., Rivas-Torres, A., Sanmartín-Villar, I., Wellenreuther, M., Bybee, S. M., Velasquez-Vélez, M. I., Realpe, E., Chávez-Ríos, J. R., Dumont, H. J., and Cordero-Rivera, A. (2020). The evolutionary history of colour polymorphism in Ischnura damselflies (Odonata: Coenagrionidae). *Odonatologica* **49**(3/4): 333–70.

Sánchez-Guillén, R. A., Van Gossum, H., and Cordero Rivera, A. (2005). Hybridization and the inheritance of female colour polymorphism in two ischnurid damselflies (Odonata: Coenagrionidae). *Biological Journal of the Linnean Society* **85**(4): 471–81.

Sánchez-Guillén, R. A., Wellenreuther, M., Chávez-Ríos, J. R., Beatty, C. D., Rivas-Torres, A., Velasquez-Velez, M., and Cordero-Rivera, A. (2017). Alternative reproductive strategies and the maintenance of female color polymorphism in damselflies. *Ecology and Evolution* **7**(15): 5592–5602.

Sander, K. (1976). Specification of the basic body pattern in insect embryogenesis. In: *Advances in insect physiology*, **12.** Treherne, J. E., Berridge, M., and Wigglesworth, V. B. (eds), pp. 125–238. Academic Press, Cambridge.

Sander, K. (1996). Pattern formation in insect embryogenesis: The evolution of concepts and mechanisms. *International Journal of Insect Morphology & Embryology* **25**(4): 349–67.

Schilder, R. J. and Marden, J. H. (2007). Parasites, proteomics and performance: effects of gregarine gut parasites on dragonfly flight muscle composition and function. *Journal of Experimental Biology* **210**(24): 4298–4306.

Schultz, Tom D., Christopher N. Anderson, and Laurel B. Symes. "The conspicuousness of colour cues in male pond damselflies depends on ambient light and visual system." *Animal Behaviour* 76.4 (2008): 1357–1364.

Schurch, N. J., Schofield, P., Gierliński, M., Cole, C., Sherstnev, A., Singh, V., Wrobel, N., Gharbi, K., Simpson, G. G., Owen-Hughes, T., Blaxter, M., and Barton G. J. (2016). How many biological replicates are needed in an RNA-seq experiment and which differential expression tool should you use? *RNA* **22**: 839–51.

Seehausen, O. (2004). Hybridization and adaptive radiation. *Trends in Ecology & Evolution* **19**(4): 198–207.

Shanku, A. G., McPeek, M. A., and Kern, A. D. (2013). Functional annotation and comparative analysis of a zygopteran transcriptome. *G3: Genes, Genomes, Genetics* **3**(4): 763–70.

Sharkey, C. R. (2015). *Ontogeny of the vision in the Emperor dragonfly*. PhD Thesis, University of Bristol. ISNI: 0000 0004 5917 3707

Sharkey, C. R., Fujimoto, M. S., Lord, N. P., Shin, S., McKenna, D. D., Suvorov, A., Martin, G. J., and Bybee, S. M. (2017). Overcoming the loss of blue sensitivity through

opsin duplication in the largest animal group, beetles. *Scientific Reports* **7**, 8.

Sherk, T. E. (1978). Development of the compound eyes of dragonflies (Odonata). I. Larval compound eyes. *Journal of Experimental Zoology* **203**: 183–200. doi:10.1002/jez.1402030202

Silberglied, R. E. (1979). Communication in the ultraviolet. *Annual Review of Ecology, Evolution, and Systematics* **10**: 373–98.

Simon, S., Sagasser, S., Saccenti, E., Brugler, M. R., Schranz, M. E., Hadrys, H., Amato, G., and DeSalle, R. (2017). Comparative transcriptomics reveal developmental turning points during embryogenesis of a hemimetabolous insect, the damselfly *Ischnura elegans*. *Scientific Reports* **7**(1): 1–14.

Stavenga, D. G., Leertouwer, H. L., Hariyama, T., De Raedt, H. A., and Wilts, B. D. (2012). Sexual dichromatism of the damselfly *Calopteryx japonica* caused by a melanin-chitin multilayer in the male wing veins. *PloS One* **7**(11): e49743.

Stoks, R., Johansson, F., and De Block, M. (2008). Life-history plasticity under time stress in damselfly larvae. In: *Dragonflies and damselflies*. Córdoba-Aguilar, A. (ed), pp. 39–50. Oxford University Press, Oxford.

Suvorov, A., Jensen, N. O., Sharkey, C. R., Fujimoto, M. S., Bodily, P., Wightman, H. M. C., Ogden, T. H., Clement, M. J., and Bybee, S. M. (2017). Opsins have evolved under the permanent heterozygote model: insights from phylotranscriptomics of Odonata. *Molecular Ecology* **26**: 1306–22.

Suvorov A., Nolte V., Pandey R.V., Franssen S.U., Futschik A., and Schlötterer C. (2013). Intra-specific regulatory variation in *Drosophila pseudoobscura*. *PLoS One* **8**: e83547.

Suvorov, A., Scornavacca, C., Fujimoto, M. S., Bodily, P., Clement, M., Crandall, K. A., Whiting, M. F., Schrider, D. R., and Bybee, S. M. (2021). Deep ancestral introgression shapes evolutionary history of dragonflies and damselflies. *Systematic Biology* **71**(3): 526–46. https://doi.org/10.1093/sysbio/syab063

Suzuki, K., Watanabe, Y., and Tojo, K. (2020). Embryogenesis of the damselfly *Euphaea yayeyamana Oguma* (Insecta: Odonata: Euphaeidae), with special reference to the formation of their larval abdominal gill-like appendages. *Entomological Science* **23**(3): 280–93.

Svensson, E., Abbott, J., and Hardling, R. (2005). Female polymorphism, frequency dependence, and rapid evolutionary dynamics in natural populations. *The American Naturalist* **165**: 567–76.

Takahashi, M., Takahashi, Y., and Kawata, M. (2019). Candidate genes associated with color morphs of female-limited polymorphisms of the damselfly Ischnura senegalensis. *Heredity* **122**(1): 81–92.

Takahashi, Y., Morita, S., Yoshimura, J., and Watanabe, M. (2011). A geographic cline induced by negative frequency-dependent selection. *BMC Evolutionary Biology* **11**(1): 1–11.

Tautz, D., Friedrich, M., and Schröder, R. (1994). Insect embryogenesis–what is ancestral and what is derived? *Development* **1994**(Suppl): 193–9.

Todd, E. V., Black, M. A., and Gemmell, N. J. (2016). The power and promise of RNA-seq in ecology and evolution. *Molecular Ecology* **25**: 1224–41.

Umbers, K. D., Fabricant, S. A., Gawryszewski, F. M., Seago, A. E., and Herberstein, M. E. (2014). Reversible colour change in Arthropoda. *Biology Review* **89**: 820–48.

Ungaro, A., Pech N., Martin, J.-F., McCairns, R. J. S., Mévy, J.-P., Chappaz, R., and Gilles, A. (2017). Challenges and advances for transcriptome assembly in non-model species. *PLoS One* **12**: e0185020.

Van Gossum, H., Sánchez-Guillén, R. A., and Cordero-Rivera, A. (2003). Observations on rearing damselflies under laboratory conditions. *Animal Biology* **53**(1), 37–45. doi: 10.1163/157075603769682567

Van Gossum, H., Stoks, R., and De Bruyn, L. (2001). Frequency-dependent male mate harassment and intraspecific variation in its avoidance by females of the damselfly *Ischnura elegans*. *Behavioral Ecology and Sociobiology* **51**: 69–75.

Van Gossum, H., Stoks, R., Matthysen, E., Valck, F., and De Bruyn, L. U. C. (1999). Male choice for female colour morphs in *Ischnura elegans* (Odonata, Coenagrionidae): testing the hypotheses. *Animal Behavior* **57**(6): 1229–32.

Veron, J. E. N., O'Farrell, A. F., and Dixon, B. (1974). The fine structure of Odonata chromatophores. *Tissue Cell* **6**: 613–26.

Vijay, N., Poelstra J. W., Künstner A., and Wolf J. B. W. (2013). Challenges and strategies in transcriptome assembly and differential gene expression quantification. A comprehensive in silico assessment of RNA-seq experiments. *Molecular Ecology* **22**: 620–34.

Waterhouse, R. M., Seppey M., Simão F. A., Manni M., Ioannidis P., Klioutchnikov G., Kriventseva E. V., and Zdobnov E. M. (2018). BUSCO Applications from quality assessments to gene prediction and phylogenomics. *Molecular Biology and Evolution* **35**: 543–8.

Wellenreuther, M., Sanchez-Guillen, R. A., Cordero-Rivera, A., Svensson, E. I., and Hansson, B. (2011). Environmental and climatic determinants of molecular diversity and genetic population structure in a coenagrionid damselfly. *PLoS One* **6**(5): e20440.

Wellenreuther, M., Svensson, E. I., and Hansson, B. (2014). Sexual selection and genetic colour polymorphisms in animals. *Molecular Ecology* **23**(22): 5398–5414.

Willink, B., Duryea, M. C., Wheat, C., and Svensson, E. I. (2020). Changes in gene expression during female reproductive development in a color polymorphic insect. *Evolution* **74**(6): 1063–81.

Yang, E. C. and Osorio, D. (1991). Spectral sensitivities of photoreceptors and lamina monopolar cells in the dragonfly *Hemicordulia tau*. *Journal of Comparative Physiology A* **169**(6),663–9.

Yang, E. C. and Osorio, D. (1996). Spectral responses and chromatic processing in the dragonfly lamina. *Journal of Comparative Physiology A* **178**(4): 543–50.