A COMPARATIVE STUDY OF PARASITISM IN INSECTS: WHY SOME ODONATA SPECIES HAVE PARASITES AND OTHERS DO NOT?

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ABSTRACT

Parasites are one of the most diverse groups of animals, capable of infecting virtually all other organisms on the planet. They are a strong evolutionary force, influencing genetic diversity and thereby affecting individuals, populations and entire species. Studies of host-parasite interactions have frequently examined how host individuals and their parasites interact, but this focus on the host individual offers a very narrow perspective on the general dynamics of hosts and their parasites. To understand the dynamics of hosts and their parasites on an evolutionary scale, examination has to be moved beyond the individual, to include multiple host species, multiple parasites, and various host species traits.

To this end, I decided to expand our knowledge by studying the host-parasite interactions of a large number of damselfly and dragonfly species and their endo- and ectoparasites. I evaluated specific physical, behavioral and distributional traits of the host species along with their parasitism in order to understand what traits affect parasitism. In addition, I examined these associations using the known evolutionary tree of the different damselfly and dragonfly hosts. Using this method I was able to get a deeper understanding on the co-evolution between damselflies, dragonflies and their endo- and ectoparasites.

In paper I, I confirmed that there is huge variation in endo- and ectoparasitism between different damselfly and dragonfly species. I also found that damselfly females had more ectoparasites than males did, but there was no difference between sexes in dragonflies. In paper II, I found that there is significant variation between different damselfly and dragonfly species in their strength of immune response and body mass. Using the evolutionary tree of these host species I also discovered that closely related species are more similar in their parasitism and in their two evaluated traits than would be expected if the species were drawn at random. In paper III, I discovered that both endo- and ectoparasites tend to infect the same host species which are relative small, live in high density and are common. Paper IV continued my investigation and I found that territorial or large species have fewer ectoparasites than non-territorial or small species and I also found that northern species have more ectoparasites than southern ones.

It seems that the larger size of damselfly and dragonfly species lowers their susceptibility to parasitism. However, whether this is the cause of the host's physical traits or due to the infection mechanism and/or preference of the parasite, remains unknown. Further studies are required to understand how the size of the host influences other traits and subsequently co-evolution between damselflies, dragonflies and their endo- and ectoparasites.

TIIVISTELMÄ

Loiset ovat monimuotoinen eliöryhmä ja ne kykenevät hyödyntämään miltei kaikkia muita eliöitä. Loiset ovat vahva evoluutiota ohjaava voima, ja ne voivat vaikuttaa geneettiseen monimuotoisuuteen ja näin ollen myös yksilöihin, populaatioihin ja lajeihin. Loistutkimus on usein keskittynyt isäntäyksilön ja sen loisten välisen vuorovaikutuksen tutkimiseen, mutta tämä yksilöä korostava tutkimus antaa hyvin yksipuolisen ja kapean kuvan isäntien ja loisten välisestä vuorovaikutuksesta. Ymmärtääksemme isäntälajien ja loisten välistä vuorovaikutussuhdetta riittävän kattavasti, tulee tutkimuksissa käyttää suuria määriä isäntälajeja ja niiden loisia sekä mahdollisimman monia isäntälajin ja loisten ominaisuuksia.

Lisätäkseni ymmärrystä tältä osa-alueelta, tutkin suuren määrän aito- ja hentosudenkorentolajeja ja vertailin niiden ulko- ja sisäloisten määriä. Tutkin näiden sudenkorentolajien fyysisiä, maantieteellisiä ja käyttäytymiseen pohjautuvia ominaisuuksia selvittääkseni, mitkä näistä ominaisuuksista vaikuttavat kyseisten isäntälajien sisä- ja ulkoloisten määrään. Yhdistämällä nämä vuorovaikutussuhteet uusimpaan tutkimustietoon sudenkorentojen sukupuusta, pyrin myös selvittämään, millä tavalla sudenkorentoisännät ja niiden loiset ovat kehittyneet yhdessä.

Ensimmäisessä työssäni totesin, että eri sudenkorentolajien välillä on suuria eroja niin sisä- kuin ulkoloisten määrässä. Huomasin myös, että hentosudenkorentonaaraille on selvästi enemmän loisia kuin koirailla, mutta tätä sukupuolten välistä eroa ei löytynyt aitosudenkorennoilta. Toisessa työssäni löysin sudenkorentolajien väliltä selkeitä eroja niin immuunipuolustusvasteessa kuin ruumiin koossa. Hyödyntämällä sudenkorentojen sukupuuta havaitsin myös, että läheistä sukua olevat lajit ovat keskenään todennäköisemmin samanlaisia sekä sisä- ja ulkoloisinnan, että immuunivasteen ja koon osalta kuin sukupuusta otetut satunnaiset isäntälajit. Kolmannessa työssäni havaitsin, että niin sisä- kuin ulkoloisetkin tuntuvat hyödyntävän todennäköisimmin pieniä, tiheästi eläviä ja yleisiä sudenkorentolajeja. Viimeisessä työssäni kävi ilmi, että sisäloisia on pienemmällä todennäköisyydellä isoilla, reviirillisillä ja pohjoisemmilla sudenkorentolajeilla kuin pienillä, ei-reviirillisillä tai pohjoisemmilla lajeilla.

Vaikuttaa siltä, että sudenkorentolajin suuri koko pienentää lajin todennäköisyyttä olla loisittu. Ei kuitenkaan vielä tiedetä, johtuuko tämä isäntälajin fyysisistä ominaisuuksista vai loisten tartuntatavasta ja/tai halusta valita tietty isäntälaji. Jatkotutkimuksia tarvitaan, jotta ymmärretään, miten isäntälajin koko vaikuttaa sen muihin ominaisuuksiin sekä sen yhteiskehitykseen loisten kanssa.

LIST OF ORIGINAL ARTICLES

(I)   Ilvonen, J.J., Kaunisto, K.M. & Suhonen, J. (2016) Are sexes equally parasitized in damselflies and dragonflies? *Oikos*, **125**, 315-325. DOI: 10.1111/oik.02437

(II)   Ilvonen, J.J. & Suhonen, J. (2016) Phylogeny affects host's weight, immune response and parasitism in damselflies and dragonflies. *Royal Society Open Science*, **3**, 160421. DOI: 10.1098/rsos.160421

(III)   Ilvonen, J.J., Kaunisto, K.M. & Suhonen, J. (2018) Odonates, gregarines and water mites: why are the same host species infected by both parasites? *Ecological Entomology*, DOI: 10.1111/een.12634

(IV)   Ilvonen, J.J., Hasik, A., Siepielski, A.M. & Suhonen, J. Patterns of parasitism: the role of host species size, territoriality and latitude. Manuscript.

1.   INTRODUCTION

Parasites are a main group of organisms capable of infecting virtually all other organisms (e.g. Price, 1980). However, parasitic interactions have received less attention from the scientific community than e.g. predation and competition has (Price, 1980). That being said, the advances in parasitology have shown that it is a strong evolutionary force, influencing genetic diversity and thereby affecting individuals, populations and entire species (Haldane, 1949; Oppliger et al., 1997; Berenos et al., 2011). Studies of host-parasite interactions have frequently examined how host individuals and their parasites interact. However, studying how host individuals and their parasites interact is a limited point of view and offers a very narrow perspective on the general dynamics of hosts and their parasites (e.g. Kortet et al., 2010). To understand the dynamics of hosts and their parasites on an evolutionary scale, examination has to be moved beyond the individual, to include multiple host species, multiple parasites, and various host species traits.

To this end, I decided to expand my current knowledge on parasitism by using a large number of closely related insect host species along with several host species traits that require additional research. Damselflies (Zygoptera) and dragonflies (Anisoptera) were chosen as the model organisms, because they are relatively well known (e.g. Corbet, 1999), data of them is easily acquired and they often harbor two types of parasites which are easily assessed. The host species traits I chose to examine represent physiological, behavioral and distributional traits, all of which have been previously studied but the level of research is far from adequate or the effect of these traits on parasitism is conflicting.

Sex of the host is one of the physical traits that seem to affect parasitism. There are studies on invertebrates conducted mainly with a few species of either damselflies or dragonflies that have found a male bias in parasitism (McKee et al., 2003; Lajeunesse et al., 2004; Córdoba-Aguilar & Munguía-Steyer, 2013), a female bias (Bunker et al., 2013) and then there are studies that have found no differences between sexes in their parasitism levels (Nagel et al. 2010, Forbes et al. 2012, Mlynarek et al. 2012). This level of confliction in results demands for a conclusive study that would use a large number of host species.

Another physical trait that is intimately linked with parasitism is the level of immune response of a host. In insects, the strength of immune defense is often measured by the host's ability to synthesize melanin around a pathogen (Siva-Jothy, 2000; Kanost et al., 2004; González-Santoyo & Córdoba-Aguilar, 2012). This encapsulation response appears to be the primary immunological way by

which odonates defend against ectoparasitism, therefore making it a good trait to be studied on a large scale. Previous studies have found variation in encapsulation response within and between different species (Mucklow et al., 2004; Córdoba-Aguilar et al., 2006; Abdel-latief & Hilker, 2008; Riddell et al., 2009; Córdoba-Aguilar & Munguía-Steyer, 2013) and populations (Kaunisto & Suhonen, 2013), but the range of hypothesis explaining these differences clearly demonstrate the need for an additional study using a wider range of host species.

The size of a host may be one of the most studied aspects of host-parasite interactions. Because a host is a finite resource and a habitat to the parasite, it has been suggested that the larger the host is the more parasites and parasite species it can harbor (Poulin, 1997). Indeed, this positive association has been shown in fishes (e.g. Guégan et al., 1992; Grutter & Poulin, 1998; Cable & van Oosterhout, 2007; Cardon et al., 2011), in birds (e.g. Scheuerlein & Ricklefs, 2004; Santiago-Alargon et al., 2016), and in mammals (Lindenfors et al., 2007) although in some mammals this association is complicated (e.g. Patterson et al., 2008; Sackett, 2018) or non-existent (Krasnov et al., 2004). However, in insects the role of host size is perhaps even more complicated, because there are studies that have found larger hosts to have more parasites (Müller et al. 1996), larger hosts to have fewer parasites (Häckermann et al., 2007; Sánchez et al., 2015; Hughes et al., 2016), and there are also studies that have not found any associations between host size and their parasite numbers (Durrer & Schmid-Hempel, 1995; Abé et al. 2015). Given this variation in the effect of host size, especially in insects, it seems necessary to investigate this further using a large number of different sized insect hosts.

Besides physical host traits, behavioral traits are also important in host-parasite interactions. A trait that has been investigated in many animal groups is the territoriality of a host. Territoriality, i.e. the aggressive behavior between males to compete for high quality breeding sites, is a physically demanding behavior (e.g. Marden & Waage, 1990). Males that take part in these territorial contests strain themselves and thus increase their susceptibility to parasitism. On the other hand, males that succeed in establishing territories and survive these contests have a high fitness (e.g. Siva-Jothy, 1999; 2000), indicating that they are also good at defending against parasites or tolerating them. However, the role of territoriality in determining parasitism is inconclusive, because it has been shown to correlate positively with parasitism in mammals (e.g. Ezenwa, 2004; Nunn & Dokey, 2006; Poirette et al., 2017), but in insects territoriality has correlated both negatively (Marden & Cobb, 2004) and positively with parasitism (Tsubaki & Hooper, 2004). Therefore additional research is required to determine whether insect territoriality affects parasitism and if it does, what the relationship is.

When assessing the large scale patterns in host-parasite interactions, distributional traits, e.g. the latitudinal range and distribution center of the host species are often used. Because the distance from the equator correlates with average environmental temperature, photoperiod, and overall species richness (Corbet, 1999; Hillebrand, 2004), it is expected to influence parasitism as well. In fish, an increase in latitude decreases parasitism (Poulin & Rohde, 1997), and this correlation can also be seen in primates to a certain extent (Nunn et al. 2005). In contrast, an increase in latitude has been shown to increase parasitism in rodents, carnivorous mammals and in bivalves (Krasnov et al., 2004; Lindenfors et al., 2007; Studer et al., 2013). In insects, parasitoids have been shown to decrease as latitude increases (Gröbler et al., 2008; Santos & Quicke, 2011), and a similar relationship was found in the parasitism between different populations of a host damselfly species (Kaunisto et al., 2015). There is an obvious need for a study assessing whether such trends exist in insects and their parasites.

Damselflies (Zygoptera) and dragonflies (Anisoptera) that together form the order Odonata, are a globally widespread insect group absent only in the Antarctica and showing the highest species diversity in tropical streams and declining as latitude increases (Kalkman et al., 2008). It is a relatively well know insect group and display a wide range of different behavioral and physical traits making them ideal model organisms for ecological and evolutionary studies.

Damselflies and dragonflies come in all sizes, ranging from tiny damselflies to large dragonflies and the species size functions as an important trait affecting several aspects of an odonate's life. Size influences population densities, meaning that large species have fewer individuals in a given area than smaller species (e.g. White et al., 2007). Even though adult odonate densities have not been studied enough to make widespread conclusions, it is safe to assume that a similar pattern exists in the Odonata order as well. If an odonate species has a low population density, it can be considered locally rare and this may decrease their parasitism, because water mites have been known to prefer common hosts over rare ones (Grant & Samways, 2007). Large size may also be beneficial in general fitness, demonstrated as an increased mating rate and lifetime mating success of odonate males and larger clutch sizes and increased longevity in odonate females (Sokolovska et al., 2000; Waller & Svensson, 2007). Odonate species differ also in their ability to resists parasitism, particularly water mite parasitism. Odonates have the ability to encapsulate the feeding tube of larval water mites by using melanin which is controlled by the activity of phenoloxidases (POs) (e.g. Nagel et al., 2011 and references therein), a substance also used in the hardening of the cuticle (Bennett, 1993; Sánchez et al., 2015) and in the formation of wing spots that serve as secondary sexual characters (Hooper et al., 1999; Rantala et al., 2000). The strength of this encapsulation response varies within species (Siva-

Jothy et al., 2001; Córdoba-Aguilar & Mendez, 2006; Contreras-Garduño et al., 2011; Kaunisto & Suhonen, 2013) and between species (Conteras-Garduño et al., 2008; Nagel et al., 2011; Nagel et al., 2014; Ilvonen & Suhonen, 2016) and it may result in resistance differences between odonate individuals (Yourth et al., 2002) and odonate species (Lajeunesse et al., 2004; Nagel et al., 2011; Nagel et al., 2014; Worthen & Hart, 2016).

2.   AIMS OF THE THESIS

My personal field observations indicated that there were significant differences in the parasitism levels between different damselfly and dragonfly species. However, there were no large scale studies that could have offered a satisfactory answer into taxon level interaction between insect hosts and their parasites. Therefore I created a study plan with two primary aims: (i), to confirm previous observations that there are significant differences between damselfly and dragonfly species in their endo- and ectoparasitism, and (ii) to evaluate what traits of host species and parasites affect the interaction between hosts and their parasites and subsequently cause these differences between different damselfly and dragonfly species. In paper I, I wanted to see if the so called "sicker sex" exists, i.e. whether sex as a host trait affects parasitism significantly. In paper II, I wanted to establish whether host species size, strength of immune response and phylogeny affect the parasitism of the host species. In paper III, I investigated whether an association existed between the endo- and ectoparasites. The study culminated in paper IV, where I assessed a range of host species traits and wanted to find out how territoriality, size or latitude influences the endo- and ectoparasitism patterns of the host species.

Specific research questions of each paper:

(I)   Do male and female odonates differ in their prevalence of endo- and ectoparasitism? Is there a general trend in odonates, i.e. is there a "sicker sex"?

(II)   Does average body mass (proxy for species size) or encapsulation response of a host species correlate with its prevalence of endo- or ectoparasitism? Does either parasitism show a pattern that would be visible in the odonate phylogeny? Are host species body mass, immune response and endo- or ectoparasitism more similar between closely related species than with species drawn at random?

(III)   Is there an association between the prevalence of endo- and ectoparasitism within the Odonata order? Why? Do phylogenetic trees constructed using different methods give different results? Does the number of investigated individuals per species affect its parasitism?

(IV) Does host species territoriality, wing length (proxy for species size), latitudinal range or latitudinal center associate with the prevalence of endo- or ectoparasitism of the host species? Why do these associations exist or don't exist? Are there phylogenetic signals in these traits? How does the relationship of these traits and parasitism influence the co-evolution of odonate and their parasites?

3.   MATERIAL AND METHODS

3.1.   Study species

3.1.1. Damselflies and dragonflies

Damselflies and dragonflies, that together form the order Odonata, are relatively well known insects that display a wide range of different behavioral and physical traits making them ideal for ecological studies. They are semi-aquatic insects, meaning that females lay their eggs in water and after a larval period a sub-adult emerges from water, takes flight and begins its terrestrial adult phase (Corbet, 1999). The length of the larval period is positively associated with both latitude and species size (Waller & Svensson, 2017) meaning that the larval stages develop from a few months in the tropics to several years in the north (Corbet, 2006). This results in large differences in the number of generations produced each year between odonate species. After completing their maturation period that most often occurs outside the water, mature odonates return to water body to reproduce. In odonates, male reproduction is mainly governed by two different breeding strategies: territorial and non-territorial behavior (e.g. Tsubaki et al. 1997; Suhonen et al., 2008) of which non-territorial behavior can be further divided into "sneaking" and "satellite" tactics (Forsyth & Montgomerie, 1987). Territorial males defend a favorable breeding site, e.g. an aerial space or a vegetation floating in water, against other competing males in order to improve their chances of mating. Territorial males have been observed to have a higher reproductive success than non-territorial males (Grether, 1996; Plaistow & Siva-Jothy, 1996; Tsubaki et al., 1997), showing how beneficial being territorial is to a male. However, these territorial contests are energetically very demanding and they have often been referred to as "energetic wars of attrition" (Marden & Waage, 1990), leading to a situation where only the fittest males can obtain and hold territories. It has been shown that territorial males have higher fat reserves (Marden & Waage, 1990; Plaistow & Siva-Jothy, 1996; Marden & Cobb, 2004; Contreras-Garduño et al. 2006) and immune response levels (Koskimäki et al., 2004; Contreras-Garduño et al., 2006; Córdoba-Aguilar & Mendez, 2006) than their non-territorial counterparts.

3.1.2. Larval water mites

Water mites (Acari) are aquatic predators that have a parasitic larval stage often using damselflies and dragonflies as their definite host (Di Sabatino et al., 2000; McDevitt-Galles et al., 2018). Larval water mites of *Arrenurus* sp. actively seek and find their hosts, e.g. odonate larvae, in the aquatic environment and tune their

peak host-seeking activity to match the time when their "preferred" odonate hosts emerge from water and begin their adult life (Mitchell, 1967; Smith, 1988). Upon finding a larval odonate, the larval water mite rests on the host and waits until the odonate emerges from water and moves from the hosts larval exoskeleton (naiad skin) to the newly emerged adult skin (Mitchell, 1961). Then, while the odonate's cuticle is still soft, the larval water mite uses its feeding tube (stylostome) to pierce the skin of the host and starts feeding on the host's body fluids (Figure 1) and after engorgement drops back to water (Åbro, 1982). The water mite larvae of *Limnochares americana*, on the other hand, climb onto emergent vegetation and wait for adult odonates (Forbes & Robb, 2008), often creating a situation where *L. americana* numbers increase on odonate hosts as the season progresses (Smith & Cook, 1991; Lajeunesse et al., 2004). However, it seems probable that there are other water mite species besides *L. americana* that are capable of infecting adult odonates, because these similar increases in water mite numbers as the season progresses have been found across the Atlantic in Norway (Åbro, 1982; 1990).

Figure 1. *Larval water mites attached to a damselfly.*

Water mites can be very opportunistic parasites when it comes to odonate hosts: a single water mite species can attach to several different host species (Zawal & Szlauer-Łukaszewska, 2012; Zawal & Buczyński, 2013; Mlynarek et al., 2013), but on the other hand a single odonate species may be parasitized by several different water mite species (Zawal & Szlauer-Łukaszewska, 2012; Mlynarek et al., 2013; Zawal et al., 2017). However, the attachment and piercing of the

odonates cuticle does not necessarily mean successful engorgement by the water mite, because the odonate host's immune defense may be able to encapsulate the feeding tube and thus kill the larval water mite (Forbes et al., 1999; Mlynarek et al., 2014).

Since larval water mites drain the body fluids of their hosts and they can be very numerous to the extent where they hinder the host's movements, they can cause deleterious effects on their hosts and decrease host survivorship (Åbro, 1982; Robinson, 1983; Forbes & Baker, 1991).

It has to be noted, that given the importance of the odonate larval stage to *Arrenurus* sp. parasitism, the dynamics of odonate larvae are likely to affect the subsequent adult parasitism. However, too little research exists regarding e.g. the density or individual size of odonate larvae for making any conclusions.

3.1.3. Gregarines

Gregarines (Apicomplexa: Eugregarinorida) are protozoan parasites often parasitizing damselflies and dragonflies, among other insect hosts. They appear to be opportunistic parasites capable of using multiple odonate species as hosts (e.g. Åbro, 1974; Locklin & Vodopich, 2010b, Cook & Smith-Herron, 2014) but on the same time a single odonate species can be parasitized by several different gregarine species (Åbro, 1974; Cook & Smith-Herron, 2014). After being ingested by the host, the infective stage of the gregarine, the oocyst, enters the insect's midgut where it raptures into male and female gametes, which then fuse into gametocysts. These cysts then leave the host imbedded in the fecal matter and eventually become infective oocysts (Åbro, 1976). Gregarines have been found in odonate larvae (McDevitt-Galles et al., 2018) and adult odonates have been experimentally infected via contaminated drinking water (Córdoba-Aguilar & Munguía-Steyer, 2013), indicating that odonates can become infected via water. However, newly emerged odonates have not had any gregarines even though their larval forms have had them (Åbro, 1974; Locklin & Vodopich, 2010; Bunker et al., 2013), indicating that gregarine species may be stage-specific and therefore restricted to either larval or adult form of the odonate host. In addition to getting infected via drinking water, odonates may contract infective oocysts via prey items. Åbro (1976) found that among the various prey insects consumed by adult damselflies, medium-sized flies (Chrinomidae) and midges (Ceratopogonidae) had oocysts attached to their legs, therefore serving as an infective vector. However, it is unknown as to which transmission mechanism is more common and causes more infections in odonate hosts.

Because infected insect hosts spread the gregarine gametocysts to their surrounding environment, accumulation of oocysts and the subsequent increase

in infection probability occurs. This has been observed both in damselflies and dragonflies (Åbro, 1974; Locklin & Vodopich, 2010).

Gregarines may consume the nutrients in the digestive tract, therefore impacting negatively on the host if the host can't compensate this lack of food (Tsubaki & Hooper, 2004) Gregarines may also cause local ulcerations and ruptures of the gut wall and they can even block intestinal tract when their numbers are great (Åbro, 1974; 1987; 1990). These effects may further reduce the fat contents of the host therefore reducing the host's general condition (Siva-Jothy & Plaistow, 1999).

3.2. Field work

Field collected specimens were collected for papers I, II and IV. For papers I and II, field data were collected mainly from Central and Southern Finland from 2009 to 2013 consisting of 12 damselfly and 10 dragonfly species. For paper IV, field work was carried out at nine different lakes around Fayetteville, Arkansas, USA in 2017, consisting of 54 odonate species.

For papers I and II, specimens for each species were collected within a few days from the same location to minimize spatial and temporal variation. The aim was to collect an equal number of adult males and females for each damselfly and dragonfly species to reduce the difference between sexes in the measured host traits. Individuals were collected between 10:00 and 16:00 using a sweeping net, sexed, placed in an individual container with a moist paper towel to avoid dehydration and transported to a laboratory for further tests.

For paper IV, each lake was sampled bi-weekly throughout the flight season (mid-May to early August) at three different times (morning, noon, and afternoon), as some species are more active at different times of day (Abbott, 2011). In total, each lake was sampled three times per week sampling. Each sampling round lasted six person-hours, during which time researchers caught mature male odonates using aerial sweep-nets and then preserved them in 95% ethanol.

Only adult odonates were collected because individual age is likely to affect parasitism (Åbro, 1982; 1990; Hecker et al., 2002). Adult were separated from juveniles by the stiffness of their wings (Plaistow & Siva-Jothy, 1996).

3.3. Lab work

For papers I and II, the analysis of the field collected specimens began with immune defense assessment. By inserting a 3mm long nylon filament, henceforth called an implant, into the abdomen of the odonate, we were able to simulate the encapsulation response that naturally occurs when an odonate defends against the feeding tube of a larval water mite (Rantala & Roff, 2007; Suhonen et al., 2010; Nagel et al., 2011). After the removal of the implants, each individual was killed, measured, their attached water mites counted following the removal and dissection of the digestive tract after which the gregarines within were counted. Then each implant was photographed (Figure 2) and the melanization of each implant was assessed using ImageJ software. Paper IV's lab work followed that of I and II, except for the immune defense assessment.

Figure 2. *A nylon implant: the non-melanized section on the top and the larger melanized section in the bottom.*

For papers III and IV, the average wing length of a species was primarily based on the field collected individuals and museum specimens photographed in the Helsinki Natural History Museum (Figure 3), London Natural History Museum and Stockholm Natural History Museum. Whenever possible, the wing lengths of at least 3 male individuals were measured using ImageJ and the average wing length was calculated from these.
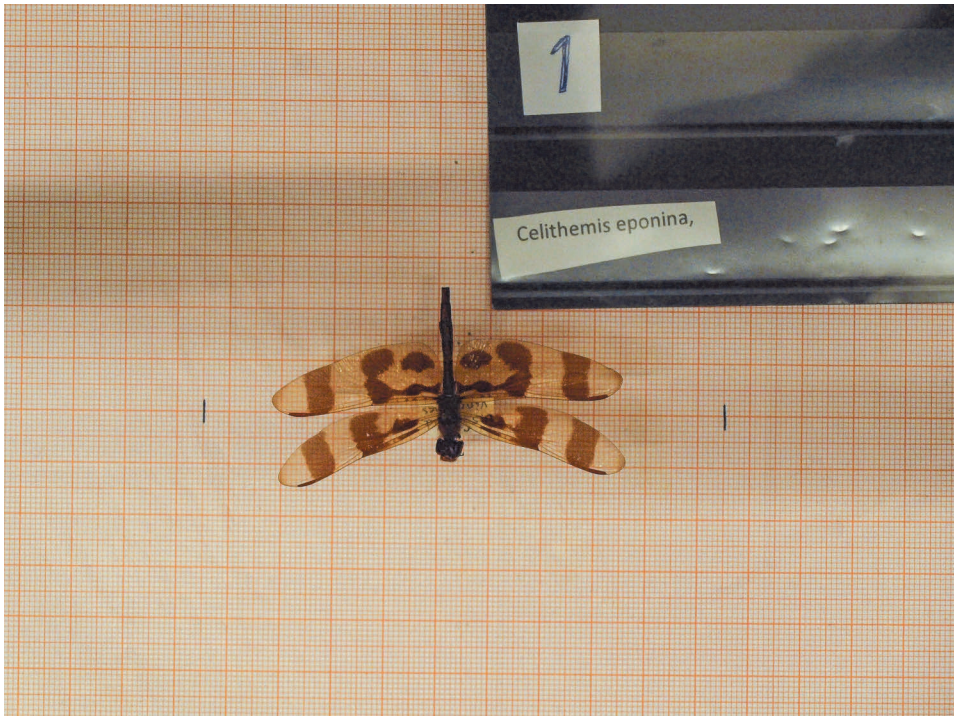
Figure 3. *A* Celithemis eponina *odonate individual in the Helsinki Natural History Museum waiting to be photographed and its wings measured.*

3.4. Data mining

To maximize the number of host species in studies I, III and IV, we also included data from previously published studies. To this end, we first searched for relevant studies from repositories such as Google Scholar, Web of Science, Scopus, and ResearchGate using key words such as "damselfly AND water AND mite", "dragonfly AND gregarine" or "Odonata AND parasitism". For a study to have usable data, it had to meet the following criteria: 1) a damselfly or a dragonfly species had to have individuals examined for either gregarines and/or water mites, 2) the number (and sex in paper I) of examined and infected individuals had to be clearly stated and 3) examined individuals had to be mature. For paper I, III and IV, when data for a single species was available from multiple sources, all examined and infected individuals were pooled and the prevalence was calculated from this data set. In papers III and IV, for an odonate species to be usable in our analyses, it had to have at least 10 individuals examined for either parasite.

In paper IV, we also assessed the territoriality of an odonate species using either previously published studies or field observations. For an odonate species to be deemed territorial or non-territorial, we preferred a clear statement of either

behavior, a sufficiently detailed description of breeding behavior of which territoriality could be determined, or a personal comment made by a scientist. Unfortunately, detailed data was not available for every species. In these situations we estimated the territoriality status based on the territoriality of their sister species or on general statements such as "all Coenagrionidae are non-territorial". However, due to the nature of territoriality, even experienced scientists find it difficult at times to establish whether males of a species exhibit territorial behavior or not. This was clearly seen in conflicting statements of a species' territoriality: one study stated a species was territorial and another study that is was non-territorial. In these situations we assessed the status of close relatives and put more weight on the statement of the most recent study, because the understanding of territoriality has increased substantially since e.g. the 1960's.

3.5.   Statistics

Prevalence (i.e. the proportion of infected individuals within a given population, Bush et al., 1997), was chosen as the measure of odonate parasitism for all the papers. It accurately describes the overall parasitism of a species and it is one of the most commonly used methods in the literature.

In paper I, where sex differences were analyzed, non-parametric tests were used because the assumptions of parametric tests could not be fulfilled. In the later papers (II, III and IV), generalized linear models (GLMs) were used to assess the relationship between host traits and their parasitism. Due to the close relatedness of the odonate species, it seemed probable that the host species traits would be more similar between closely related species than if the species were drawn at random due to the shared evolutionary ancestry. This was tested by analyzing the phylogenetic signal of each host species trait and for both parasite prevalences using the phylogenetic tree of the odonates, and because phylogenetic signals were present, it had to be accounted for in the analyses of the host traits. To this end, phylogenetic generalized least square (PGLS) models were used in papers II, III and IV and the necessary phylogenetic trees had to be constructed. For paper II, the phylogeny of the 22 species was crafted using previously published phylogenies, but due to the different construction mechanisms by which these tree had been made, branch lengths had to be standardized to 1. In paper III, a standardized tree was constructed using the aforementioned mechanism, but we also created a time-calibrated tree using an existing larger tree originally created by Willink & Svensson (2017). Because this time-calibrated tree is more accurate than the standardized tree and the results did not significantly differ between these trees in paper III, we used only

the time-calibrated tree in paper IV. RStudio (version 1.1.423) was used in the phylogenetic analyses with the **ape** (version 5.1), **phytools** (version 0.6.44), and **geiger** (version 2.0.6) packages (Paradis et al., 2004; Revell 2012; Harmon et al., 2008).

4. RESULTS AND DISCUSSION

In the first paper, I confirmed my field observations and determined that significant differences exist between different odonate species in their parasitism. I also found that in odonates a "sicker sex" (Zuk, 2009; Córdoba-Aguilar & Munguía-Steyer, 2013) does not exist, in contradiction to previous studies. In the second paper I continued my investigation into odonate parasitism and found that there are significant differences in immune defense between odonate species and odonate phylogeny has to be taken into account in future studies. In the third paper, I confirmed my previous preliminary results of an association between endo- and ectoparasitism in odonates and discussed about the role of parasite transmission and infection. This was followed by the fourth paper, in which I found that host species territoriality, size and latitudinal center affect ectoparasitism, but not endoparasitism in odonates.

4.1. Does the "sicker sex" exist? (I)

While setting out to determine whether there are significant differences between odonates in their parasitism, I also wanted to confirm whether a previously reported "sicker sex" (either gender has more parasites than the other) exists in odonates (Zuk, 2009; Córdoba-Aguilar & Munguía-Steyer, 2013). I found three main results in our first paper (Figure 4). Firstly, most of the odonate host species showed no differences between sexes in either gregarine or water mite prevalence and intensity. The only exception was higher gregarine prevalence and intensity of female damselflies as compared to males. Secondly, damselflies had higher prevalence and intensity levels both in gregarine and water mite parasites compared to dragonflies. Finally, we found a strong pattern in parasitism between female and male odonates: a certain level of gregarine or water mite parasitism in one sex was matched with a similar parasitism level on the other.
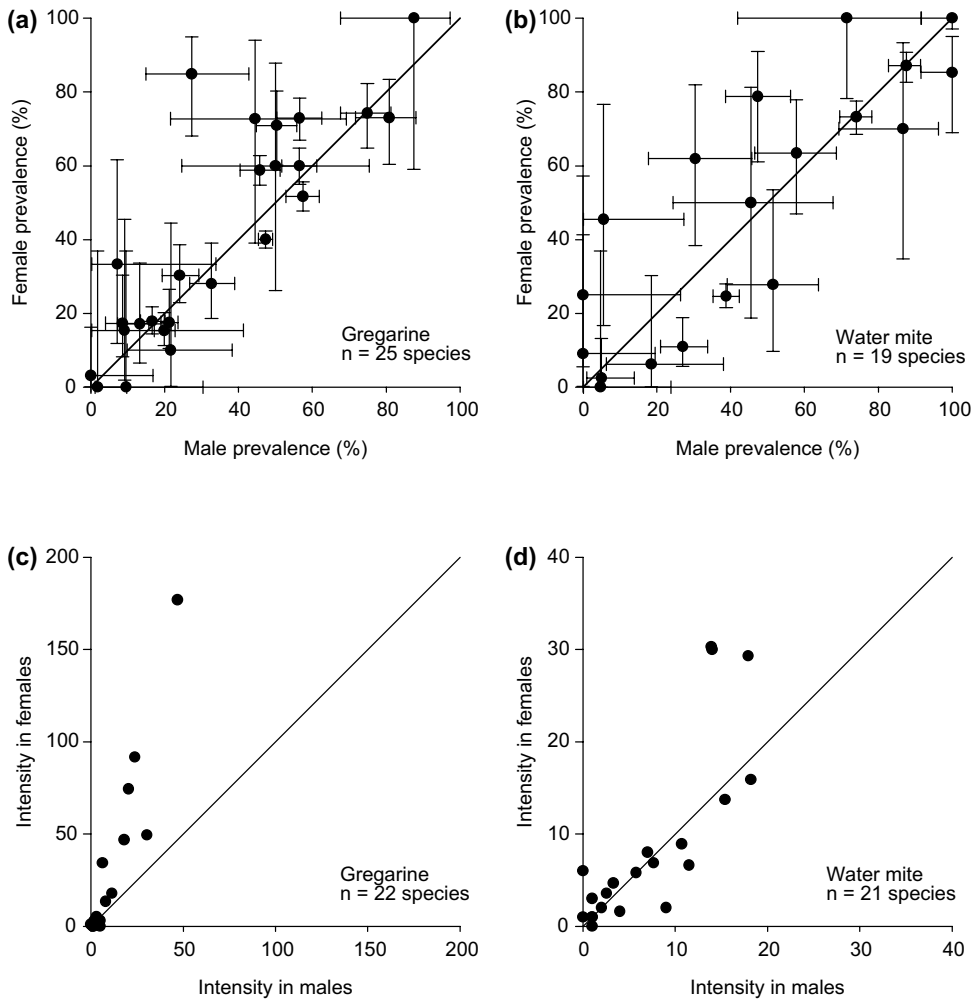
Figure 4. *Relationship between male and female parasitism in (a) gregarine prevalence, (b) water mite prevalence, (c) gregarine intensity and (d) water mite intensity in the odonate order. A dot represents a host species with male and female prevalence or intensity values with 95% confidence intervals. Gradient line in the middle represents a situation, where the parasitism of males and females is the same.*

The reason why female damselflies had more gregarines than males, is most likely due to behavioral differences between the sexes. Females prioritize eating in order to increase their egg production, and because gregarines infect via digested prey items or via drinking water, difference in consumption rate between sexes may explain the difference in gregarine parasitism.

In general, water mite parasitism did not differ between sexes, even though seven species had significant differences. It seems that because larval water mites

attach to their hosts before the hosts mature, the behavioral and physiological differences between the sexes do not affect water mite parasitism.

There was a strong and significant similarity between male and female parasitism within species. We found that whenever females had a high prevalence, so did males and vice versa. It seems that the behavioral and physiological differences between sexes do not affect parasitism strongly enough that only a single sex of a host species would be parasitized.

We also found that dragonflies had a significantly lower parasitism rates than damselflies in both endo- and ectoparasites. In general, dragonflies are larger, they have a lower population density and they have a longer larval period than damselflies. These factors may decrease dragonflies susceptibility to parasitism, therefore explaining why damselflies have a higher parasitism level.

According to my results, differences exist on a species level, but it cannot be said that either sex could be considered "sicker" or more prone to parasitism. However, the fact that female damselflies had more gregarines than males, but no difference existed in water mite parasitism, indicates that the infection and transmission mechanisms of these parasites may play a much bigger role than previously assumed. It also seems that dragonflies experience lower parasitism rates than damselflies.

4.2. From host phylogeny to immune defense - what effects parasitism? (II)

In paper II, I continued my investigation into what determines odonate parasitism by evaluating three aspects of odonate species: strength of immune defense, body mass and phylogeny. Using an artificial pathogen, a piece of nylon filament, I measured the encapsulation response of 22 different damselfly and dragonfly species and found that (i) there are significant encapsulation differences between species, (ii) body mass has a strong association with encapsulation and parasite prevalences, (iii) body mass shows a strong phylogenetic signal, whereas encapsulation response and gregarine and water mite prevalences show weak signals, and (iv) associations between the traits are affected by phylogeny.

I found considerable variation in the encapsulation response between different odonate species, but not between sexes. Without taking phylogeny into account, the encapsulation response was associated with odonate mass, but not with either parasite prevalence (Figure 5). It is possible that larger odonates may capture more prey on an absolute level compared to smaller species, indicating that they may be able to use more resources for mounting an encapsulation response. A larger body may also function as a larger energy reserve when faced with parasitism, therefore
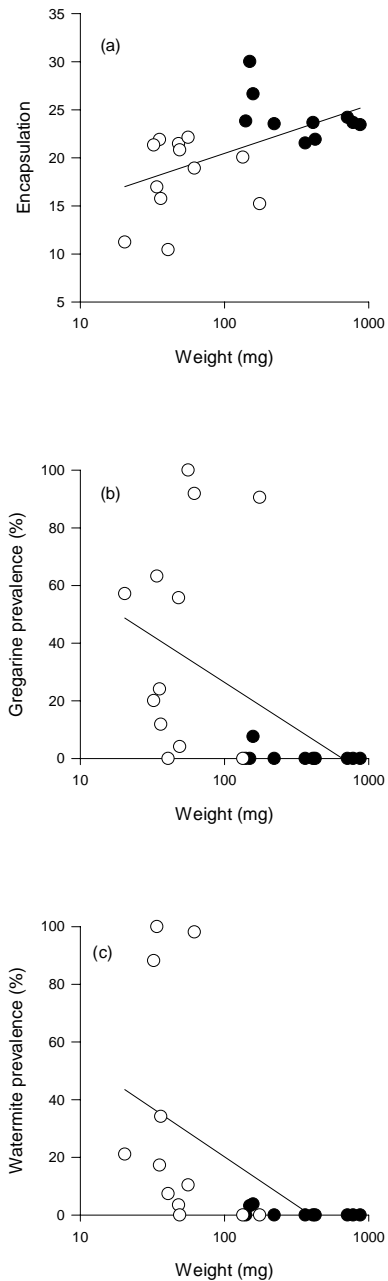
Figure 5. *Encapsulation response (a), gregarine prevalence (b) and watermite prevalence (c) in relation to the average fresh body mass (weight) of different odonate species without phylogenetic corrections. White dots represent damselflies (Zygoptera) and black dots represent dragonflies (Anisoptera).*

improving the encapsulation response of larger species. However, all four traits measured showed a phylogenetic signal, and after controlling for this, the associations between the four measured traits lost their significance. This indicates that they are more similar between closely related odonate species than would be by chance. It also indicates that other traits, either behavioral or physical, are different between large and small odonates and affect gregarine and water mite parasitism. Further research is required to understand what traits affect parasitism when taking phylogeny into account.

4.3. Ménage à trois - do both parasites prefer the same host species? (III)

The results of paper II indicated that small odonates have higher parasitism rates than larger odonates and it also indicated that there might be a correlation between gregarine and water mite prevalences. I decided to test these assumptions using a larger data set, phylogenetic analysis and two different phylogenetic trees.

I found that dragonfly and damselfly host species show great variability in their gregarine and water mite parasitism, but three distinct patterns can be observed from our dataset. First, an odonate host species was more likely to have both gregarines and water mites, rather than only one or neither. Second, water mite prevalence was positively associated with gregarine prevalence across host species and there was a similar trend when gregarine prevalence was associated with water mite prevalence,

but only in the larger standardized tree (Figure 6). In addition, there was no strong support for a difference between the average gregarine and water mite prevalences. Third, a weak phylogenetic signal was detected in gregarine prevalence and a stronger one in water mite prevalence. This indicates that, at least with water mites, closely related odonate hosts are more likely to share similar levels of parasitism than would be expected by chance. Additionally, I found that water mite prevalence was associated with the number of examined individuals per species, meaning that species with a higher number of examined individuals were more likely to have parasite prevalence than species with a low sample size. However, this did not have an effect on the relationship between the two prevalences.

Odonate parasitism seems to aggregate to small, common and densely living odonate species. It seems that ectoparasites prefer host species with these characteristics, but at the same time these species are more prone to endoparasites due to the parasites infection and transmission mechanism.
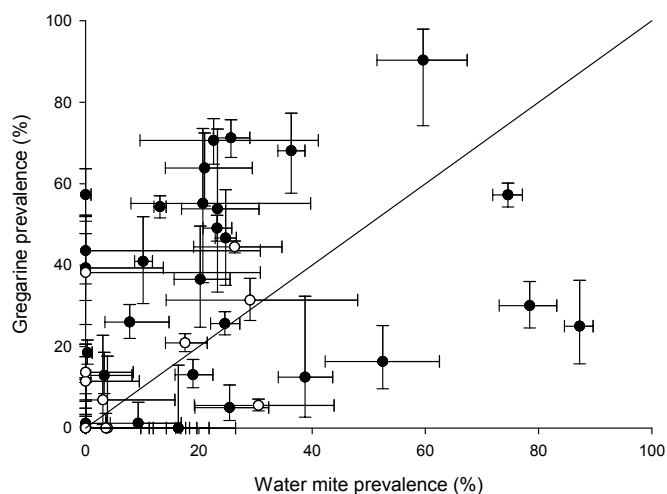

Figure 6. Gregarine and water mite prevalence (%) for each odonate species (n = 46) with 95% confidence intervals. White dots represent dragonflies and black dots represent damselflies. The continuous line indicates an equal prevalence of both gregarines and water mites. Note that seven dragonfly species have no gregarines or water mites and are therefore clumped in the origo.

4.4. Size, location and male aggression - the parasitism pattern of the odonata order (IV)

To further investigate what affects odonate parasitism, I expanded my data set to include 130 odonate species along with the host species' wing size (physical trait), territoriality (behavioral trait that considers mostly males) and latitude (geographical trait) in combination with their ecto- and endoparasitism levels.

I found that both territorial and large species have fewer ectoparasites than small or non-territorial species (Figure 7), while species from higher latitudes had more ectoparasites than species from lower latitudes. Interestingly, endoparasitism was not affected by any of these host traits.

We confirmed our previous finding from paper II that large species are less likely to be parasitized than small species. It is possible that larger species have a thicker cuticle, higher immune defense or they have a lower population density therefore making them less likely parasitized. I also found that territorial species are less likely to be parasitized than non-territorial species. This is an interesting finding, since it is only the males of a species that exhibit territoriality. It seems that because being territorial requires a high fitness, there is a selection towards resisting parasitism in territorial species, which may explain why territorial species have a lower endoparasite prevalence than non-territorial species. Thirdly, an increase in latitudinal center of a species increased the ectoparasitism of an odonate species. Odonates are ectotherms and therefore tightly linked with the latitudinal thermal gradient. Northern latitudinal center, i.e. a decrease in environmental temperature, may increase odonates susceptibility to parasitism or the number of potential host species may decrease towards the poles causing an aggregation of parasites to these northern species. Surprisingly, endoparasitism was not affected by any of the host traits measured. It seems that because endoparasites infect hosts through ingestion prey vectors or water, this method of infection is random enough not to be associated with host size, behavior or geography.

Water mite parasitism appears to be influenced by a variety of host species traits, suggesting a long lasting co-evolutionary history. Gregarines, on the other hand, seems to be more of a random event in an odonates life, therefore reducing the possibility of a co-evolution between odonates and their endoparasites.
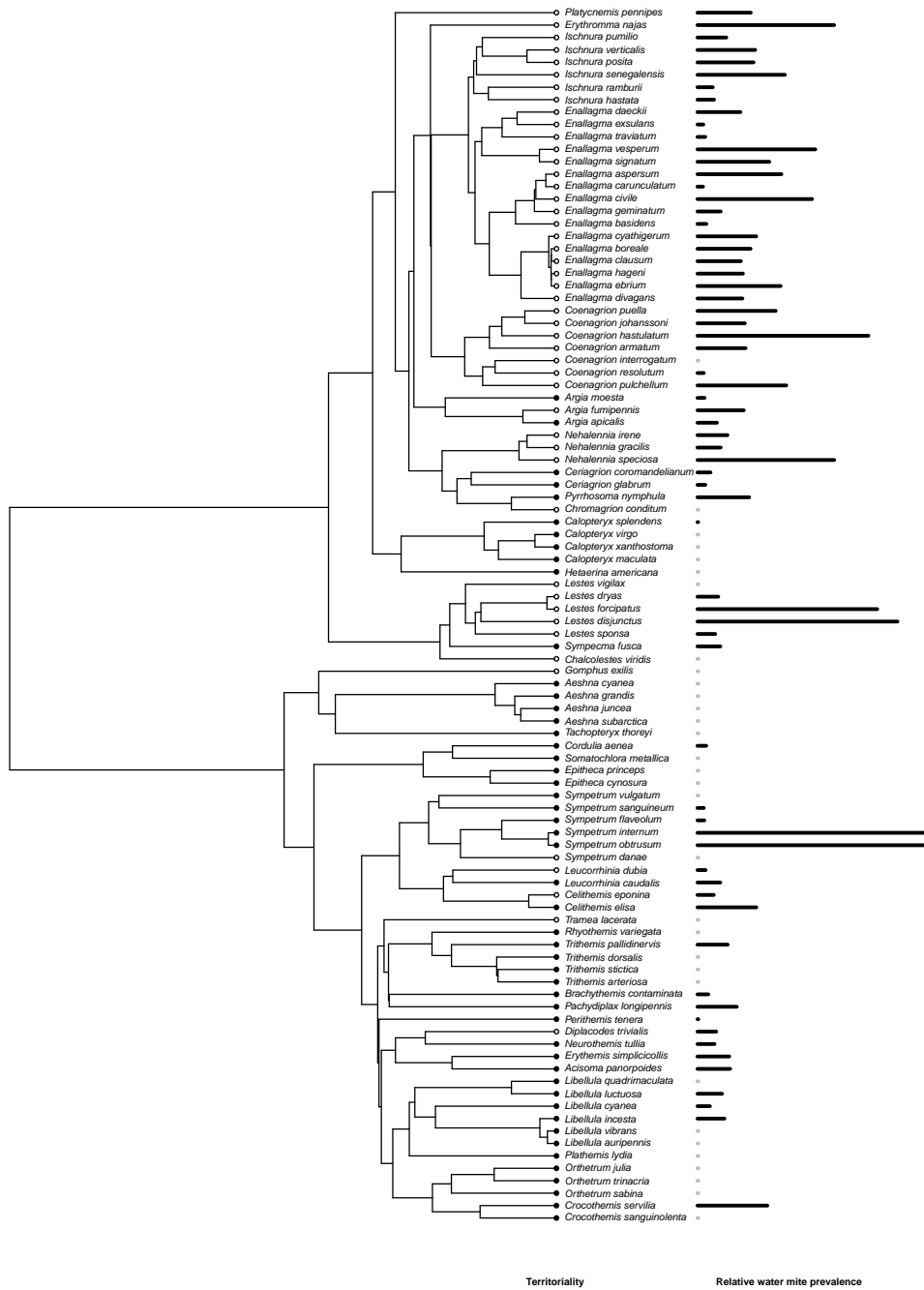
Figure 7. *The phylogenetic tree of different odonate species and their territorial status and water mite parasitism. In territoriality, a black dot represents territorial and a white dot non-territorial behavior. In relative water mite prevalence, a grey dot represents 0% prevalence and the length of the black bar indicates the value of relative prevalence.*

4.5. From host traits to parasite preferences - in the end, what determines parasitism?

In my four sub-studies (papers I, II, III and IV) I have evaluated the endo- and ectoparasitism of damselflies and dragonflies and analyzed how selected physiological, behavioral and geographical traits of host species affect their parasitism.

According to my results, gregarine endoparasitism is not affected by odonate traits (paper IV). They seem to infect hosts via prey vectors and water and it seems that this method of infection and transmission is random enough and not influenced by odonate sex, size, territoriality or geography. Endoparasitism does show a phylogenetic signal, albeit a low one, indicating that there might be an unknown odonate trait that influences endoparasitism.

Ectoparasitism by water mites is strongly influenced by the traits of odonates. Because larval water mites actively choose their hosts and odonates are able to resists water mites, co-evolution has played s substantial role in this relationship. This is visible as significant associations between odonate traits and water mite parasitism and as phylogenetic signals in the Odonata order. Odonate sex influences water mite prevalence, but only to a minor degree, and so far it is unknown why damselfly females are the most parasitized group in the Odonata order (paper I). Odonate encapsulation response, i.e. immune defense, shows significant between-species variability, which may be linked to species size and parasitism (paper II). Odonate species body size does influence water mite parasitism, but it is unclear as to why large odonate species have a lower water mite prevalence (paper IV). Territorial behavior decreases odonate species' water mite parasitism and the high fitness of territory holders is a likely explanation into this decrease in parasite prevalence (paper IV). Furthermore, there seems to be a positive association between the latitudinal center of an odonate species and its water mite prevalence (paper IV).

4.6. Methodological issues

In paper III I also tested whether the construction method of a phylogenetic tree influences the result obtained from phylogenetic generalized least square (PGLS) models. I had a time-calibrated tree that was based on the larger tree created by Willink & Svensson (2017) and a tree that had its branch lengths standardized to 1, because it was based on previous phylogenetic trees created using different methods. Even though the time-calibrated tree was technically much more accurate, my results did not differ significantly between the two trees. It is likely that if the form of the trees are the same and the number of species used is high

enough, significant differences do not emerge. Therefore using a "hand crafted" tree is acceptable if a more accurate time-calibrated tree does not exist.

I also evaluated whether the number of examined individuals per species affects its parasite prevalence in paper III. I found that if a species had a high number of examined individuals it was likely to have a higher prevalence as well. It is logical that the more individuals are examined for a given species, the likelihood of finding parasites increases regardless of how widely infected the species is. Regardless, the most likely explanation as to why there is a positive association, is the tendency of scientists to examine common, high density species, which are also the same species that have the highest water mite prevalences.

5. CONCLUSIONS AND FUTURE RESEARCH

The Odonata order displays a huge variability in both endo- and ectoparasitism, ranging from a very low prevalence in some species to very high prevalences and intensities in some other species. Species traits also show vast differences making it somewhat challenging to establish what are the traits that affect the parasitism of a species, in general. That being said, it can be concluded that endoparasitism of odonates is not affected by any of the host species traits examined in papers I, II, III or IV. On the other hand, ectoparasitism is affected by several host species traits, most likely because of the co-evolutionary arms race that exists between odonate hosts and water mites forcing water mites to actively prefer the best hosts and odonates to develop new and better ways to resist and endure infections.

My results highlight the need for further studies. Since there is a phylogenetic signal in the gregarine prevalence, what is the host trait that affects this parasitism? What mechanism in being large reduces odonate's water mite parasitism? Why is territoriality affecting water mite parasitism? Why are northern species more likely to be infected by water mites than southern ones?

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