SECTION 1

Genomics

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CHAPTER 2

Genomic insights into micro- and macro-evolutionary processes in Odonata

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Overview

Odonata (dragonflies and damselflies) present an unparalleled insect system for investigating biodiversity impacts of environmental change because of their ease of sampling, occupation of environmental gradients, and rapid movement dynamics. In addition, due to their ancient phylogenetic position, research into this order provides fundamental insights into key evolutionary processes such as the evolution of flight, vision, and sexual behavior. The chapter investigates how emerging genomics-based studies have provided novel insights into micro- and macro-evolutionary processes, namely: 1) movement dynamics; 2) local adaptation; and 3) species boundaries. It covers how genomic studies are starting to reveal the micro- and macro-evolutionary processes that underpin form and function in this order. It concludes that future work needs to taxonomically and geographically broaden the species investigated to capture the full extent of diversity in this order, and to enable comparative work.

2.1 Introduction

Odonata (dragonflies and damselflies) present an unparalleled insect system for investigating eco-evolutionary processes (Bybee et al., 2016). With many odonates exhibiting philopatric behavior, narrow habitat requirements, and patchy distributions, the Odonata are an ideal insect taxon to study processes of local adaptation and other responses to environmental change. Furthermore, the diverse color patterns and variation in the capability for dispersal makes odonates valuable for gaining insight into the evolutionary mechanisms underlying geographically structured patterns of morphological variation. With a significant reduction in the costs of next-generation sequencing (NGS) approaches in recent years, reduced representation and whole-genome approaches have become a cost-effective research tool for developing genomic insights into these ecological and evolutionary processes in charismatic non-model taxa (Ellegren 2014), including odonates.

This cost reduction has enabled the study of hitherto little explored questions in Odonata using a genomic lens. Due to the documented range-shifts of many populations in response to shifting thermal regimes (Hickling et al., 2006a, 2006b) much of the research in this area has been done in the demographic context of range expansion, and offers supportive evidence for the use of odonate systems as sentinels of global change. For example, identifying genomic patterns of neutral and adaptive processes across a species' range is a core aspect of predicting which species will persist or perish under current and future environments (Sánchez-Guillén et al., 2016). Recent studies on this and other topics have applied reduced representation sequencing approaches (e.g. RADseq, ddRAD, GBS [see Glossary for an explanation of these and other technical terms]), transcriptomics (e.g. RNAseq), and whole-genome sequencing (Swaegers, Mergeay et al., 2015; Lancaster et al., 2016; Fitt and Lancaster 2017; Dudaniec et al., 2018) to generate genome-wide panels of single nucleotide polymor-

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phisms (SNP) from which to analyze the allelic basis of trait and demographic variation in odonates. These contemporary approaches commonly outperform previously used population genetic methods that used microsatellites or other markers (e.g. RFLPs, AFLPs) due to the high density of data that can be acquired across the genome, and capacity to identify both neutral and adaptive regions, genes or markers (Andrew et al., 2013).

The generation of this new "omic" toolbox has facilitated the generation of data to interrogate the "natural history" of life of Arthropoda (e.g. i5K initiative to sequence 5,000 arthropod genomes). Additionally, increasing whole-genome comparative functional tools can provide novel conclusions about the origins and maintenance of key traits for radiations and major evolutionary transitions. However, while conscious efforts to develop "omic" resources across the *Tree of Life* have been made, major gaps remain, and include aquatic insects generally, and Odonata specifically (Hotaling, Kelley, and Frandsen 2020). Despite the Odonata comprising almost 6,000 extant species, genome assemblies are available for only three odonate species (as of 01 July 2021): scarce chaser *Ladona fulva*, (1.16 Gbp, BioProject PRJNA194433), banded demoiselle *Calopteryx splendens* (1.63 Gbp, Ioannidis et al., 2017) and the blue-tailed damselfly *Ischnura elegans* (1.67 Gbp, Chauhan et al., 2021). Reasons for the lack of odonate genome assemblies are unclear, but may be related to the size of genomes, which commonly exceed 1 Gbp, the high repeat content (e.g. nucleotide sequences that are duplicated), no strong economic justifiers (e.g. invasive and pest species are rare among odonates), and the difficulty of extracting high-genomic weight DNA from individuals.

This chapter provides a summary and synthesis of the insights that genomics-informed studies have provided about the genome-scale architecture of complex traits to disentangle the interplay between selection, gene flow and recombination. It focuses on both micro- and macro-evolutionary processes and genomic responses in Odonata, such as during development and range shifts, and in the maintenance of phenotypic diversity, such as color polymorphisms. The three focus areas in this chapter are: 1) Genomic insights into population processes, including dispersal and connectivity and biogeographic processes, such as range shifts; 2) Local adaptation and adaptive trait evolution; and 3) Genomic variation associated with hybridization and speciation. Each section first outlines the background, including relevant insights from pre-genomic studies, and then goes on to highlight how genomic tools have provided additional support, refuted, or added nuance to our understanding. The chapter ends with a discussion about the general conclusions and the future directions.

2.2 Genomic insights into population processes

2.2.1 Dispersal and connectivity

Genetic connectivity in odonates have a long history of study, and have often used markers from microsatellites, mitochondrial markers, allozymes, and amplified fragment length polymorphisms (AFLPs) to assess barriers to gene flow and identify dispersal corridors. Such population genetic studies overwhelmingly focus on damselflies (Zygoptera), with a few studies from dragonflies (Anisoptera)—the latter often limited to assessing variation in genetic diversity in threatened species (Keller et al., 2010). Genetic studies of dispersal in odonates demonstrate that, being generally strong fliers and typically habitat generalists with rapid (re)colonization rates (De Block et al., 2005), odonates often exhibit weak or absent isolation by distance (Low et al., 2017), though exceptions exist (Watts et al., 2007), but the degree to which this is evident depends on ecological conditions and past biogeographic and demographic processes (Wellenreuther et al., 2011; Johansson et al., 2013). However, genetic isolation by distance may be difficult to capture in snapshot studies, as these signals can fluctuate rapidly in time, under strong extinction-re-colonization dynamics, in many odonate species (Abbott et al., 2008; McCauley et al., 2014).

Many odonates exhibit a conspicuous lack of barriers to dispersal by landscape features such as agricultural areas and human infrastructure (with the exception of urbanization) (Sato et al., 2008; Lorenzo-Carballa et al., 2015) or tree cover (except for species with poor flight ability; Watts, Thompson, and Kemp 2004). However, shorter- versus longer-distance movements might be differentially regulated by fine-scale features of the landscape, such as access to waterways (Purse et al., 2003; Lorenzo-Carballa et al., 2015). Moreover, populations of lentic species may exhibit lower connectivity than lotic species, due to greater habitat patchiness of standing water compared with rivers and streams (Grewe et al., 2013), although this has not been evaluated in odonates using genetic studies. Aspects of climate, such as precipitation, often correlate with genetic structure in damselflies (Wellenreuther et al., 2011; Herzog and Hadrys 2017).

Topographic shifts remain a key exception to odonates' general lack of landscape dispersal barriers, with changes in elevation often posing significant barriers to movement and thus gene flow in some odonates, and mountains in particular posing significant barriers to the connectivity of both lowland (Watts et al., 2005; Iserbyt et al., 2010; Keller et al., 2012) and upland (Torres-Cambas et al., 2017) species. Patterns of population and genetic structure corresponding to topography may be particularly evident in the Zygoptera, which tend to be poorer fliers than dragonflies (Nagy et al., 2019), although differences in landscape barriers to gene flow remain to be formally tested across these two taxonomic groups. Elevational barriers are likely imposed by temperature differentials between lowland and upland areas rather than the topography itself; for example, mountains can be surmounted by lowland species during warmer, interglacial periods (Huang and Lin 2011; Xue et al., 2019). As oceans typically pose a strong barriers to dispersal by zygopterans, damselflies have been used as a model to unravel effects of historic sea level changes on island biogeography (Jordan et al., 2005; Jones and Jordan 2015). Finally, competition may also be important for limiting gene flow in odonates; for instance, a study using AFLPs in sympatric species of *Enallagma* suggests that interspecific reproductive interference may strongly limit colonization at both local and regional scales in odonates (Bourret, McPeek, and Turgeon 2012), and indicating that damselflies in particular are good model systems for understanding the ecological and evolutionary causes and consequences of competitive exclusion.

More recent genomic studies present the opportunity to track spatial processes using a greatly expanded set of genetic markers, enabling high power tests for dispersal and demographic processes impacting connectivity (Balkenhol et al., 2017). Such studies in odonates typically confirm weak isolation by distance (Fitt and Lancaster 2019; Dudaniec et al., 2021), presumably because land cover rarely presents a barrier to dispersal (Fitt and Lancaster 2019; Dudaniec et al., 2021). Using SNP markers from reduced-representation genome sequencing (RAD-seq), Fitt and Lancaster (2019) confirmed that mountains represent a primary dispersal barrier to the damselfly *Ischnura elegans* in Scotland, and that the strongest signals of directional dispersal were to areas of high population density and between climatically similar habitat, suggesting that establishment success is a limiting step of dispersal success in this species, and that temperature overwhelmingly drives this. Dudaniec and colleagues (2021) further studied patterns of landscape resistance to dispersal across a non-mountainous coastal cline of *I. elegans* in the northern part of its range and found complementarily that low temperature posed the greatest barrier to movement. Furthermore, Kahilainen and colleagues (2014), using microsatellite data, and Fitt and colleagues (2019), using RAD-seq data, each found a strong role for intra- and inter-specific density as a predictor of population genetic patterns. Genetic diversity was positively associated with population density (as expected), but Kahilainen and colleagues (2014) show that diversity can also be impacted by density of heterospecific competitors. Fitt and Lancaster (2019) found that genetic diversity was further associated with patterns of gene flow (higher genetic diversity *and* higher population densities exhibited in sites receiving abundant gene flow from multiple sources; Fitt and Lancaster 2019) suggesting density- or competition-dependent settlement behaviors. Genomic studies continue to provide additional insights established from genetic marker approaches, by providing stronger inference from more markers in order to infer finer-scale patterns in individual movements (Dudaniec et al., 2021) and better discrimination of environmental gradients in movement and resistance in response to biotic and abiotic drivers. Genetic and genomic studies alike continue to suggest that temperature and biotic interactions are highly important drivers of landscape connectivity on odonates.

2.2.2 Range shifts and other spatial processes

Odonates are shifting their geographic ranges more rapidly in response to a warming climate than almost any other taxonomic group (Hickling et al., 2006a, 2006b; Hassall and Thompson 2008). Indeed, genetic analyses of odonates had revealed a signal of rapid demographic and spatial expansion (Lorenzo-Carballa et al., 2012; Hassall et al., 2014). As such, odonates are excellent model systems to quantify the ecological and eco-evolutionary processes occurring at a range margin. Such studies enable new insights into how patterns of neutral and adaptive genetic diversity may facilitate or result from very rapid spatial shifts (Wellenreuther et al., 2011; Swaegers, Mergeay et al., 2015; Swaegers, Mergeay et al., 2013).

Typically species' are expected to exhibit declines in genetic diversity but increases in genetic differentiation toward range edges, where populations tend to be more isolated, recently colonized, or subject to stronger colonization-extirpation dynamics (Hewitt 2001; Eckert, Samis, and Lougheed 2008). Loss of genetic diversity and/or an increase in genetic differentiation in populations at range margins is a common features of odonate species (Iserbyt et al., 2010; Swaegers, Mergeay et al., 2013; Swaegers, Mergeay et al., 2014; Dudaniec et al., 2018; Sánchez-Guillén and Ott 2018), although these patterns may be tempered by historic and ongoing range shift processes (Swaegers, Mergeay et al. 2014; Dudaniec et al., 2021). For instance, patterns of declines in genetic diversity and increases in genetic differentiation toward the range edge may be stronger during periods of active range shifting, such as during contemporary climate warming (Iserbyt et al., 2010). Alternatively, declines in genetic diversity toward range edges may be reduced during expansions, given that directional gene flow associated recent colonizations can temporarily increase genetic diversity at the expanding range edge (Fitt and Lancaster 2019; Dudaniec et al., 2021), and the expansion front may further admixed with lineages from multiple sources (Sánchez-Guillén and Ott 2018; Fitt and Lancaster 2019). By comparing core and edge populations in species that are expanding their ranges, microsatellite markers have identified expected patterns of genetic diversity associated with poleward range expansions in *Coenagrion scitulum* (Swaegers, Mergeay et al., 2014) and *Erythromma viridulum* (Watts et al., 2010), but find no signatures of bottlenecks. These studies are consistent with a model of an "expanding wave" or highly admixed pattern of colonization, and indicate a "pushed" rather than "pulled" range expansion process (Dahirel et al., 2021). Thus, odonate range limits appear to be likely set by migration load instead of Allee effects and bottlenecks at range edges (Watts et al., 2010; Swaegers, Mergeay et al., 2013; Swaegers, Mergeay et al., 2015).

More recent studies using genomic methods have supported expected patterns of reduced genetic diversity and increased genetic differentiation in the populations at range margins (Swaegers, Mergeay et al., 2015; Dudaniec et al., 2018; Xue et al., 2019) and provide additional evidence for how these patterns are driven by expansion processes. For instance, the *C. scitulum* range fronts in France, Germany, and Belgium are genetically different, indicating that these represent independent expansion events from a shared gene pool in the core (Swaegers, Mergeay et al., 2015). In contrast, the Scottish range front of *I. elegans* exhibits signals of admixture from multiple colonization routes, originating from the east versus the west of the insurmountable Cairngorm *massif* in the central Scottish Highlands (Fitt and Lancaster 2019). In central Sweden, in contrast, the expanding range front of *I. elegans* exhibits genetic differentiation across climate gradients (Dudaniec et al., 2018). These three contrasting results suggest that the topography, geographic, and environmental gradients over which the expansion wave occurs can strongly influence the degree of genetic differentiation at the front (Möbius et al., 2015).

Use of genomic methods provide additional nuance in understanding spatial genetic patterns of gene flow in odonates (Figure 2.1). For instance, Takahashi and colleagues (2016) used a combination of mitochondrial marker sequencing and reduced-representation DNA sequencing (MIG-seq) to identify genetic patterns associated with distance to geographic range limits in the damselflies, *Ischnura senegalensis* and *Ischnura asiatica*. These species occur in sympatry in part of their distributions; a signal of northward gene flow, perhaps assisted by dominant wind directions, was nonetheless associated with a reduction in genetic diversity at the range edge. This result may imply that gene swamping from core populations limits adaptation in marginal populations. Asymmetrical gene flow toward the north was also observed in Scottish and Swedish populations of *Ischnura elegans* (Fitt and Lancaster 2019; Dudaniec et al., 2021), which is further consistent with this species' recent and rapid poleward range expansion in a northerly direction. Interestingly, in Scotland, mountain ranges, which limit dispersal in many damselflies (mentioned earlier), can limit this gene swamping process (Fitt and Lancaster 2019).

Mapping genomic variants to environmental conditions at range margins (using gene-environment analysis) indicates that dispersal evolution is a strong component of the process of range shifting in odonates (Swaegers, Mergeay et al., 2015; Dudaniec et al., 2018) and that some species may be pre-adapted to range shift by exhibiting high dispersal propensities already in the range core (Swaegers, Mergeay et al., 2015). Intriguingly, Dudaniec and colleagues (2021) found that sex-specific dispersal processes may limit range shifts. Using gene classification analysis of 3,554 putatively neutral SNPs from RAD-seq data to identify populations of origin for migrant individuals, they found that the rapidly advancing poleward range shift in *I. elegans* is predominantly driven by long-distance male migration and provides additional evidence that sex-specific selection and sexual conflict may mediate the rate of range change. These genomic studies therefore leverage copious data from neutral markers to better illuminate spatial patterns and drivers as well as population consequences of range shifts in odonates.

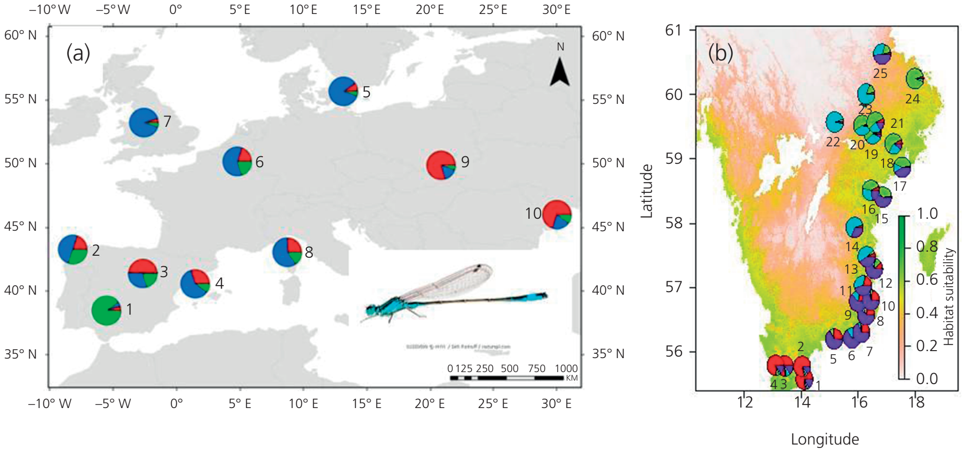

Figure 2.1 Contrasting genetic structure for *Ischnura elegans* from (A) microsatellites at the continental scale, and (B) evidenced from RAD-seq derived SNPs more locally at the Swedish range margin. A: Green indicates hybridizing congener *I. graelsii*, while blue and red represent *I. elegans*; population divergence is relatively low at the continental scale. B: Despite genomic evidence for high gene flow, distinct genetic clusters emerge at the cooler and more climatically variable range margin. Genomic approaches enable sampling a larger number of neutral loci in contrast to traditional markers, providing better fine-scale characterization of neutral genetic diversity (although in this instance the spatial scale of sampling also impacts inference). Figure credits: A: Wellenreuther et al., 2011; B: Dudaniec et al., 2018.

2.3 Adaptation and adaptive trait evolution

2.3.1 Environmental adaptation

Early genetic studies of odonates were very limited in their capacity to detect adaptive genetic variation as they used neutral genetic markers such as microsatellites or AFLPs that were unable to detect loci under selection. However, non-genetic studies previously found phenotypic evidence for selection in odonates (McPeek 1997; Hassall 2013), and local adaptation using reciprocal transplant experiments (Siepielski et al., 2016). Such studies provide evidence that odonates are genetically responsive to local environmental conditions and are likely to be subject to spatially varying selection pressures. The effects of these selection pressures also appear to be readily measured at the molecular and morphological level in odonates (Watts et al., 2007; Alvial et al., 2019). Genetic and genomic studies have added mechanistic detail to these findings and have been increasingly used over the past decade to identify candidate genes under selection from large SNP datasets (Frichot et al., 2015; Ahrens et al., 2018; Forester et al., 2018).

Genomic signatures of adaptive evolution in association with spatially variable thermal regimes has been investigated in two range expanding European damselfly species. In *Coenogrion scitulum*, genotype by sequencing (GBS) data combined with outlier detection approaches found that flight period temperatures were associated with multiple loci under putative selection (Swaegers, Mergeay et al., 2015). *Ischnura elegans* has its northern range edge located in Sweden, and Dudaniec and colleagues (2018) found evidence for rapid adaptation along environmental gradients tracking the species' range expansion. This was observed even under conditions of moderate to high gene flow (Figure 2.2; see also Dudaniec et al., 2018), and a panel of genes under putative selection was identified that were mapped to gene functions associated with increasing temperature tolerance (e.g. heat shock proteins). These genes under selection showed co-varying, non-linear selection signatures that differed in magnitude, indicating environmental thresholds of local adaptation (Dudaniec et al., 2018). In the north-east of Scotland, where *I. elegans* has recently expanded its range, Fitt and Lancaster (2019) identified that mountain ranges limit dispersal and gene swamping to promote local adaptation, suggesting that, counter to some predictions (Hill et al., 2011), adaptation during range shifts may be more likely to occur at elevational than latitudinal range fronts (Fitt and Lancaster 2019). Here, in isolated, high-elevation sites, genome-wide allele frequencies were correlated with landscape-driven spatial heterogeneity in local temperatures.

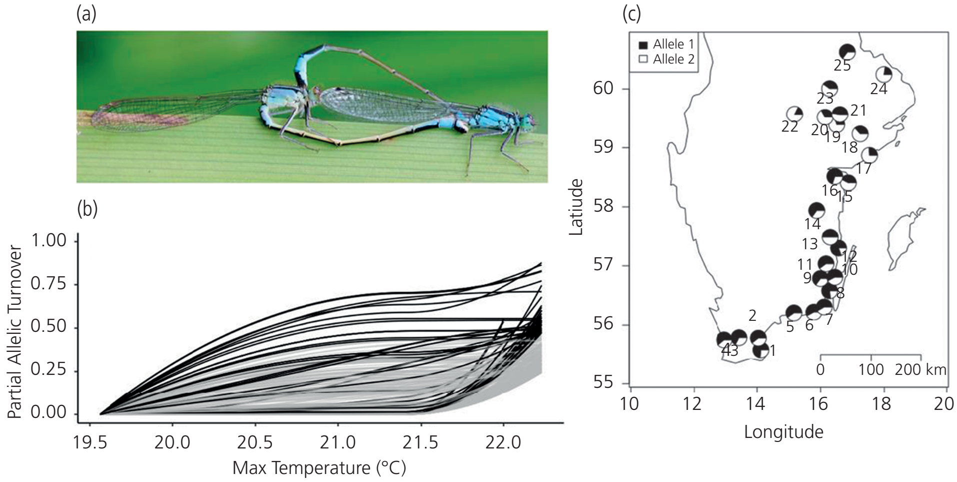

Figure 2.2 A: *Ischnura elegans* shifts in allele frequency (or partial allelic turnover) is shown for putatively adaptive SNPs in response to B: mean maximum summer temperature (°C) along a range expansion gradient in Sweden. C: Allelic turnover for a single SNP that was annotated to Heat Shock Protein 70. Pie charts show the shift in the frequency of each allele within the sampled population from the southern core to the northern range edge in Sweden. Figures are from Dudaniec et al., 2018. Photo credit: Rachael Dudaniec.

An increased plastic response to temperature (i.e. thermal plasticity) toward the range limit has been indicated via differential gene expression analysis (Lancaster et al., 2016). It was found that *I. elegans* adults showed evolutionary "switching" of transcriptomic responses between warmer core and cooler range limit regions (Lancaster et al., 2016). Some of the genes that were differentially expressed were also found to be under selection in response to environmental variables (i.e. temperature, wind speed, precipitation) using SNP data from individuals sampled from the same sites (Dudaniec et al., 2018). In *I. elegans* larvae, Swaegers, Spanier, and Stoks (2020) confirm a role for plasticity in adaptation during range shifts, demonstrating that differential gene expression in response to warming for core versus edge populations of *I. elegans* was better explained by genetic compensation (i.e. evolution to overcome a maladaptive plastic response to novel conditions) rather than genetic assimilation (i.e. evolution to canalize a beneficial plastic response). These studies demonstrate how odonates can contribute toward understanding how plasticity and evolution affect rates of local adaptation in response to climate change.

2.3.2 Morphological adaptation

Extensive morphological diversity within odonates, particularly with respect to body size, color polymorphisms, and sexual dimorphism (Blow, Willink, and Svensson 2020), makes them an obvious subject for examining biotic and abiotic selection on morphology across environmental gradients. Body size metrics have been the focus of some odonate studies investigating spatial selection processes (Taylor and Merriam 1995; Therry et al., 2014). Patterns of neutral genetic diversity and structure have also been reflected in observed patterns of morphological variation within odonate species (Alvial et al., 2019; Vega-Sánchez, Mendoza-Cuenca, and González-Rodríguez 2019), but this congruence is not always clear (Dudaniec et al., 2021) or may be absent (Hassall et al., 2014). While odonates have attracted much interest in determining the genetic basis and evolutionary significance of color polymorphisms, genomic analyses of selection on other morphological traits in odonates have otherwise been scarce. Much work in odonates has been done on sexual size dimorphism in the context of mating systems and sexual selection (reviewed in Bybee et al., 2016), but the genetic basis of sexual size dimorphism, including the role of natural selection pressures, is little known (Svensson, Eroukhmanoff, and Friberg 2006). Observation of reduced sexual size dimorphism toward the cooler poleward range limit in *I. elegans* suggests that environmental conditions may significantly impact the strength of sexual selection and evolution of odonate mating systems (Dudaniec et al., 2021). Several relevant genes for body size and developmental processes have been identified in odonates (D'Amico, Davidowitz, and Nijhout 2001; Chauhan, Wellenreuther, and Hansson 2016), which may serve as targets for studies of adaptive processes in the evolution of sexual size dimorphism.

While the evolution of color polymorphism in odonates and the role of sexual selection is discussed elsewhere in this book, the role of natural selection in maintaining these polymorphisms is little known. Shifts in the frequencies of particular color morphs have been observed along the range expansion axis of *I. elegans* in Sweden and Spain (Gosden, Stoks, and Svensson 2011; Sánchez-Guillén et al., 2011), and these shifts are linked with fitness via color morph-specific thermal tolerances and frequency-dependent social feedback (Lancaster et al., 2017). An earlier study found a latitudinal cline in andromorph morph frequency in *Ischnura senegalensis*, indicative of natural selection acting on color, with a concurrent observation of increasing andromorph fitness with latitude (Takahashi et al., 2011). Despite the importance of sexual selection in the maintenance and evolution of color polymorphisms in odonates (e.g. Andrés, Sánchez-Guillén, and Cordero-Rivera 2000) such studies suggest that environmental variation and change, may lead to new color morph distributions that are the result of a balance between multiple selection pressures. Genomic approaches to detect selection (Ahrens et al., 2018) combined with environmental and ecological data are yet to offer further insights into the sources of selection that maintain color polymorphisms (Rojas et al., 2020).

2.3.3 Life stage-specific adaptation

The complex life-cycles of odonates, which involves the occupation of both aquatic and terrestrial habitats, brings constraints to optimize adaptive responses to contrasting environmental selection pressures (Johansson, Śniegula, and Brodin 2010). Over longer time periods, such responses are subject to the additional effects of climate change, which will vary in impact across these two habitats (Kingsolver et al., 2011). Gene-by-environment interactions, and thus polygenic responses, are therefore likely to be different according to the life stage in odonates (Freda et al., 2019), despite research highlighting how different selection pressures differentially act on genes in larval and adult life stages (Fellous and Lazzaro 2010). By identifying adaptive genes, together with high resolution, life-stage-specific data on environmental conditions, we may ascertain the appropriate life stages and environmental conditions that should be measured when predicting evolutionary responses to changing climates.

2.4 Genomic variation associated with hybridization and speciation

Hybridization has been documented in various odonates (Monetti, Sánchez-Guillén, and Cordero-Rivera 2002; Sánchez-Guillén et al., 2011; Sánchez-Guillén, Córdoba-Aguilar et al., 2014), and the likelihood of species mixing appears to be related to the degree of genetic differentiation between species pairs (Sánchez-Guillén, Córdoba-Aguilar et al., 2013). Such a correlation between the level of reproductive isolation and genetic divergence is a phenomenon that is referred to as the "speciation clock," and has previously been documented in a comprehensive meta-analysis in *Drosophila* fruit flies (Coyne and Orr 1989), and since then in various other groups including birds, frogs, and butterflies (e.g. Presgraves 2002). It has been argued that hybridization in odonates may be linked to the low level of niche differentiation that is commonly observed across many closely related species (Wellenreuther, Larson, and Svensson 2012; Wellenreuther et al., 2018). Indeed, some damselfly radiations are thought to have diversified via non-adaptive processes unrelated to niche exploitation, and thus where reproductive isolation is not intrinsically linked to the build-up of ecological niche diversification (Siepielski et al., 2010; Siepielski et al., 2011, Wellenreuther and Sánchez-Guillén 2016). Candidate odonate genera for non-adaptive radiation include *Enallagma*, *Calopteryx*, and *Ischnura*, which share mostly generalist diets and habitat niches, with large niche overlap between species from the same genus, and yet these species often exhibit strongly divergent sexual behaviors and genital morphologies (Wellenreuther and Sánchez-Guillén 2016). However, it should be noted that non-adaptive and adaptive speciation are not categorical variables and should rather be treated as extremes along a continuum, where allopatric non-adaptive speciation could represent an initial step that might be followed by adaptation to, for example, changing habitats.

Hybridization is thought to be likely for species that show niche conservatism and previously existed in allopatry but are now in the process of expanding their geographic ranges to inhabit new areas of *de novo* sympatry (Sánchez-Guillén, Cordoba-Aguilar et al., 2016). In these areas of contact, hybridization may be likely because learned as well as genetic premating recognition behaviors were not reinforced during the allopatric phase (Wellenreuther et al., 2012; Sánchez-Guillén, Cordoba-Aguilar et al., 2016). The genomic impacts of these hybridization events are starting to become uncovered with the use of affordable sequencing techniques, yielding insights into the processes that facilitate and prevent species formation through a genomics lens. The genomic signatures of hybridization among *I. elegans* and *I. graellsii* in southern Europe were studied in detail by Sanchez-Guillen and colleagues (2021). Extensive and recent niche overlap in the studied *de novo* sympatric zones of this species pair have been fueled by climate-induced range shifts of *I. elegans* into the previously allopatric zone of *I. graellsii* (Wellenreuther et al., 2018), and reproductive isolation between this sister species pair is known to be incomplete (Sánchez-Guillén et al. 2012; Sánchez-Guillén, Córdoba-Aguilar et al., 2014). In these newly created sympatric zones, hybridization and subsequent introgression have become chronic, particularly when population densities are high (Sánchez-Guillén et al., 2011). Through the use of a detailed genome-wide SNP panel, Sanchez-Guillen and colleagues (2021) were able to partition species-specific and shared SNPs to allopatric and sympatric populations of the two introgressing species. Using over 5,000 SNP markers in replicated populations across three Spanish hybrid zones they were able to detail evidence for chronic and ongoing hybridization between the species, and individual introgression analysis of species-specific SNPs showed a high degree of bidirectional backcrossing in the hybrid regions (e.g. via the presence of F_2 hybrids). The study also revealed that the older hybrid zone showed higher levels of genetic mixing, as demonstrated by the almost completed absence of "pure" species compared to the younger hybrid zone (Sanchez-Guillen et al., 2021). This finding is in line with the extent and directionality of the introgression being consistent with the intensity of the reproductive barriers in each hybrid zone (Sánchez-Guillén et al., 2011). Further investigation of fixed SNP markers in each hybrid zone revealed a surprisingly low level of parallelism when compared to other species groups such as Tilapia (Syaifudin et al., 2019). While only relatively few shared fixed SNPs could be identified, these fixed SNPs provide important tentative candidates for further investigation, as these may link to genomic areas that behave neutrally or pinpoint to areas that are mutually advantageous, as such alleles are expected to introgress easily (Harrison and Larson 2014). Identification of such advantageous genomic regions may lead to improved insights into the genomic underpinnings that improve performance in these habitats, and further work could test if these originated from *I. elegans* and allowed this species to expand its habitat over recent years. Conversely, SNPs under divergent selection or responsible for reproductive isolation are predicted show little or no introgression. The identification of both unique and in parallel fixed alleles together creates an important future platform to further investigate the genomic underpinnings of introgression in this species pair. Evidence is also emerging that introgression among other odonate species is more common than was historically thought. Using a phylogenomics approach to understand patterns of introgression along the evolutionary history of Odonata, Suvorov and colleagues (2022) demonstrated that introgression is a pervasive evolutionary force across various taxonomic levels within this order.

2.4.1 Insights from genome assemblies into species- and order-specific functional traits

Insights into odonate evolution have also been gained with the assembly of the first odonate genomes offering new opportunities for molecular-level evolutionary, ecological, and behavioral studies. Importantly, these assemblies lend themselves to comparative analyses because they represent non-holometabolan insects and thus occupy an early-branching phylogenetic position within insects in general and within winged insects in particular, making them valuable for comparative studies (Grimaldi and Engel 2005).

The first odonate genome was sequenced in 2013 by the i5K-pilot project and was that of the dragonfly *Ladona fulva* (Family: Libellulidae). This first genome provided an important NCBI data resource for researchers wishing to conduct comparative work. Subsequent genome assemblies were conducted on the damselflies *Calopteryx splendens* and *Ischnura elegans*, with both assemblies being analyzed in a comparative framework. The *C. splendens* (Family: Calopterygidae) genome analyses provided the first publicly available genome of a paleopteran and highlighted certain interesting aspects of the biology of this insect, such as the discovery of a cytochrome P450 CYP enzyme that has not been previously found in insects (Ioannidis et al., 2017). Overall, the analyses identified a relatively small repertoire of detoxification genes, which could explain the species' previously noted sensitivity to habitat pollution (Ioannidis et al., 2017). Another equally interesting aspect of the *C. splendens* genome was that the genome contained few odorant receptors (ORs) and a large complement of species-specific gustatory receptors. Analysis of chemosensory genes revealed the presence of both gustatory and ionotropic receptors, as well as the insect odorant receptor coreceptor gene (Orco), but no partner ORs. Since formation of an OR/Orco complex is generally thought to be indispensable for odor perception in insects (e.g. for the detection of predators), this finding is intriguing. The *C. splendens* immune gene repertoire appears relatively complete and features several genes encoding novel multi-domain peptidoglycan recognition proteins. Moreover, the immunity-related proteins belonging to the PGRP family appear to have a peculiar structure, containing up to six PGRP domains, never observed before in an animal genome. These findings suggest that the underlying molecular mechanism of common insect traits, such as detoxification of xenobiotics, immunity, and olfaction, can be very different in clades other than the well-studied Holometabola.

The recent genome assembly of *I. elegans* (Family: Coenagrionidae) was accompanied by the characterization of the X chromosome in this species, and by analyzing transcriptome-wide gene expression in adult males and females. The subsequent analysis focused identifying homologies in the X chromosome of this old insect with evolved insect orders, the presence of dosage compensation, and which genes exhibit sex-biased expression and are enriched on the X chromosome (Chauhan et al., 2021). Characterization of the X chromosome in representatives of the first winged insects allowed an unprecedented exploration of orthologous relationships between the sex chromosomes of other insects with available genomes (Pease and Hahn 2012). *Ischnura elegans* has a male hemigametic sex chromosome system (XX females and X0 males, Frydrychova et al., 2004), a feature shared with many other odonate species (Kiauta 1969). The identification of sex-linked genes in *I. elegans* demonstrated the deep phylogenetic conservation and synteny of genomes and sex chromosomes in insects, and for the first time, provided evidence of dosage compensation in odonates. Together, these analyses revealed a highly dynamic insect genome evolution with deep conservation on the one hand, and frequent chromosome rearrangements, translocations, and sex chromosome turnovers on the other.

2.5 Conclusions and future directions

With an increasing number of genomic resources becoming available and a parallel decrease in costs to obtain and analyze genomic data, research on odonate population processes, local adaptation, and speciation will continue to advance our understanding of evolutionary dynamics. Due to their ease of sampling, occupation of variable and shifting environmental gradients, and a propensity for rapid range expansion, many odonate species are prime candidates for monitoring the evolutionary consequences and biodiversity impacts of local and global climate change (Miguel et al., 2017). In particular, opportunities exist for looking at parallel evolutionary processes within and across species (Kahilainen et al., 2014), examining spatially varying selection across multiple loci, and corroborating results from gene expression and whole genome sequencing data with experimental data on different life stages.

Genomic investigations into adaptive processes have hitherto been on a modest selection of odonate species (Hotaling et al., 2020). Understanding how color polymorphisms are maintained in odonates is a topic that could benefit from genomic analysis in future research. This could involve analyzing allele frequencies of candidate genes under environmental selection in relation to color morphs along latitudinal gradients, and corroborating these relationships with information on gene functions, as well as ecological and behavioral observations. Furthermore, selection detection methods may assist with locating the genes or genetic correlates that result in specific color morphs or their divergence across variable environments (e.g. in grasshoppers: Yadav et al., 2019, in frogs: Rojas et al., 2020), which would provide further opportunity for selection experiments on odonates. Another compelling context for examining adaptive genomic responses in odonates is in the detection of pollution and toxicity in freshwater environments. Odonates have been heralded for being effective bio-indicators of metal contaminants due to their apparent tolerance (Park and Kwak 1986, Selvi et al., 2015) or developmental responses (Hardersen and Frampton 1999). Much of this work has been done at the benthic larval stage, which is most exposed to bioaccumulation effects of water contaminants. Therefore, life-stage-specific genome scans for loci associated with such tolerance or response traits in polluted environments may provide significant insights and applications for monitoring. As for all such studies, a replicated methodological approach is needed to reduce false positive signals of selection, and improve the determination of selection pressures, gene functions, and their relevance for long-term fitness. It is clear that studies on odonates can provide timely and compelling findings about how organisms respond to different environments and pave the way for future research in this area.

Studies on odonates have demonstrated that species boundaries are a lot more fluid than previously thought, and that, in general (e.g. *Ischnura*), introgression can be rampant. An important future component of studies should be an increased effort to understand the role of plasticity in evolution and the influence of epigenetic effects. Insights into these two areas will help to gain an understanding about the capacity of odonates to rapidly respond to changes to their environment (e.g. within a few generations or even within a single generation). Research on odonates in these areas is necessary to fill key knowledge gaps surrounding the genetic and ecological processes that govern local adaptation. So far, research that has explored the genomic processes leading to speciation in odonates is limited, although the study of genomics in general is increasing in this order. Future work could contribute to the general knowledge of speciation through identifying genes related to development or phenotypic pattern.

Notably, studies to date have been largely focused on damselflies, particularly on a few well-studied species in the northern hemisphere. Future efforts should try to taxonomically and geographically broaden the species investigated to enable comparative work so that general insights can be gained. The future of odonate research looks bright, as more resources are being developed to enhance genomic research capability in this group, enabling researchers to dive deeper into the question of how the nature and evolution of genome structure creates and maintains diversity.

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