



Changes in effective population size of Odonata in response to climate change revealed through genomics

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Research Article

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Published: 18 December 2023

Received: 8 September 2023

Accepted: 9 December 2023

Citation:

Tolman, Bruchim, Driever, Jordan, Kohli, Montague, Park, Park, Rosario, Ryu & Ware (2023): Changes in effective population size of Odonata in response to climate change revealed through genomics. International Journal of Odonatology, 26, 205–211
doi:10.48156/1388.2023.1917241

Data Availability Statement:

All relevant data are within the paper.

Abstract. The advent of third generation sequencing technologies has led to a boom of high-quality, chromosome level genome assemblies of Odonata, but to date, these have not been widely used to estimate the demographic history of the sequenced species through time. Yet, an understanding of how lineages have responded to past changes in the climate is useful in predicting their response to current and future changes in the climate. Here, we utilized the pairwise sequential markovian coalescent (PSMC) to estimate the demographic histories of *Sympetrum striolatum*, *Ischnura elegans*, and *Hetaerina americana*, three Odonata for which chromosome-length genome assemblies are available. *Ischnura elegans* showed a sharp decline in effective population size around the onset of the Pleistocene ice ages, while both *S. striolatum* and *H. americana* showed more recent declines. All three species have had relatively stable population sizes over the last one hundred thousand years. Although it is important to remain cautious when determining the conservation status of species, the coalescent models did not show any reason for major concern in any of the three species tested. The model for *I. elegans* confirmed prior research suggesting that population sizes of *I. elegans* will increase as temperatures rise.

Key words. Dragonfly, *Hetaerina americana*, *Ischnura elegans*, *Sympetrum striolatum*, demographic histories, pairwise sequential markovian coalescent

Introduction

Managing species in the Anthropocene is an immense task. Climate change is a major threat to insects, and many species do not have the physiological capability to withstand continuous exposure to ever increasing maximum temperatures in their distributions (González-Tokman et al., 2020). Micro-environmental changes, such as loss of plant cover, can further exacerbate changes in temperature, and change the assemblage of Odonata communities specifically, and communities of Insecta more generally (Castillo-Pérez et al., 2022). By identifying species and populations that are more at risk, it is possible to more efficiently allocate resources. The studies of thermal limitations, in conjunction with known range sizes, preferred habitat (lentic or lotic), and phylogenetic context have been an important tool to identify Odonata at risk of extirpation. For example, in North America, risk of regional extinction has been linked to use of lotic habitats, narrow thermal limits, and a large body size

(the latter is only the case for Zygoptera) (Rocha-Ortega et al., 2020). The cold temperate to sub-boreal North-eastern United States and the transversal neo-volcanic system are regions of special conservation concern for Odonata on the North American continent (Rocha-Ortega et al., 2020). Despite these recent advances, there remains an urgent need to gather data from more species to understand the resiliency of Odonata to environmental changes more generally (Castillo-Pérez et al., 2022).

Another important, but underutilized, tool for understanding the response of Odonata to climate change is whole genome sequencing (Newton et al., 2023). High-quality reference genomes can be used to model the change in effective population size (N_e) of a species through time. Although present levels of climate change are much more accelerated than events in the recent geologic past (Osman et al., 2021), such models can be used to determine how species have responded to past changes in the climate, and thus predict how they might respond to future change (Newton et al., 2023). In Odonata, a coalescent analysis generated with SMC++ (Terhorst et al., 2017) using the reference genome of *Pantala flavescens* (Fabricius, 1798, Libellulidae, aka the wandering glider) (Liu et al., 2022) and resequencing data from two individuals demonstrated that the N_e of *P. flavescens* has declined in the agricultural and industrial period, and may not be as stable as previously thought (Liu et al., 2022). The pairwise markovian coalescent (PSMC) generated for *Tanypteryx hageni* (Sélys, 1879, Petaluridae, aka the black petaltail) (Tolman et al., 2023) showed that this species had a higher effective-population size (N_e) before the Pleistocene ice ages, implying that warming may not be an immediate risk to this species (Tolman et al., in preparation). A far from comprehensive list of work done using genomics to model historical change in N_e outside of Odonata includes studies involving lions (Armstrong et al., 2020), clouded leopards (Bursell et al., 2022), flycatchers (Nadachowska-Brzyska et al., 2016), and Lepidoptera (García-Berro et al., 2023).

The advent of third generation sequencing technologies has led to a boom of high-quality, chromosome level genome assemblies of Odonata (Liu et al., 2022; Newton et al., 2023; Price & Allan, 2023; Price et al., 2022; Tolman et al., 2023), opening the door for a broader study of the response of N_e to climate change across Odonata. As of August 1, 2023, six chromosome level genome assemblies for Odonata were available on genbank. To date, models of historical N_e have only been generated for two of these species. Here we present PSMC models for an additional three species, *Sympetrum striolatum* (Charpentier, 1840, Libellulidae, aka the common darter), *Ischnura elegans* (Vander Linden, 1820, Coenagrionidae, aka the common bluetail), and *Hetaerina americana* (Fabricius, 1798, Calopterygidae, aka the American rubyspot). We provide background on the species, discuss the models in context of what is known about how these species will respond to climate change and explore conservation implications.

Ischnura elegans

Ischnura elegans is widely distributed in the palearctic, occurring as far north as the north coasts of Scotland (Price et al., 2022) and Sweden (Boudot & Kalkman, 2015). Its eastern extremity is in Japan, with a western extremity in Spain, and Iran to the south (Boudot & Kalkman, 2015). Prior research suggests that this species may adapt well to a warming climate. *Ischnura elegans* thrives in temperatures as high as 30°C, displaying increased immune function, higher fat content, and greater muscle mass (Van Dievel et al., 2017). When exposed to a heat wave, *I. elegans* had also displayed an accelerated growth rate at higher temperatures as compared to another Coenagrionidae, *Enallagma cyathigerum*, which is similarly widely distributed (Van Dievel et al., 2017).

Sympetrum striolatum

Sympetrum striolatum is a palearctic Anisoptera characterized by a one year life cycle and can be identified by its bright orange-red features (Horne, 2012). It has been historically observed in southern Europe and the Mediterranean (Borkenstein & Jödicke, 2022). An analysis of amplified fragment length polymorphism (ALFP) markers comparing genetic signatures from different individuals concluded that salt water barriers inhibit intra-species breeding within *S. striolatum* populations, leading to decreased genetic diversity (Parkes et al., 2009). This finding raised concerns regarding rising sea levels that could represent barriers to gene flow of this species.

This species also has a unique method for dealing with heat. Obelisk posture is a commonly seen phenomenon in both dragonflies and damselflies, in which the individual assumes a posture similar to a handstand, raising its abdomen until the tip points towards the sun (Borkenstein & Jödicke, 2022). This is generally done in order to minimize the amount of surface area hit by solar radiation, thus avoiding overheating on sunny days (Borkenstein & Jödicke, 2022). *Sympetrum striolatum* very rarely adopts this posture and it is presumed that this species avoids overheating by seeking shade in the woods (Borkenstein & Jödicke, 2022). This could imply that *S. striolatum* is at an increased risk for overheating, especially in areas lacking forest cover, exposing a potential vulnerability to climate change. However, further research is needed to fully investigate the impact of climate change and other anthropogenic pressures on this species.

Hetaerina americana

Hetaerina americana is a Zygoptera from the family Calopterygidae, found throughout continental United States. Damselflies of the genus *Hetaerina* are of a neotropical origin, and have migrated into the nearctic over time (Pritchard, 2008). Habitats of *H. ameri-*

cana include streams and rivers, where they perch at low to medium height above the water (Rosenthal, 2014). *Hetaerina americana* requires consistent access to the water as adults can quickly desiccate without it (Grether, 2023).

Although *Hetaerina* originated in the Neotropics, plasticity in certain morphological traits of *H. americana* seem to be adaptive for survival in differing temperatures, an example being forewings and hindwings developing differently between summer and spring months (Rosenthal, 2014). Due to warmer water temperatures during the summer, larvae metabolism accelerates, resulting in a broader wingspan in the adults (Rosenthal, 2014).

The influence of climate change upon *H. americana* remains understudied. Therefore, it is important to analyze how this damselfly species adapts to climate change-driven environmental patterns.

Materials and methods

Data acquisition

We identified all chromosome length genome assemblies for Odonata on genbank and searched google scholar for all papers citing the genomes, and then selected papers with which an analysis was conducted to estimate the effective population size of the species through time. We identified the genomes of *I. elegans* (Price et al., 2022), *H. americana* (Grether et al., 2023), *S. striolatum* (Crowley et al., 2023), and *Platycnemis pennipes* (Pallas, 1771) (Price & Allan, 2023) as chromosome-length genome assemblies for which demographic modeling had not been conducted. All four genome assemblies were assembled with PacBio HiFi reads, and scaffolded to chromosome level with HiC illumina reads (Crowley et al., 2023; Newton et al., 2023; Price et al., 2022; Tolman et al., 2023). At the time of submission, the PacBio Hifi reads for *P. pennipes* were not available for download, therefore this assembly was not included in the manuscript.

Quality assessment

Quality has previously been assessed for *I. elegans* (Price et al., 2022), and *H. americana* (Grether et al., 2023). To ensure the genome of *S. striolatum* (Crowley et al., 2023) was of comparable quality to other published genomes, we calculated contiguity statistics with assembly-stats (v.0.4) (Trizna, 2020) and ran BUSCO (v.4.1.1) (Manni et al., 2021) with the Insecta ODB10 database, in genome mode with the flag—long to retrain BUSCO for more accurate identification of genes.

Coalescent modeling

Many techniques exist for estimating historical changes in N_e from genomic data, but the pairwise sequential markovian coalescent (PSMC) (Liu & Hansen, 2017)

best suits the purposes of this work. Techniques based on the site frequency spectrum (Gutenkunst, 2021; Jouganous et al., 2017; Noskova et al., 2020, 2023) generally require a large sampling of resequencing data in addition to a reference genome, and the multiple sequentially markovian coalescent (MSMC) and SMC++ both require resequencing data from several individuals (Schiffels & Wang, 2020). Although it is cannot infer N_e in the very recent past, the PSMC (Liu & Hansen, 2017) only uses the two genomes from one diploid individual to estimate demographic history, thereby allowing us to model historical N_e for our three chosen species without the generation of any new data.

To perform PSMC on the three species selected, the PacBio HiFi reads were mapped to the reference assembly for each species using minimap2 v2.1.1 (Li, 2018) with default filtering settings. We converted the resulting bam files to sorted bamfiles with samtools v1.16.1 (Danecek et al., 2021), and performed basecalling with samtools v1.16.1 and bcftools v1.6 (Danecek et al., 2021) with a minimum depth of five, and a maximum depth of 45 with default filtering settings. We then used psmc v0.6.5-r67 (Liu & Hansen, 2017) to convert the basecalling output to psmcfa format, and calculate the PSMC with 100 bootstraps for each species. We visualized the PSMC with the command psmc_plot.pl from the psmc module (Liu & Hansen, 2017) and gnu-plot v5.2 (Phillips, 2012) using mutation rates of 1e-9, 2e-9, 3e-9, and 4e-9, spanning known genome wide mutation rates in insecta (Liu et al., 2017), and tested values in previous coalescent analyses in Odonata (Liu et al., 2022). Based on prior estimates of the generation time for the genera tested in this paper (Nakanishi et al., 2018; Nicolai & Carchini, 1985; Pritchard, 2008), we used a generation time of one year for *S. striolatum* (Nakanishi et al., 2018) and *I. elegans* (Nicolai & Carchini, 1985), and half a year for *H. americana* (Pritchard, 2008).

Results

Quality assessment

Similar to the other genomes used in this paper, the genome of *S. striolatum* was highly contiguous (N50 = 103,201,359; L50 = 6; Gaps = 350; N_count = 70,000), and the BUSCO score (C: 97.5% [S: 96.5%, D: 1.0%], F: 0.2%, M: 2.3%, n: 1367) showed the genome to be highly complete.

Demographic modeling

Palaearctic taxa

Although the palaearctic *S. striolatum* and *I. elegans* have overlapping distributions, they have responded to past climate change differently. The effective population size of *I. elegans* decreased with the onset of the Pleistocene ice ages (Fig. 1A) and may be primed to climb with an increasingly warming climate. PSMC

estimates of *Ischnura elegans* showed the population increased steadily until it reached a peak N_e between 2 and 8 Ma depending on the genome wide mutation rate used (Fig. 1A). The respective mutation rates of 2e-9, 3e-9, and 4e-9 peaked in order from lowest to highest mutation rate succeeding the 1e-9 mutation rate. The lowest mutation rate of 1e-9 had the highest level of variance in bootstrap replicates and thus the largest confidence interval out of the four differing mutation rates (Fig. 1A). The highest mutation rate of 4e-9 had the lowest level of variance in bootstrap replicates and thus the smallest confidence interval out of the four mutation rates (Fig. 1A).

In *S. striolatum*, peak populations were predicted in the order of slowest (1e-9) to fastest (4e-0) mutational rates, with all effective population size estimates converging around 100,000 years ago (Fig. 1B). This convergence correlated with the beginning of a period characterized by low population variation which endured at least until one thousand years before present (Fig. 1B). This lineage has fared well in the Pleistocene and appears to be highly adaptable to changes in the climate.

Nearctic taxa

The PSMC for *H. americana* suggests population decline has stabilized since the last glacial maxim, consistent with previous suggestions that this genus is limited by cooler temperatures (Pritchard, 2008) *Hetaerina americana* had a peak effective population size across all rates 1 Ma around an N_e of 500,000–900,000, and steadily decreased for about 900,000 years until it plateaued between 10,000 to 100,000 years ago (Fig 2). Rates of 2e-9, 3e-9, and 4e-9 tracked similarly, while 1e-9 had higher confidence intervals and fluctuated notably more compared to the other rates (Fig. 1B).

Discussion

Palaearctic taxa

Ischnura elegans

The differing mutation rates varied in the timing and size of their N_e peaks (Fig. 1A), however, the pattern ob-

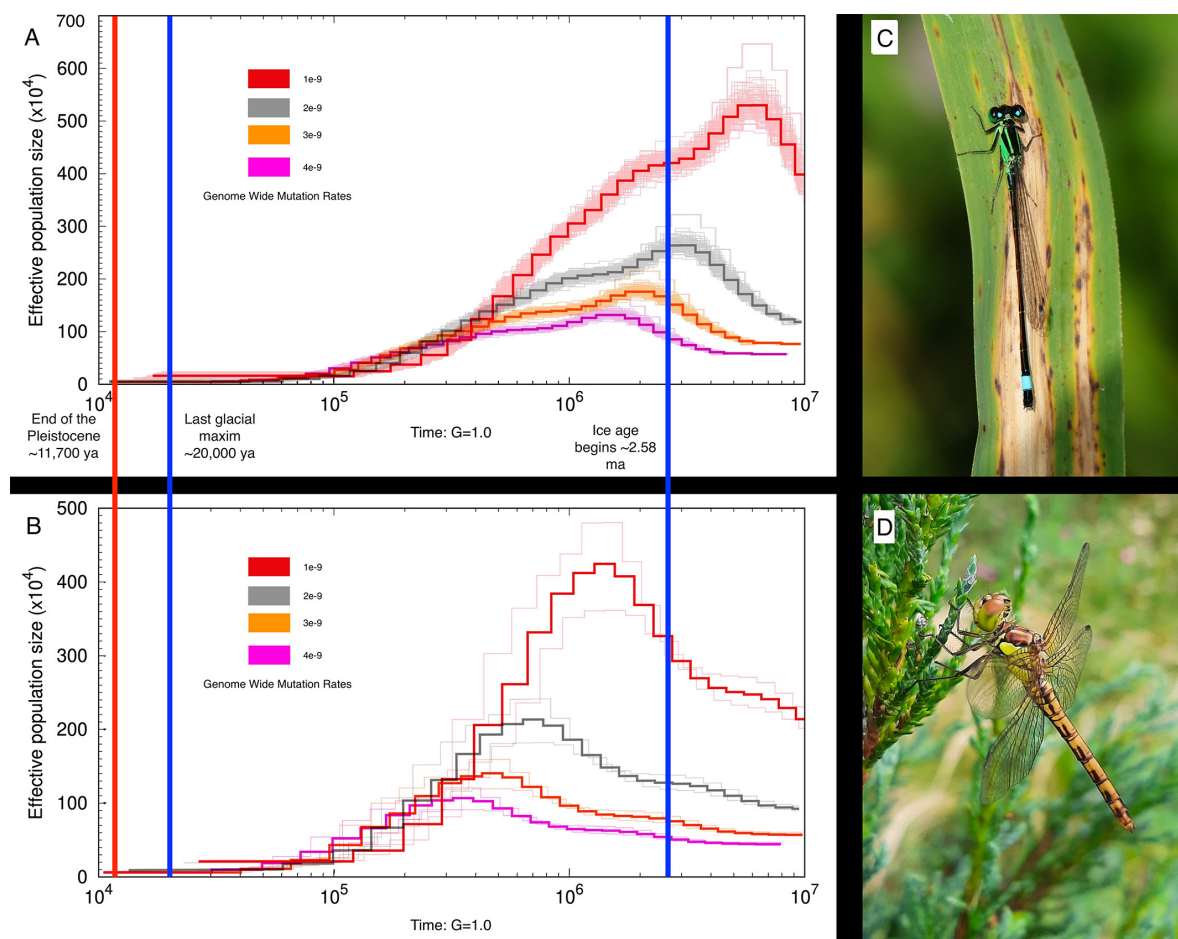


Figure 1. PSMC of palaearctic taxa, *Sympetrum striolatum* and *Ischnura elegans*, which have an overlapping distribution. PSMC estimates for *I. elegans* (A) and *S. striolatum* (B) were generated with mutation rates of 1e-9 (red), 2e-9 (grey), 3e-9 (orange), and 4e-9 (pink). Time interval is shown on a logarithmic scale on the x-axis, so 10^4 means 10,000 Years Before Present (YBP), 10^5 means 100,000 YBP, and so on. The estimated effective population size is on the y-axis. Bootstrap replicates are shown with lighter lines. The start of the Pleistocene, last glacial maxim, and the end of the Pleistocene are highlighted on the graph. (C) Adult *Ischnura elegans* (©Hayden Waller, some rights reserved (CC-BY-NC)), and (D) adult *Sympetrum striolatum* (©Christopher Moning, some rights reserved (CC-BY)) are also shown.

served across all four was largely equivalent (Fig. 1A). The data displays an increase in effective population size in each mutation rate up until the Pleistocene (~2.58 ma), peaking during the beginning of the Pleistocene, then consistently decreasing in a linear manner, with the decline zeroing out near the end of the Pleistocene Age ~11,700 years ago (Fig. 1A). The effective population size from each genome wide mutation rate converged to about 10,000 individuals at that time.

The N_e of *Ischnura elegans* increased steadily before the Pleistocene Age began and peaked near the onset of the Pleistocene ice ages. Like all organisms, *I. elegans*' physiological activities thrive at certain temperatures. As a general rule, Zygoptera are sensitive to global warming, which thus causes a fluctuation in physiological activities (Janssens et al., 2014). However *I. elegans* is primed to thrive in warmer temperatures,

with increased physiological performance at higher temperatures (Janssens et al., 2014). This is reflected in our analysis. Prior to the Pleistocene Age, the ancestral lineage to *I. elegans* showed an elevated N_e . During the Pleistocene ice ages temperatures decreased substantially, as did the N_e of *I. elegans* (Fig. 1A).

While general warming may be beneficial to this species, and increase its distribution, as with many other Zygoptera in a warming climate (Corser et al., 2015), exposure to extreme low and high temperatures lessens the physiological response of *I. elegans* to the alternate extreme, negatively affecting their survivability (Smith & Lancaster, 2020). Thus, there is some concern that *I. elegans* may be negatively influenced in localities experiencing more extreme weather events, but it should thrive in regions that are warm, but do not experience large temperature fluctuations.

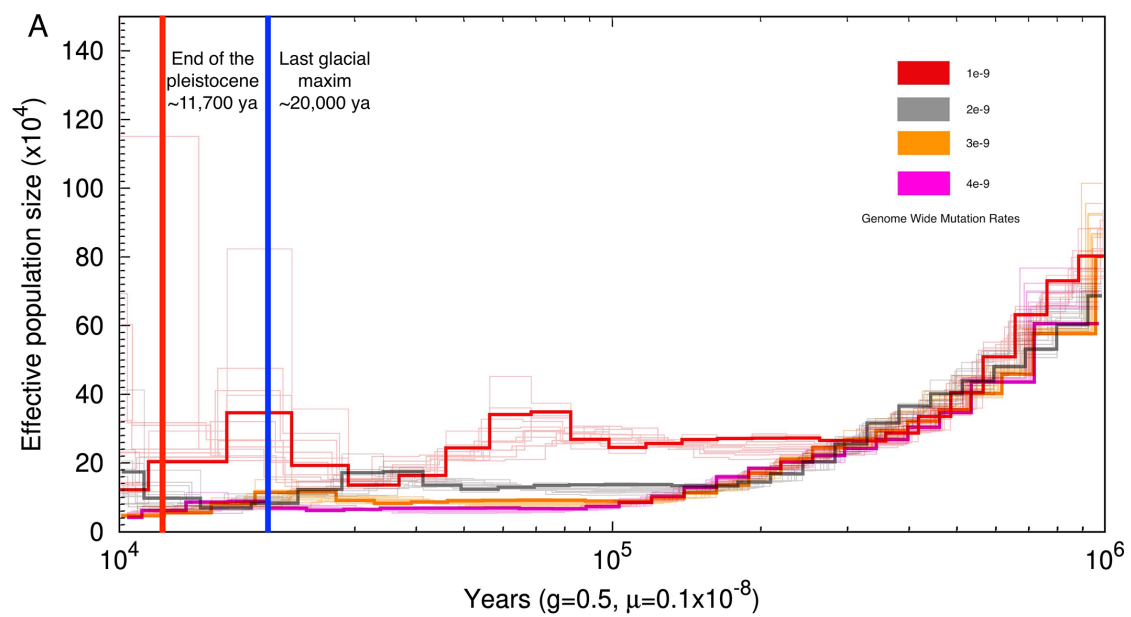


Figure 2. PSMC of *Hetaerina americana*, the only nearctic taxon, generated with mutation rates of $1e-9$ (red), $2e-9$ (grey), $3e-9$ (orange), and $4e-9$ (pink). Time interval is shown on a logarithmic scale on the x-axis, so 10^4 means 10,000 Years Before Present (YBP), 10^5 means 100,000 YBP, and so on. The estimated effective population size is on the y-axis. Bootstrap replicates are shown with lighter lines. The start of the Pleistocene, last glacial maxim, and the end of the Pleistocene are highlighted on the graph. (B) Adult *Hetaerina americana* (©Steve Pelikan, some rights reserved (CC-BY-NC)).

Sympetrum striolatum

Sympetrum striolatum was the only species we analyzed which did not negatively correlate with historical shifts in temperature, a finding attesting to the formidable ability of this lineage to adapt and survive in varying environments over time. The bootstraps were largely indistinguishable from the main estimate (Fig. 1B), leading to high confidence in our interpretation of this plot, which does not show that this species has been strongly affected by the average surface temperature. Previous research has suggested increased temperature is correlated with faster egg development, increased breeding rates, and a higher likelihood of geographic intermixing of *S. striolatum* (Borkenstein & Jödicke, 2022), so there is little concern about the impact of climate change on this particular species.

Nearctic taxa

Hetaerina americana

All four genome wide mutation rates showed a much higher overall species population size 1 ma (Fig. 2). The PSMC model shows a steady decrease in the overall species population size for about 900,000 years until the population size plateaus at approximately 100,000. This could be caused by fluctuation in temperatures. It has also been suggested that *H. americana* has high levels of population differentiation, and may even contain at least two cryptic species (Vega-Sánchez et al., 2019). The decreasing N_e could reflect an increasing isolation between populations of this species through time. Notably, the four different mutation rates remain nearly consistent with each other. The PSMC did suggest that *H. americana* will fare worse in warming temperatures.

Conservation implications and species management

There is an urgent need to develop more conservation plans for Odonata (Khelifa et al., 2021). Our analyses do not suggest that any of the three species are immediately threatened, but we must acknowledge that this should not be a reason to delay action for any of these three species should more information come to light. While no single analysis is going to accurately assess risk for a species, we believe that coalescent analysis, and other estimates of historical population demographics are a powerful tool for identifying potentially high-risk species in the face of climate change. Coalescent models can be used alongside environmental distribution models (Goodman et al., 2023), models of gene flow, ecological and behavioral data, functional genomics and molecular and physical estimates of the current effective population size to provide a more accurate and holistic picture of species risk. As more genomes of Odonata are sequenced, analyses relating to the response of the species to climate change should be prioritized (Newton et al., 2023). We hope that the field of Odonatology will embrace these methods as we race to fight global extinction.

Footnotes and acknowledgements

We would like to thank the Boise WaterShed for facilitating this project, Kristin Gnojewski, and the board of the Conservation Connection Foundation.

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