WESTERN SYDNEY
UNIVERSITY

Hawkesbury Institute
for the Environment

Sex allocation in Kelly’s citrus thrips, *Pezothrips kellyanus*: mechanisms, constraints and the role of endosymbionts

Alihan Katlav
BSc, MSc

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

Supervisors: A/Prof. Markus Riegler and Prof. James M. Cook

Hawkesbury Institute for the Environment
Western Sydney University

February 2021

Acknowledgements

First, I would like to sincerely acknowledge my principal supervisor, Associate Professor Markus Riegler, for his constant support and untiring leadership over the course of my PhD journey. Markus's inspirational approach in science and his patience and wisdom in finding solutions for problems is second-to-none. Beyond great scientific knowledge, his admirable managerial skills have immensely contributed to the success of my scientific achievements in the last four years. I am also indebted to my PhD co-supervisor professor James Cook for his contributions in conceiving the experimental designs and great feedback in my writing drafts which helped me improve my academic writing skills. James' great knowledge in evolutionary concepts of sex allocation was a huge asset.

Secondly, great thanks to Dr. Duong Thi Nguyen for her advice about Kelly's citrus thrips lab population culturing and her conceptual and practical advice during my research, and to Dr. Jennifer Morrow for sharing her great experience and knowledge in laboratory molecular biology. A special gratitude goes to Geraldine Tilden for her kind help in thrips laboratory culture maintenance and other technical assistance in the entomology laboratory, and Dr. Marcus Klein for his great technical advice in the PC2 molecular laboratory. I am also thankful to great advice on spermathecal analyses provided by Dr. Stephanie Krueger and Prof. Gerald Moritz from the University of Halle. Furthermore, I acknowledge Dr. Laurence Mound for a meeting at the Australian National Insect Collection which I found very inspirational. Many thanks also to Dr. Andrew Gherlenda, Dr. Jeff Powell (Hawkesbury Institute for the Environment, Western Sydney University) and Dr. Mohammad Ameri (Department of Biological Sciences, Macquarie University) for their all helpful advice on statistical analyses.

I would like to earnestly acknowledge the Western Sydney University for granting me the Postgraduate Research Award and Tuition Fee Waiver Scholarship (International) and the Hawkesbury Institute for Environment for providing me with a supportive environment with most sophisticated facilities that helped me thrive in scientific research. My thanks also to the Hawkesbury Earth Care Centre, Richmond, and Ross Hitchcock, Kulnura, for the supply of lemons and access to their lemon orchards. I also acknowledge the Hawkesbury Foundation

for the 2018 F.G. Swain Award that supported my project with additional research funding; the Australian Entomological Society for providing me a travel grant to support my attendance and presentation of a part of my research at the Society’s 50th AGM and Scientific Conference in Brisbane, QLD in 2019, and the Organising Committee of The Entomological Society of America for providing me the opportunity to showcase my research with the international entomological community at ESA's 2019 (physical attendance; St. Louis, MO) and 2020 (Virtual attendance) Annual Meetings and awarding me the prestigious the prestigious John Henry Comstock Award (International Branch) providing the travel funding for attending the upcoming ESA's 2021 meeting (Denver, CO). I also express my gratitude to the Australian Distinguished Talent Program of the Australian Department of Home Affairs which generously granted me an Australian Distinguished Talent Visa allowing me to live the rest of my life in beautiful Australia to achieve a research career and my life objectives, and to continue to serve in Australian agricultural research.

A heartfelt thank you goes to all may lab mates for all their support during the last four years. You are the kindest and the most down-to-earth fellows that everyone would wish to work with. I am particularly grateful to my faithful friend, Alexander Robertson, for his substantial help in my field trips and all the inspiring chats we had in our coffee breaks.

Last but not least, I owe a lot to my beautiful partner Sarah for all her unwavering love and being always there for me in my PhD journey. I am very lucky to have you in my life, Sarah, and I should confess that without you this story could not have happened.

Finally, I would like to dedicate this piece of work to the most magnificent mum, I could have ever asked for – Shams Paryav. You have always been my backbone and the reason for all my achievements in life. Words cannot express how much I love you, and I humbly thank you for giving me the courage to follow my chosen path.

Statement of authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.

[redacted]

Alihan Katlav

February 2021

Preface

This PhD thesis comprises five chapters, including a general introductory chapter providing a thorough literature review, research aims and objectives, and overarching hypotheses of the thesis (Chapter 1), followed by three experimental data chapters (Chapters 2 to 4), and the final general discussion chapter (Chapter 6) which discusses the key findings of the thesis and outlines the current research caveats and remaining unanswered questions that warrant several important future studies. The three experimental data chapters are structured in the format of stand-alone manuscripts for publication in peer-reviewed international research journals and thus repetition of some concepts is unavoidable. Each experimental data chapter encompasses the following sections: abstract, introduction, materials and methods, results, discussion as well a supporting information and appendices, at the end of each chapter. Chapter 2 has already been published in the journal *Functional Ecology*; Chapter 3 has been submitted to the journal *Evolution*, has been revised according to two anonymous reviewers, and the revised manuscript (minor revision) is under review with the same journal; and Chapter 4 is planned to be submitted to a peer-reviewed journal after submission of this thesis.

I am the main author of all chapters. I conceptualised and designed all experiments under the guidance of my supervisory panel, Assoc. Prof. Markus Riegler (principal supervisor) and Prof. James Cook (co-supervisor). I conducted all the laboratory experiments, collected and analysed all data, wrote the individual chapters with contributions and guidance of my supervisory panel. Chapters 3 and 4 received further conceptual contributions and technical advice of Dr. Duong Nguyen who will be an additional co-author of these chapters.

This thesis was completed during the SARS-CoV-2 (COVID-19) pandemic. Although the campus and laboratory lock-down did not hinder the completion of the current experimental data chapters, it resulted in the completion of data analyses and thesis writing in my small college accommodation. Furthermore, one additional data chapter was omitted from the thesis albeit a large data set for it had already been collected. This data collection effort follows up on Dr Nguyen’s previous work and covers in more detail the genetic diversity of *P. kellyanus* and infection frequencies of *Cardinium* and *Wobachia* across the native and invasive ranges.

This research will be prepared as a separate manuscript for submission to a peer-reviewed journal after the submission of the PhD thesis.

The thesis structure is as below:

Chapter 1: **General Introduction**

Chapter 2: **Egg size-mediated sex allocation and mating-regulated reproductive investment in a haplodiploid thrips species**. Alihan Katlav, James M. Cook, Markus Riegler (2020) *Functional Ecology* (online early view version; DOI: 10.1111/1365-2435.13724).

Supporting information and supplementary figures are provided in Appendix I at the end of the chapter. The published version of the paper is embedded at the end of the thesis in Appendix IV.

Chapter 3: **Constrained sex allocation after mating in a haplodiploid thrips species depends on maternal condition**. Alihan Katlav, Duong T. Nguyen, James M. Cook, Markus Riegler. Revised manuscript currently under peer-review with the journal *Evolution*.

Supporting information and supplementary figures and tables are provided in Appendix II at the end of the chapter.

Chapter 4: **Endosymbionts affect sex allocation and fitness through egg-size manipulation in a haplodiploid host species**. Alihan Katlav, Duong T. Nguyen, James M. Cook, Markus Riegler. Manuscript in preparation for submission to a peer-reviewed journal.

Supporting information and supplementary figures and tables are provided in Appendix III at the end of the chapter.

Chapter 5: **General Discussion**

Table of Contents

List of Tables

List of Figures

ABSTRACT

Kelly’s citrus thrips, *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae), native to Australia and invasive in New Zealand, other Pacific islands and Mediterranean countries, is an important pest of citrus. Despite its wide distribution, not much is known about its biology and reproductive strategies. Insecticide application is the predominant control measure (in particular in the invasive range) for this pest. Recently it has been shown that *P. kellyanus* is associated with two maternally inherited endosymbiotic bacteria, *Cardinium* and *Wolbachia* that can manipulate the reproductive biology of this species. In this haplodiploid species, these two bacteria cause cytoplasmic incompatibility (CI), which, in its simplest way, occurs when an infected male mates with an uninfected female. CI can lead to the mortality of fertilised embryos. In diplodiploid insects this results in no offspring, however, in haplodiploid insects this can result in production of male offspring and a sex ratio distortion. Endosymbiont induced CI is a mechanism by which endosymbionts can invade host populations, however, invasion of endosymbionts in haplodiploid species can be hindered by the production of large numbers of uninfected males as an outcome of incompatible matings. Furthermore, CI offers a modern environmentally friendly control strategy known as incompatible insect technique (IIT) that has been tested for the control of mosquitoes and fruit flies and could potentially be developed for thrips control in the future. However, in order to understand the endosymbiont invasion dynamics, and prior to the development of IIT programs, the reproductive strategies of host species and the endosymbionts’ effects on host reproductive biology and other life history traits need to be scrutinized. Understanding the mechanisms of sex and resource allocation, the sex ratio adjustment patterns and their effects on life history traits of parents and offspring can provide insights into the population dynamics of endosymbionts which can subsequently help the development of new pest management strategies in the future.

This PhD project aims to disentangle the mechanism of sex allocation in *P. kellyanus* and the effect of *Cardinium* and *Wolbachia* in the reproductive biology of this species. This PhD thesis encompasses five chapters. Chapter 1 provides a comprehensive literature review about sex and resource allocation strategies and their consequences on evolution of sexual selection and mating systems in arthropods with a focus on species with haplodiploid reproduction systems, as well as relevant concepts about the factors that can manipulate reproductive biology of insects such as paternal sex ratio (PSR) elements and reproductive parasites. It also introduces the potential roles of the endosymbiotic bacteria *Cardinium* and *Wolbachia* in evolution of sex allocation in haplodiploid insects and their potential application in biological control of haplodiploid arthropods. This chapter also covers the aims and scope of this PhD thesis.

Chapter 2 establishes sex allocation mediated by egg size for the first time for any haplodiploid insects. Using *P. kellyanus* it investigates the link between the size of eggs and the sex of the developing embryos within, and unravels the mechanisms underlying the sex ratio adjustment and sex-specific egg size dimorphism in this species. It shows that females can control the sex of offspring by regulating the resource allocation towards eggs prior to fertilisation. Thus far, very little research has been undertaken to study egg size effects on offspring sex and fitness in insects, and in arthropods more widely, and therefore this chapter delivers ground-breaking findings for the study of sex allocation in haplodiploid insects. It also raises interesting questions regarding the evolutionary forces driving reproductive strategies in haplodiploid species.

Chapter 3 studies the mechanisms underlying constrained sex allocation and split sex ratios that have previously been discovered in *P. kellyanus*. This species has a bimodal reproduction mode, i.e. the majority of mated females generally produce extremely female-biased broods, but still a considerable proportion of mated females produce extremely male-biased brood. Controlled mating experiments revealed that production of male-biased broods was not due to endosymbionts nor influenced and inherited by fathers, as sons of constrained females still produced female-biased brood. Therefore, constrained sex allocation in *P. kellyanus* is not associated with PSR elements that can cause such sex ratio bias in other species. However, constrained sex allocation in *P. kellyanus* was found to be a maternal effect, as large females produced large eggs that were fertilised and became female offspring. This chapter suggests that constrained sex allocation may be a fitness-based strategy to counterbalance the highly female-biased population sex ratio in *P. kellyanus*.

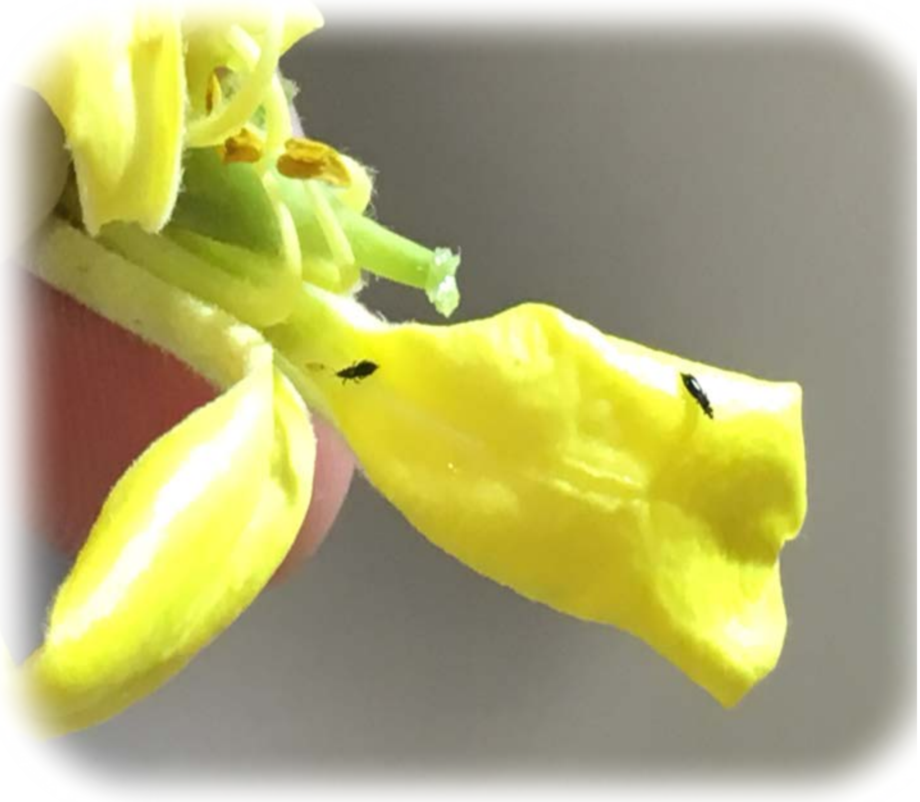
Chapter 4 addressed the role of *Cardinium* and *Wolbachia* in the process of resource allocation and manipulation of egg size-mediated sex allocation in *P. kellyanus*. It detected a complex fitness cost-benefit interaction between both endosymbionts, with indirect (via maternal resource allocation) and direct impacts on offspring fitness. *Cardinium* was found to be beneficial to the host, whereas *Wolbachia* appeared to act antagonistically. Interestingly, previously published research on endosymbiont-induced CI in this species found that the CI induced by *Cardinium* may be less favourable to endosymbiont invasion than the CI phenotype caused by *Wolbachia*, and therefore the *Cardinium* fitness benefit could have evolved to overcome this invasion barrier. The findings of this chapter also support the different prevalence of *Cardinium* and *Wolbachia* across the native and invasive range of *P. kellyanus.*

Chapter 5 summarises the key findings of this thesis and proposes future research directions. Overall, this thesis expands the knowledge about the evolution and mechanisms of sex allocation in thrips and haplodiploid insects; the mechanisms of constrained sex ratio in thrips and haploidploid insects; and the role of maternally inherited endosymbionts in modulating egg size and thereby sex allocation favouring their invasion of host populations.

Finally, the findings of this PhD project contribute fundamentally to future studies of the population dynamics of *Cardinium* and *Wolbachia* in haplodiploid species and their use in the development of IIT programs to manage *P. kellyanus* and other haplodiploid pests.

GENERAL INTRODUCTION

> *"It is not the strongest of the species that survives,*
> *not the most intelligent that survives.*
> *It is the one that is the most adaptable to change."*
> **Charles Darwin**



Individuals of *Pezothrips kellyanus* on flowers of Australian native frangipani, *Hymenosporum flavum*

1.1 What is sex allocation?

Decision on how to allocate energy budget to reproduction at any given time is a fundamental aspect of life history of organisms (Schmidt-Nielsen 1984; Stearns 1992; Charnov 1993; Davies et al. 2012). This allocation towards offspring production must be traded-off against other biological expenditures such as growth rate, lifespan and future reproductive efforts (Noordwijk & Jong 1986; Flatt & Heyland 2011), and can involve adjustment of offspring size and/or number (Stearns 1989; Olsson & Shine 1997), and, in some species, parental and/or social care. The resource pool available for these allocations is limited, thus any changes in one allocation is likely to result in a concomitant change in another (Zhang & Jiang 2002).

A key aspect of reproductive decision is sex allocation which involves the adjustment of resource allocation to any particular offspring sex, and offspring sex ratio is an important aspect of this (Charnov 1982; Bulmer 1994). Sexually reproducing species are generally expected to produce female and male offspring at an approximately equal ratio. This equal sex ratio theory was first conceptualised by Carl Düsing (1884) assuming that mating opportunities for individuals of both sexes and fitness return on investment towards production of the two sexes are equal. Fisher (1930) further developed this principal in ‘The Genetical Theory of Natural Selection’ without references to Düsing (1884) and no other book of the same period treated the subject and therefore Düsing’s contribution remained unknown to the English literature and was then attributed to Fisher and became commonly known as Fisherian principal (Edwards 1998).

In many cases the principle of equal sex ratio is not met. When sex-specific fitness returns of investment into female versus male offspring vary among individuals depending on conditions or resource availability, variation in the sex ratio can appear (Hamilton 1967; Trivers & Willard 1973). When reproductive fitness returns of investment into one sex are higher than the other, it is advantageous for individuals to shift their reproductive efforts towards the more rewarding sex, if they have sufficient resources to do so (Trivers & Willard 1973). For example, in many haplodiploid species sex allocation can be female-biased depending on their population structure. Such a bias may arise under local mate competition (LMC) which occurs when founder females are scarce in natal batches and male siblings may compete over mating with their sisters, and therefore earn less return on investment. In this situation, selection may favour higher investment towards female production to compensate for the paucity of females in the population (Hamilton 1967). However, the patterns of allocation are not always similar among females resulting in variation in sex ratio among mothers experiencing different conditions (Trivers & Willard 1973). More interestingly allocation patterns do not always have the same direction between sexes, and this may generate an evolutionary arms race between male and female interests (sexual conflict) over mating preferences, frequencies and offspring sex ratios (Parker 1979; 2006; Shuker & Day 2001; Chapman 2018).

1.2 Genetic regulation of sex allocation

Sex allocation in organisms can be regulated at three levels (Werren & Beukeboom 1998). The first level occurs before fertilisation (pre-zygotically), when a mother's sex allocation genes may affect sex of offspring. At this level, strong biases in sex ratios are rarely found in diplodiploid heterogametic systems, and most species meet the equal sex ratio hypothesis of equal investment toward both sexes (Werren & Beukeboom 1998). At the remaining two levels, sex allocation is influenced by post-zygotic genetic processes (Beukeboom & Perrin 2014): this can involve expression of maternal genes that act in the developing zygote, or genes that are expressed by the developing zygote itself, which then impact the sex of zygote (Werren & Beukeboom 1998). It is, however, worth mentioning that epigenetic factors linked with different biotic and abiotic factors can also manipulate the regulation of gene expression at any level. Selection on genes in the first two categories is associated with parental conditions, while the last stage is further affected by conditions experienced by the zygote itself (Beukeboom & Perrin 2014).

1.3 Sex ratio at different life stages

Sex ratio is generally defined as the proportion of individual males to the total number of individuals which can be assessed at different life stages: (1) sex ratio upon fertilization (primary sex ratio); (2) sex ratio after hatching of eggs and before emergence of adults (secondary sex ratio); (3) sex ratio upon emergence of adults (tertiary sex ratio); and (4) ratio of sexually active females to males in the population (operational sex ratio) (Majerus 2003; Terry & Kelly 1993; Bondy & Hunter 2019). The primary sex ratio is generally the most reliable stage for the analysis of sex allocation (West 2009). However, most sex allocation studies assess the sex ratio at adult stages instead of primary sex ratios prior to or at fertilisation, or at least at oviposition. Sex-specific developmental mortality of male and female offspring can potentially confound the accurate sex allocation analyses (Bondy & Hunter 2019). Moreover, sex allocation is frequently (and superficially) considered synonymous with only sex ratio adjustment; however, it must be acknowledged that parental investment towards offspring may extend beyond sex ratio adjustment (Shuker et al. 2009). An alternative strategy for parents (mainly mothers) to adjust sex allocation is modifying the quality of the eggs, via different investment of resources into the eggs that develop into different sexes (Clutton-Brock 1991; Berthouly et al. 2008). Different size of eggs that develop into females or males is another strong indicator for sex allocation other than a biased sex ratio (Macke et al. 2011). If eggs that develop as the more frequent sex are larger, then resource allocation towards this sex might be higher than anticipated in a situation where egg size does not differ (Cordero et al. 2000). On the other hand, allocation of resources can be even or smaller if eggs that develop into the more frequent sex are smaller (Macke et al. 2011). This biased investment toward eggs via egg size might be regarded as another evolutionary consequence of the genetic arms race between females and males, driving another mode of sexual conflict that is not seen if one was to only look at the sex ratio (Macke et al. 2011).

1.4 Sex allocation in haplodiploid animals

Haplodiploidy is a sex determination system that occurs in many arthropod taxa. In this system sex is defined by an individuals' ploidy status (number of chromosome sets), with females being diploid and males haploid, and females develop from fertilised eggs while males develop from non-fertilised eggs. Therefore, males only possess the maternal genome which they pass on to female offspring only (Otto & Jarne 2001; Normark 2003; Engelstädter & Hurst 2006). It is remarkable that this sex determination system has independently evolved (at least ten times) in at least 15% of animal species: several lineages of arthropods such as insect orders Hymenoptera (e.g. ants, bees, wasps), Coleoptera (bark beetles), Thysanoptera and Hemiptera, as well as in the arachnid order of Acari (mites and ticks) (Andersson 1984; Normark 2003; 2004). Arrhenotoky is the most common form of haplodiploidy, in which males are haploid and develop from unfertilised eggs and females are diploid, resulting from fertilisation of the female's haploid ova with the male's haploid sperm (Normark 2003; Engelstädter & Hurst 2006).

In arrhenotokous haplodiploidy females can have an adaptive control over fertilisation (and therefore over sex allocation) in response to population density and environmental factors (King 1987; West & Sheldon 2002). However, the underlying mechanisms that allow females to regulate the fertilization process and sex ratio adjustment has not been well studied (but see Macke et al. 2011). Understanding such mechanisms in haplodiploids can provide clearer predictive models about population dynamics (Helms et al. 2005), the evolution of sociality (Rautiala et al. 2018) and the selective forces that trigger variation in phenotypic traits (e.g. body size, polyandry, mating rate and resource allocation) that affect offspring sex ratios (Trivers & Willard 1973; Macke et al. 2012a; Schärer & Pen 2013).

Another unusual, less common form of haplodiploidy is pseudoarrhenotoky which is also called ‘paternal genome elimination’ (PGE) in which zygotes that develop into males lose their paternal genome during early embryonic development (Normark 2003). It is suggested that PGE is an evolutionary precursor of arrhenotoky (Schrader & Hughes-Schrader 1931; Cruickshank & Thomas 1999).

Another rare form of haplodiploidy is thelytoky which has so far been recorded for about 1,500 arthropod species (e.g. in aphids and thrips) (White 1984; Nguyen et al. 2015). This system drives the evolution of asexuality in which females produce unfertilized diploidised eggs resulting in all-female offspring (Stenberg & Saura 2009). Thelytokous parthenogenesis can be defined by the host genome, and polyploidy is one example of this (Nguyen et al. 2015). In some haplodiploid species thelytoky is induced by maternally inherited bacterial endosymbionts, such as *Wolbachia, Cardinium* and *Rickettsia,* which cause complete diploidisation of the egg by host gamete duplication resulting in a diploid homozygous individual (Weeks & Breeuwer 2001; Provencher et al. 2005; Schneider et al. 2011). In this system elimination of the bacteria from host via antibiotic treatment can (but not always) restore arrhenotoky and male production in the host species (Stouthamer et al. 1990). However, in some parasitic wasp species removal of endosymbionts from thelytokous lineages causes failure in oogenesis and reproductive impairment (Dedeine et al. 2001).

1.5 Sex allocation drives sexual conflict between parents

The evolution of anisogamy, i.e. sexual reproduction that involves two types of gametes (Dusenbery 2009), has promoted distinct roles as well as sex-specific reproductive interests in males and females (Chapman et al. 2003). It is well ascertained that when selection works in two opposite directions, it often leads to a conflict (Keller 1999). A profound example is the discrepancies in evolutionary outcomes between males and females that can result in a sexual conflict (Parker 1979; Arnqvist & Rowe 2005). This conflict is a key driving force in male–female antagonistic coevolution (the evolution of adaptation in one sex and counter-adaptation in the other) (Arnqvist & Rowe 1995). Sexual conflicts can lead to evolution of traits that are beneficial to one sex but not to the other, and can occur for traits relevant to courtship, mating, fertilisation and parental investment into offspring (Parker 1979; Chapman 2006; Boncoraglio & Kilner 2012).

Generally, traits related to reproduction in females versus males may have different optima due to the differential cost and benefit of reproduction between male and female in terms of parental investment, establishing a conflict (Williams 1966; Chapman 2006). This conflict results in situations where reproductive traits evolve towards an optimum in either sex, however this cannot simultaneously be realised for both sexes, i.e. one sex cannot reach its optimal trait. Therefore, the adversely affected sex may evolve counter-adaptation to compensate for this fitness reduction (Lessells 2006). For example, multiple mating of females confers more fitness advantage on females than on males through increased chances for fertility, increased number of compatible matings and greater genetic diversity of their offspring (Bateman 1948; Andersson 1994; Tregenza & Wedell 2000; Hosken & Stockley 2003). However, males benefit from mating with monogamous females, as this ensures them to sire all the females' offspring. This sexual conflict can lead to adaptation by males and counter adaptations by females

(Hammerstein & Parker 1987; Clutton-Brock & Parker 1992). For example, males can transfer seminal toxins that reduce female receptivity to re-mating with other males, while females may evolve resistance to this coercive trait to restore polyandry (reviewed in Simmons 2001).

It has been suggested that differing sex allocation and biased sex ratios are amongst the most important factors driving sexual conflict between females and males (Shuker et al. 2009). Different reproductive systems experience sexual conflict at different levels. Best example of this is haplodiploidy in which genes are not evenly transmitted to different sexes, with paternal genes only being transmitted to daughters while sons have no reproductive value to males. However, females transmit their genes to both sons and daughters (West 2009). Thus, in this genetic system the reproductive investment returns from female and male offspring can be markedly different for mothers and fathers, leading to possible sexual conflicts over sex ratio (Trivers & Hare 1976). Such an evolutionary arms race can give rise to the evolution of secondary traits in males (e.g. male weapons and ornaments, mate guarding, and morphology of signalling traits such as antennae) (Eberhard 1985; Cameron et al. 2003; Snook et al. 2013), which can result in the evolution of antagonistic traits in females that counter the males' mating decision and sex allocation strategy (Parker 1979). Moreover, in some haplodiploids, males can transfer sperm together with accessory gland compounds that increase fertilization and production of daughters in females (Colonello & Hartfelder 2005; Gillott 2003). Theoretically, females may resist this manipulation to recuperate the sex ratio control (Parker 2006), yet what adaptive traits are involved in females needs to be explored in different taxa.

1.6 Factors affecting and constraining sex allocation

Identifying the mechanisms that drive sex ratio bias can help improve our understanding of sex allocation models and associated evolutionary theories. Moreover, elucidating factors affecting sex ratio adjustment and their fitness consequences on both parents and offspring can provide valuable information on how individuals optimize their reproductive efforts together with investment into other biological traits (West & Sheldon 2002; Pike & Petrie 2003; Shuker & West 2004). Although sex determination follows primarily genetic pathways (which can be altered by epigenetic factors such as thermal condition as in reptiles; see Janzen 1994), several abiotic and biotic factors can profoundly affect the sex ratio at different levels, from the primary to the operational sex ratio (Bondy & Hunter 2019). Trivers and Willard (1973) first developed the concept of conditional sex allocation that suggests parents should adjust their offspring sex ratio in response to abiotic factors. This concept has so far received the highest attention in sex allocation empirical studies (e.g. study of environmental factors such as temperature; photoperiod and pH; Cook 2002; Göth & Booth 2005; Xiao et al. 2016).

Sometimes biotic factors such as mating opportunity, resource availability (Ghislandi et al. 2018) as well as mating interference can affect sex allocation (De Barro et al. 2011; Liu et al. 2012). In some haplodiploid species, a fraction of females can be constrained to only produce male offspring. This impediment in sex ratio adjustment, generally called constrained sex allocation, results from various factors (Godfray 1990). One major source of constrained sex allocation occurs when some females fail to acquire a mate and remain virgin; hence they could only produce unfertilized eggs that all develop to male offspring (Higgins & Myers 1992; Guertin et al. 1996; Kranz et al. 2000). Virgin females in some species are shown to be less fecund than mated females (Tagawa 1987; Shukla et al. 2013; Macke et al. 2012a) or have a lower oviposition rate (eggs per female per day). This is probably a strategy to withhold their resources for female production after possible future matings (Macke et al. 2012a). This may also arise from higher resource availability for mated females specially in species where female receive a male nuptial gift that boost the reproductive budget (Van den Beuken & Smallegange 2018). In the haplodiploid beetle *Coccotrypes dactyliperda*, virgin females extend their lifespan until their first clutch of sons hatch, so that they can mate with them (Gottlieb et al. 2011).

Constrained sex allocation can even occur at the post-copulation stage. In some parasitoid wasps of the families Braconidae, Aphididae and Pteromalidae, mated females produce male-only offspring after mating (see Godfray 1990). For example, in *Nasonia vitripennis* (Ptromalidae), male-only production even after two-time mating is due do the blockage of spermathecal duct that prevents leakage of sperm to the oviposition chamber (Van den Assem 1977). In many other parasitic wasps females become unreceptive after the first mating and they may become constrained to produce male-only offspring even if mating doesn't secure successful insemination owing to mating disturbance or mating with sperm depleted males (Van den Assem 1970). In some parasitoid wasps, females produce male and female offspring in different hosts, therefore their sex allocation is constrained by resource availability, i.e. availability of either hosts (Godfray & Waage 1990).

Sex allocation can also be constrained by both paternal and maternal physiological traits and fitness (Trivers & Willard 1973; Charnov 1982; Henter et al. 2004). For example, in the parasitoid wasp *Uscana semifumipennis* females mated with smaller males produce more male-biased sex ratios (Henter 2004). Henter (2004) hypothesized that this could be due to their lower quantity or quality of sperm in smaller males. On the other hand, in some other species, smaller females produce more male-biased broods than larger females, which is hypothetically associated with their lower capability in foraging and finding larger hosts required for daughter production (Heinz 1996; Santolamazza-Carbone et al. 2007). However, the major physiological constraints that prevent smaller females from female offspring production remain unknown. Overall, in all mentioned examples excess of male production by constrained mothers lead to a frequency-dependent selection that drives unconstrained mothers to produce female-biased offspring to maintain the population sex ratio at the Fisherian equilibrium. This frequency-dependent selection can cause a split sex ratio in the population (Kranz et al. 2000), which is mostly studied in hymenopterans, yet the factors underlying constrained sex allocation remain elusive in other haplodiploid species.

Another important biotic source that can profoundly affect or constrain sex allocation patterns in arthropods is selfish genetic elements (SGEs) which include paternal sex ratio (PSR) elements and meiotic drivers (Werren et al. 1988; Werren & Stouthamer 2003; Wedell 2020) as well as reproductive parasites such as several endosymbiotic bacteria (Zchori-Fein et al. 2001; Perlman et. al. 2006; Hagimori et al. 2006; Werren et al. 2008; Giorgini et al. 2009; 2010) which are discussed in detail in the next section.

1.7 Role of SGEs in sex allocation

The study of the role of SGEs in biased sex ratios was commenced by Charnov (summarised in Charnov 1982) and gained profound attention in more recent sex allocation studies (Hurst 1992; Hurst & Werren 2001; Wild & West 2009). SGEs can include genes, organelles or endosymbionts, ubiquitously occurring within the genome or cells of eukaryotic organisms (Hurst & Werren 2001; Hurst & Frost 2015). They can manipulate host reproduction and normal patterns of inheritance with the outcome of representation at a higher frequency in subsequent generations and thereby facilitate their spread through populations (Hurst & Werren 2001; Burt & Trivers 2006; Wedell & Price 2015). Therefore, gametes are the most frequent cells targeted by SGEs as their manipulation may allow a bias for increased SGE inheritance (Wedell & Price 2015). For example, SGEs can give rise to reproductive incompatibilities in crosses between SGE-carrying males and non-carrying females. In some situations, this can lead to distortions of the population sex ratio (sex ratio distorters) and cause intra-genomic conflict (Zeh & Zeh 1996; Werren 2011). Such SGEs can have a major impact on the evolution of sex determination and reproductive systems (Hurst & Werren 2001; Jaenike 2001; Burt & Trivers 2006; Werren 2011). The non-Mendelian inheritance of SGE generates strong opposing selective forces for suppression of SGEs in host genomes to ensure a more equitable transmission of genes and restoration to equal sex ratios (Werren 2011; Wedell & Price 2015).

The role of SGEs, however, extends beyond mere changes in sex allocation and may involve a multifaceted impact on evolution of sexual selection (Price & Wedell 2008). Recent studies have shown that SGEs can reduce fertility and sperm competitive ability in males harbouring these elements (Wedell 2013). It is also suggested that the low fertility of SGE-carrying males promote polyandry as a female strategy to bias paternity against male carriers and reduce the frequency of SGEs in populations (Wedell 2013).

According to the stage at which they affect sex ratios, SGEs are classified into two categories: segregation distorters; post-segregation distorters (Wedell & Price 2015):

1.7.1 Segregation distorters

In diplodiploid species, during meiosis, sex chromosomes usually segregate evenly and each sex chromosome has a 50 % chance of being in a gamete. This generally results in the production of an equal sex ratio (1:1) (Düsing 1884; Fisher 1930), albeit females can skew this sex ratio towards the preferable sex in response to local conditions (Hamilton 1967, Trivers & Willard 1973). Sometimes, selfish genes can interfere with the equal segregation of sex chromosomes during meiosis to ensure the selfish gene’s presence in more gametes and increase their inheritance to the subsequent generations, resulting in biased sex ratio of offspring if they are linked to sex chromosomes or sex-specific inheritance (Burt & Trivers

2006). In species with heterogametic sex chromosomes (X and Y chromosomes), these selfish genes, called meiotic drivers, more commonly target males (except some species in which the heterogametic sex is female) where they can increase the inheritance of the genes of the sperm that carries them over those of the sperm that does not (Jaenike 2001). Maternally inherited meiotic drivers, and the biased sex ratio they induce, can generate a genetic conflict with the rest of genome of the host harbouring them (Burt & Trivers 2006; Werren 2011; Wedell & Price 2015). B chromosomes are another example of segregation distorters which are common supernumerary non-essential chromosomes in eukaryotes, that can ensure their transmission by eliminating the paternal chromosomes (Nur et al. 1988; Beukeboom 1994). An example of B chromosomes in haplodiploids are paternal sex ratio (PSR) distorters that so far have been found in a limited number of parasitic wasp species (Hunter et al. 1993). These SGEs can cause elimination of paternal genes in females mated with males carrying PSR elements resulting in all-male offspring production (Dobson & Tanouye 1996; Werren & Stouthamer 2003). An intriguing example of a segregation distortion has been found in *Eurema mandarina* butterflies (Kern et al. 2015; Kageyama et al. 2017) where a single *Wolbachia* strain is involved in both the disruption of maternal sex chromosome inheritance (a pre-segregation effect) and feminisation (a post-segregation effect) in *Wolbachia* infected lineages.

1.7.2 Post-segregation distorters

Post-segregation distorters (PSDs) are selfish genes or microorganisms that act after the fertilisation of an egg cell has been completed and zygote development has commenced, mostly by killing the offspring that does not inherit the SGE (Wedell & Price 2015). The most ubiquitous PSDs among arthropods are maternally inherited endosymbionts (mainly endosymbiotic bacteria) that induce variety of reproductive manipulations that can result in sex ratio distortion in host (Hurst et al. 1992; Stouthamer et al. 2002; Werren 2011; Wedell & Price 2015).

1.8 Roles of endosymbiotic bacteria in host fitness and sex allocation

Bacteria have contributed considerably to the evolution of more complex life forms and evolutionary innovations in eukaryotic hosts they are associated with. Over several millions of years, bacteria have evolved from free-living life modes towards symbiotic associations with eukaryotic organisms (Moran & Wernegreen 2000; Toft & Andersson 2010; Sachs et al. 2011). These symbiotic associations can be responsible for a wide spectrum of effects on hosts, ranging from harmful impacts (i.e. parasitic associations) that decrease the fitness of the infected host, to beneficial impacts as seen in mutualistic symbioses in which hosts benefit from symbionts in terms of higher survival or reproduction (Merhej et al. 2009; Medina & Sachs 2010; Sachs et al. 2011). The diversity of these associations has long been the major focus of biological research due to their evolutionary, ecological, agricultural and medical importance (Ochman & Moran 2001).

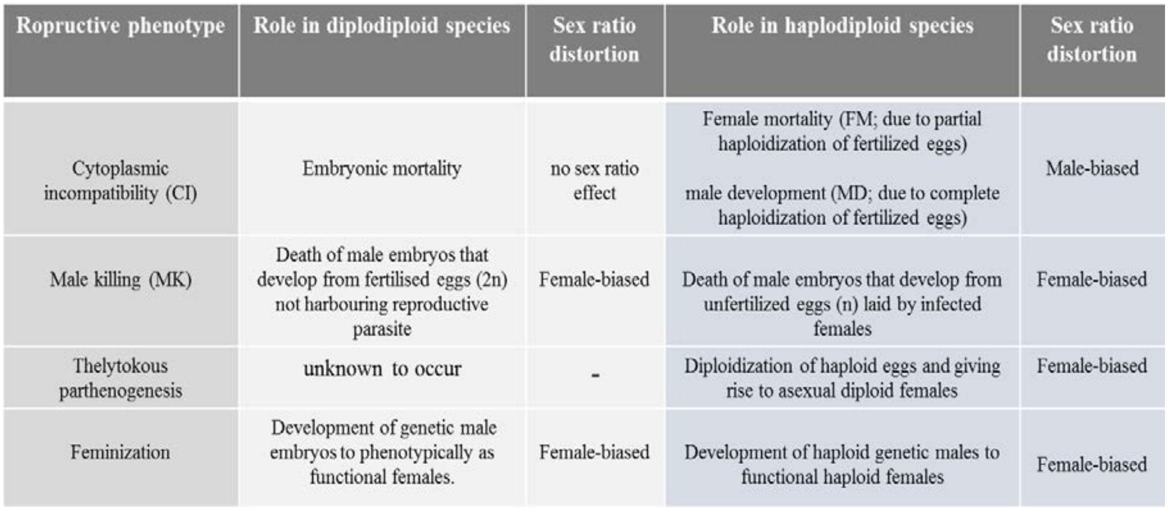
As with other living organisms, insects are closely associated with a variety of symbiotic bacteria at all stages of their lives. They play a vital role in shaping population dynamics, ecology and evolution of their hosts (Buchner 1953; Moran et al. 2008). Some endosymbiotic bacteria are categorized as obligate mutualists (primary symbionts) which occupy a distinctive host tissue, called bacteriome, contributing to provision of fundamental nutrients (such as essential amino acids) which their hosts depend on (Braendle et al. 2003; Moran et al. 2008; Wang et al. 2020). *Buchnera* and *Carsonella* are among the best examples of primary symbionts of insects, and occur in aphids and psyllids, respectively (Braendle et al. 2003;

Nakabachi et al. 2006; Tamames et al. 2007; Shigenobu & Wilson 2011; Hall et al. 2016; Morrow et al. 2017).

Unlike primary symbionts, facultative symbionts (secondary symbionts) are frequently not restricted to the bacterium, but distributed in various cell types including reproductive organs, and are not pivotal for host biology (Moran et al. 2008). It has been estimated that more than 70% of all arthropods are associated with secondary endosymbionts (Zug et al. 2012). These bacteria can play significant, but not essential, roles in host biology, including the protection of the host against pathogens and other natural enemies, resistance against heat stress and the provision of numerous biosynthetic nutrients and various other fitness modifications (Moran et al. 2008; Brownlie & Johnson 2009; Wang 2020).

Bacterial reproductive parasites are among the most ubiquitous facultative symbionts and common PSDs that can induce various reproductive manipulations to increase the production of infected daughters to increase their inheritance to the consequent generations (Hurst & Frost 2015). In some host species, these bacteria disrupt the mode of sex determination and sex allocation of their hosts because they are predominantly transmitted vertically with the female egg cytoplasm, but not via the male sperm. Therefore, males constitute evolutionary dead-ends for their inheritance. Thus, any manipulation by reproductive parasites that can lead to distortion of the host sex ratio towards infected females will be selectively advantageous (West 2009; Cordaux et al. 2011).

The best-known example of the reproductive parasites in arthropods is *Wolbachia pipientis* (commonly referred to by its genus name), a well-known representative of the class Alphaproteobacteria (phylum: Proteobacteria) and the most abundant intracellular bacterial endosymbiont (Riegler & O'Neill 2006). It has been estimated that different strains of *Wolbachia* may infect more than 65% of insect species as well as wide range of other groups of arthropods (52% of arthropods in total) and filarial nematodes (Hilgenboecker et al. 2008; Zug & Hammerstein 2012; Weinert et al. 2015). Over the last decades, *Wolbachia* has gained increasing attention largely due to its extremely high prevalence within arthropods, and its reproductive manipulations mostly involving post-segregation sex ratio distortions in its hosts. These manipulations include induction of thelytokous parthenogenesis (female-only parthenogenesis), feminization (development of genetic males into functional females), male killing (mortality of male offspring of infected females), and cytoplasmic incompatibility (crossing sterility between infected males and uninfected females) (Hilgenboecker et al. 2008; Werren et al. 2008) while pre-segregation manipulations such as disruption of chromosome inheritance as either meiotic drive (Kern et al. 2015) or chromosome elimination (Kageyama et al. 2017) have also been observed. It appears that in most hosts, *Wolbachia* causes little direct fitness cost other than the significant costs arising from the reproductive manipulations. However, there are several examples indicating that *Wolbachia* can be either deleterious or beneficial to its host's fitness (Zug et al. 2015). In haplodiploid species, reproductive manipulations induced by *Wolbachia* can result in a sex-ratio bias (via CI, male killing, parthenogenesis), whereas in some diplodiploid species only feminization and male killing can drive sex ratio distortion (Kageyama et al. 2012; Kern et al. 2015). The various impacts of reproductive parasites in haplodiploid and diplodiploid species is reviewed in Table 1.1.



Ropructive phenotype	Role in diplodiploid species	Sex ratio distortion	Role in haplodiploid species	Sex ratio distortion
Cytoplasmic incompatibility (CI)	Embryonic mortality	no sex ratio effect	Female mortality (FM; due to partial haploidization of fertilized eggs) male development (MD; due to complete haploidization of fertilized eggs)	Male-biased
Male killing (MK)	Death of male embryos that develop from fertilised eggs (2n) not harbouring reproductive parasite	Female-biased	Death of male embryos that develop from unfertilized eggs (n) laid by infected females	Female-biased
Thelytokous parthenogenesis	unknown to occur	-	Diploidization of haploid eggs and giving rise to asexual diploid females	Female-biased
Feminization	Development of genetic male embryos to phenotypically as functional females.	Female-biased	Development of haploid genetic males to functional haploid females	Female-biased

Table 1.1 Reproductive phenotypes induced by reproductive parasites in diplodiploid and haplodiploid species

CI is the most widespread form of reproductive manipulation, which occurs in crosses between infected males and uninfected females (unidirectional CI) (Werren 1997) or between males and females infected with different *Wolbachia* strains (bidirectional CI) (e.g. O'Neill & Karr 1990). According to the theoretical analysis of unidirectional CI, infected females benefit from a greater fitness advantage over uninfected females because they are compatible (and can successfully reproduce) with all males regardless of male infection status, whereas uninfected females are only compatible with uninfected males (Hoffmann et al. 1990). This manipulation can lead to a rapid spread of *Wolbachia* in panmictic populations when infected individuals occur above a threshold frequency (Stevens & Wade 1990).

The molecular mechanisms of CI are still unclear, although it has been interpreted as a 'modification-rescue' model, which was further confirmed with the recent discovery of *cytoplasmic incompatibility factor* (*cif*) genes in *Wolbachia* genomes (see overview in Shropshire et al. 2019). The modification of the infected males' sperm during spermatogenesis cannot be rescued in the zygote by the egg cytoplasm of infected females, whereas it is rescued by that of infected females (Werren 1997). If it is not rescued, the paternal chromatin complement condenses during the initial mitotic divisions during the first metaphase and exclusion during the following anaphase, rendering the embryo haploid which can lead to embryonic mortality in diplodiploid host species (Hoffmann et al. 1986; Vavre et al. 2000). Therefore, in diplodiploid hosts, embryonic mortality due to CI impacts both sexes and comes without an offspring sex ratio bias. However, in haplodiploid species CI crosses cause sex ratio distortion under two different scenarios: 1) death of the female embryos (fertilised eggs) and development of all unfertilised haploid eggs to males (FM-CI); 2) or development (or conversion) of fertilised eggs (female embryos) to functional males presumably by losing one set of chromosomes and becoming haploid (MD-CI) (Vavre et al. 2000; Bordenstein et al. 2003).

Beyond the potential of *Wolbachia* as reproductive manipulator, it can also have beneficial roles such as a protective role against some RNA viral infections (Hedges et al. 2008; Brownlie & Johnson 2009; Osborne et. al. 2009; Ross et al. 2019) and provision of essential nutrients (Brownlie et al. 2009; Zug & Hammerstein 2015; Zélé et al. 2018). In other examples *Wolbachia* may play an important role in host fitness in terms of fecundity, survival and development time (Zug & Hammerstein 2015).

“*Candidatus* Cardinium hertigii” (generally known as *Cardinium*) is another important but less widespread reproductive parasite, belonging to the phylum Bacteroidetes, and was recorded in about 13% of tested arthropod species (Zchori-Fein & Perlman 2004; Weinert et al. 2015). *Cardinium* is known to cause three types of reproductive manipulations including cytoplasmic incompatibility in sexual populations of the wasp *Encarsia pergandiella* (Hunter et al. 2003), in Hemiptera (Nakamura et al. 2012), in the Kelly’s citrus thrips, *Pezothrips kellyanus* (Nguyen et al. 2017), and in several spider mite species (e.g. Gotoh et al. 2007; Zhu et al. 2012), feminisation in one mite species (Weeks et al. 2001) and thelytokous parthenogenesis in

Encarsia parasitic wasps (Zchori-Fein et al. 2001; Zchori-Fein et al. 200 4) and possibly in a scale insect (Provencher et al. 2005). However, thus far male killing capability has not been recorded for *Cardinium*. Unlike for *Wolbachia*, few studies have addressed direct fitness effects of *Cardinium* on its hosts. However, some studies have revealed that *Cardinium* can impact oviposition behaviour, fecundity, survival rate and development time of some species (see Hunter et al. 2003; Gotoh et al. 2007).

Infections by multiple symbionts are frequent among insects (Duron et al. 2008). Co-infections of *Wolbachia* and *Cardinium* in the same host species have also been detected in several insect species (e.g. Weeks et al. 2003; Zchori-Fein & Perlman 2004; Gotoh et al. 2007; Duron et al. 2008; Nakamura et al. 2009; 2012; White et al. 2009; Zhang et al. 2016). Previous studies have shown various infection outcomes on their hosts, such as the level of CI with respect to *Cardinium* and *Wolbachia* co-infection in different species (e.g. Gotoh et al. 2007; White et al. 2009; Zhu et al. 2012; Zhang et al. 2012; Nguyen et al. 2017). However, a most recent study has shown independent, complete expression of CI in co-infected *P. kellyanus*, with *Cardinium* inducing a combination of MD and embryonic FM, and *Wolbachia* inducing embryonic FM and postembryonic mortality (Nguyen et al. 2017).

1.9 The potential role of endosymbiotic bacteria in the evolution of haplodiploidy

Recent theoretical models have suggested that haplodiploidy might have evolved as an outcome of coevolution between maternally inherited endosymbionts, including male killing and CI-inducing bacteria, and their hosts (Normark 2004; Engelstädter & Hurst 2006). Infection with male killing bacteria can lead to the elimination of the paternal chromosome set (embryo aneuploidy) in the zygote and the killing of male embryos through haploidization. This might have favoured the selection of host genes rescuing haploid embryos and haploid males that survived this phenomenon (Normark 2004). Therefore, in haplodiploids the cost of CI-inducing bacteria is only suffered by females as males do not inherit paternal chromosomes, so it leads to a male-biased sex ratio upon incompatible matings.

1.10 The invasion dynamics of CI-inducing endosymbionts in haplodiploid hosts

In CI, infected females enjoy higher reproductive fitness than uninfected females due to the fact that they are compatible to crosses with both uninfected and infected males (see Figure 1.1). Under these conditions, a CI-inducing endosymbiont can quickly spread in a host population, ultimately leading to the population-wide fixation of infection because of fitness disadvantage of uninfected females due to incompatible matings (Vavre et al. 2000). Given that CI induced by endosymbiotic bacteria is a post-zygotic phenotype, it cannot influence the primary sex ratio. However, more empirical studies are needed to unravel the role of endosymbionts on sex allocation in haplodiploid species.

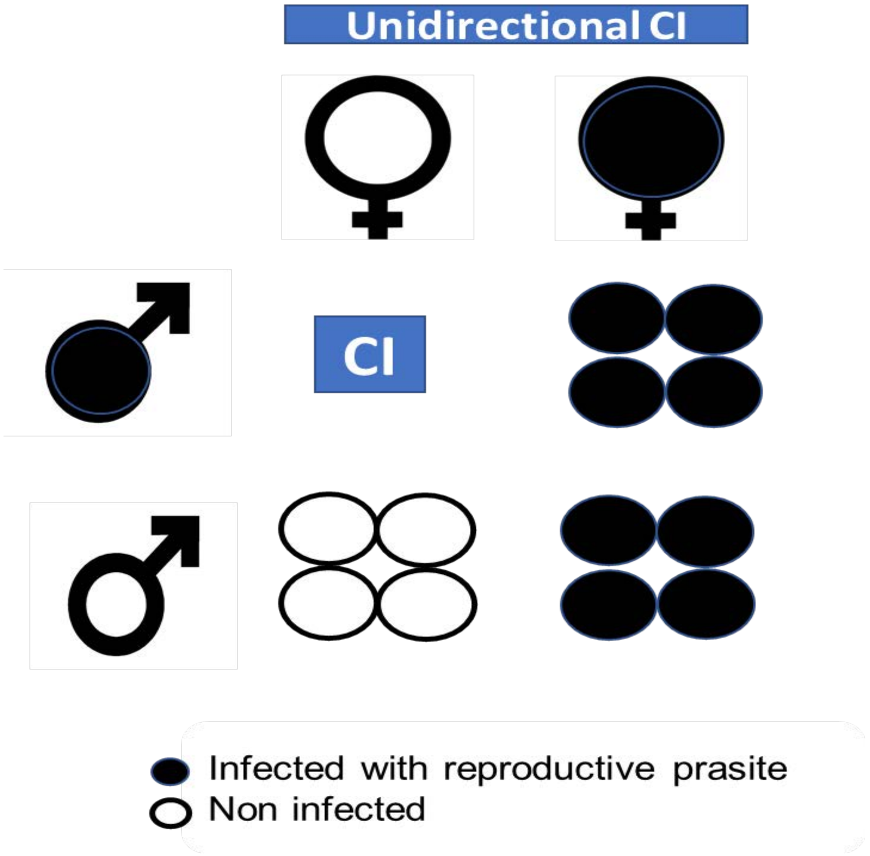


Figure 1.1 Unidirectional cytoplasmic incompatibility (CI) in incompatible crosses and higher reproductive fitness of infected females when compared to uninfected females.

Over the course of evolution, endosymbiotic bacteria like *Wolbachia* have been selected for invasiveness and increased transmission efficiency in host populations (Vavre et al. 2000). However, models suggested that CI is not as efficient a driver of endosymbiont invasion in haplodiploid species as in diploidiploid species (and this may also apply for other CI-inducing endosymbionts like *Cardinium*). The lowest invasion efficiency has been modelled for MD which can lead to production of only male offspring without any reduction in fertility (Vavre et al. 2000). The invasion efficiency is lowest in haplodiploid species with overlapping generations and does not occur when infection frequency of *Wolbachia* is lower than the equilibrium threshold.

The population dynamics of CI-inducing reproductive parasite in haplodiploid host populations was first modelled by Vavre et al. (2000) for *Wolbachia*. Given that CI can result in FM (aneuploidy of fertilized eggs) and MD (complete haploidization of fertilized eggs), and the resulting male-biased sex ratio, the frequency threshold for *Wolbachia* to spread is higher for haplodiploid than diplodiploid species and highest for the MD-inducing CI type (Vavre et al. 2000). This is explained by the fact that MD occurring in incompatible matings results in a higher production of uninfected males than in compatible matings. The lower frequency of infected males leads to lower probability of incompatible matings. However, in FM-inducing CI, all fertilized eggs die as a result of aneuploidy and the fertility of incompatible matings is reduced and the frequency threshold is lower compared to what is anticipated for MD-inducing CI. The invasion threshold for FM-inducing *Wolbachia* is more similar to CI in diplodiploid populations, though still higher, as the unfertilized haploid eggs are unaffected and some males are still produced in incompatible matings (Vavre et al. 2000; Egas et al. 2002).

Theoretical studies predict that in haplodiploid species the invasion threshold of CI-inducing endosymbionts is not only achieved by the reduction in the proportion of uninfected to infected females (Egas et al. 2002). Several promising directions for research have been proposed for the invasiveness of CI-inducing *Wolbachia* and its evolution (in general, and for haplodiploids in particular): primary sex ratio effects, population structure, and other fitness compensating effects. It has been suggested that population dynamics and evolution of these endosymbionts (like *Wolbachia*) in haplodiploid species is not related to the type of CI (FM or MD) but associated to their beneficial impact on host fitness (fitness-compensating effects) and/or increasing the transmission efficiency (Egas et al. 2002). Production of more female-biased offspring by infected mothers compared to uninfected mothers in haplodiploid insects (sex ratio effect) may be one of the more effective mechanisms (other than CI) that may facilitate invasion of *Wolbachia* in host populations, and it could hugely reduce an invasion threshold (Egas et al. 2002). Moreover, other compensatory fitness effects of *Wolbachia* such as increased fecundity and shorter developmental times could be other strategies to maximise infection prevalence (Xie et al. 2011; Sarakatsanou et al. 2011). In haplodiploid species the barrier to invasion of CI-inducing *Wolbachia* can be reduced when it is accompanied with a male killing element, having also synergic effect on CI through mortality of males produced by FM and MD (Freeland & McCabe 1997).

Host reproduction is an important component of endosymbiont fitness, thus infection costs are less favourable (Fine 1975) although it still occurs in some insects (Floate et al. 2006; Lau et al. 2020). The effects of infection on basic host life-history fitness traits (fecundity, survival) are diverse according to bacterial variants and/or host genotype and age of infection in a host species, and can range from low/absence of significant effect on host fitness (e.g. Hoffmann et al. 1994; Giordano et al. 1995; Poinsot & Mercot 1997; Zchori-Fein et al. 2000; Charlat et al. 2003; Blagrove and Arias-Goeta 2013) and reduced host fitness (e.g. Hoffmann et. al. 1990; Perrot-Minnot et al. 2002; Jiggins *et al.* 2002; Rigaud & Moreau 2004) to increased host fitness (e.g. Vavre et al. 1999; Fry & Rand 2002; Dobson et al. 2004; Joshi et al. 2014). Moreover, it has also been suggested that sex-ratio distorting phenotypes reduce the fitness of infected females since, in populations with a female-biased sex ratio, it is costly to produce less offspring of the rare, male sex (Zug & Hammerstein 2015). Therefore, it is possible that other fitness costs induced by reproductive parasites, like reduced fertility of infected females, are favourable for host populations to compensate for the scarcity of males.

Given the maternal inheritance of CI-inducing bacteria, a female-biased population can facilitate a faster increase of infection prevalence over consecutive generations (Stouthamer et al. 2002). Accordingly, another, potential complementary strategy for vertical transmission of endosymbionts is increased resource allocation towards female offspring. This is achieved by either reducing the investment of male production (with maintaining the allocation trade-off between male and female offspring based on Charnov et al. 1976) or at the expense of growth and maintenance (in the context of reproductive allocation and life-history trade-offs; see Stearns 1992). In this context, the endosymbiont can influence the reproductive decisions of their hosts toward production of more females than males through the manipulation of primary sex ratio.

Currently, there is a paucity of knowledge about the role of endosymbionts on sex allocation of their hosts and their associated mechanism. Difference in sex ratio in response to *Wolbachia* infection in arrhenotokous haplodiploid species was first reported by Vala et al. (2000) for the two-spotted mite *Tetranychus urticae*. However, this was not consistent with the data on the other *Wolbachia*-infected populations of *T. urticae*, implicating the role of host genotype in sex allocation alternation by the endosymbiont (Breeuwer 1997; Vala et al. 2000). Another study by Vavre et al. (2000) on the parasitoid wasp *L. heterotoma* infected with *Wolbachia* indicated female-biased sex ratio in offspring. Although this sex ratio difference between infected and uninfected females (when mated with uninfected males) was not revealed to be significant in this study, Egas et al. (2002) considered it as a real effect to estimate the necessary parameters for running their *Wolbachia* invasion models. Furthermore, a similar sex ratio effect was reported in another parasitoid wasp, *N. vitripennis*, where infected females produced more daughters and fewer sons than uninfected females (Bordenstein & Werren 2000).

1.11 Importance of endosymbiotic bacteria in biological control

Recent advances in arthropod microbiology have provided new avenues for biological control of pest arthropods (Huffaker 2012). Understanding the biological roles of microbial communities in host sex allocation and their ecological and evolutionary consequences on their host population may increase the success of biological control programs. For example, CI has attracted attention in fundamental and applied research, as it impacts host ecology and evolution, but also can be applied in biological control of fruit flies by suppressing uninfected field populations through the release of infected males – a strategy known as incompatible insect technique (IIT) (Boller et al. 1976; Zabalou et al. 2004). CI is currently implemented in the biological control of mosquito-vectored pathogens by spreading *Wolbachia*-conferred resistance to dengue or malaria pathogen acquisition and transmission in mosquito populations (Moreira et al. 2009). Apart from CI and the associated sex ratio distortion, reproductive parasites are responsible for a variety of manipulations of host biology. They may play pivotal roles in various reproductive traits and mating behaviour of their host species that may alter the evolutionary dynamics of sexual selection (Tregenza & Wedell 2000). Moreover, their cost-benefit effect on their hosts can significantly influence the epidemiological dynamics of infection in host population (Harcombe & Hoffmann 2002). Therefore, identifying these reproductive manipulations and corresponding fitness changes can provide better understanding about the underlying factors that are important in the success of biocontrol programs.

1.12 Thrips – suitable haplodiploid model taxa to address evolution and mechanisms of sex allocation and consequences of infections with endosymbiotic bacteria

Haplodiploidy has independently evolved in the insect order Thysanoptera (thrips). This order encompasses more than 6,000 described species in two very distinct sub-orders (Terebrantia and Tubulifera) (Mound 2011; Mound 2014), with different species showing remarkably different life strategies, from free living herbivores and predators (Lewis 1973; Mound 2005) to parasites of other insects (Cavalleri et al. 2020). Several species are important agricultural pests and some others are gall-inducers and vectors of viral pathogens (such as *Tospoviruses* and *Machlomoviruses*) of different plants species (Conti et al. 2002; Mound 2005; McLeish et al. 2007; Schneweis et al. 2017). However, compared with other haplodiploid taxa such as hymenopterans, thrips have rarely been subjected to sex allocation studies (Bondy & Hunter 2019). Understanding of the reproductive strategies and sex ratio dynamics of thrips is, however, important for the devolvement of integrated pest management programs and the efficiency of biological control applications in agriculture and horticulture against these insects (Castañé et al. 1999; Krüger et al. 2016; Bondy & Hunter 2019).

Thrips generally reproduce via arrhenotoky (male progeny arising from unfertilized, haploid eggs and female offspring from fertilized, diploid eggs) and less frequently via thelytoky (female offspring arising from unfertilized eggs) (Kumm & Moritz 2008). In arrhenotokous thrips, females have the ability to control the sex of their offspring due to controlled fertilisation of eggs. Thus, female thrips can adjust their offspring's sex ratio in response to various biotic and abiotic factors under the context of adaptive sex ratio theory (Charnov 1982; King 1987; Wrensch & Ebbert 1993; Macke et al. 2014; Krueger et al. 2016). Among the biotic factors, endosymbiotic bacteria like *Wolbachia* are known to alter reproduction and sex ratio in thrips. For example, several studies have attributed the thelytoky in some thrips species such as *Hercinothrips femoralis, Franklinothrips vespiformis* and *Taeniothrips inconsequens* to *Wolbachia,* where after elimination of *Wolbachia* by antibiotic treatment individuals of these species switch to arrhenotokous mode (Moritz 1997; Arakaki et al. 2001; Kumm & Moritz; 2008). Among abiotic factors temperature is likely another influential source, either directly affecting sex allocation in thrips or indirectly changing the sex ratio by impacting endosymbiont phenotypes or CI effect (Kumm & Moritz 2010; Pakyari et al. 2011; Nguyen 2015).

So far the study of sex and resource allocation in the context of egg size has been given little consideration in thrips. Few studies have investigated the evolution of split sex ratio and adaptive sex and resource in eusocial thrips (Kranz et al. 2002; Kranz 2005). Kranz (2005) investigated the egg size and fecundity trade-offs in eusocial gall-inducing thrips and demonstrated that the reproductive allocation patterns and gall size play a role in the social evolution of these thrips. However, studies are lacking on free-living thrips species. Moreover, the mechanism of sex allocation and the effect of endosymbionts on resource allocation has never been addressed in thrips, nor in any other haploidploid insects except for some recent studies of endosymbionts in whitefly (Wang et al. 2020).

1.13 Study system: Kelly's citrus thrips, *Pezothrips kellyanus* (Bagnall)

Kelly's citrus thrips, *Pezothrips kellyanus* (Bagnall) is an economically important pest of citrus (Webster et al. 2006; Navarro-Campos et. al. 2012a; b; Varikou et al. 2012) and is native to Australia and invasive to New Zealand and Mediterranean countries (Blank & Gill 1997; Mound & Jackman 1998; Vassiliou 2007; Nguyen et al. 2016). Adults and larvae of this species feed on leaves but generally shelter within the citrus flowers and where they also feed on pollen and very young fruits. This results in rind damage to fruitlets that is mostly caused by the larvae (Blank & Gill 1997; Navarro Campos et al. 2011). Their feeding activity on the surface of the young citrus fruits can cause patches or rings of scarred tissue and necrotic area around the apex of the fruit once they grow bigger (Mound & Jackman 1998). In cases of serious damage, the scarring may cover the entire fruit skin (Vassiliou 2010). The highest risk of injury occurs 4 to 5 weeks after petal fall (Baker et al. 2002; 2011). The damage caused by this thrips on citrus (lemon and orange species are most affected) does not degrade the internal tissue of fruit but rather downgrades the external appearance of affected fruits and reduces their value for domestic and international markets (Mound & Jackman 1998; Webster et al. 2006). Biological pest control for this species is under-developed, apart from a few studies that have demonstrated that supporting and improving a high diversity of soil organisms (like predatory mites) reduces the survival of pupal stages that dwell in the soil (Crisp & Baker 2011; Navarro-

Campos et. al. 2012b). Therefore, control of this species currently relies on insecticide applications (Webster et al. 2006). Increasing rates of pesticide applications, which risk issues with other pests, insecticide resistance, environmental and other health effects prompts the need for the development of environmentally-friendly approaches to control this pest.

Pezothrips kellyanus is a haplodiploid species, and populations within its native range (Australian populations) are generally infected with *Cardinium* and *Wolbachia*. In contrast, populations within the invasive ranges (populations of New Zealand and Mediterranean regions) are only *Cardinium* infected (Nguyen et al. 2016). A recent study has shown independent CI caused by *Cardinium* and *Wolbachia* in an Australian populations of *P. kellyanus* (Nguyen et al. 2017). However, investigating further roles of these bacteria in host sex allocation and fitness is essential for the development of highly targeted pest management programs such as Insect Incompatible techniques (IIT) (Zabalou et al. 2004). A clear understanding of sex allocation mechanisms and constraints in thrips (as a haplodiploid representative) and the mechanism whereby endosymbionts manipulate host' sex allocation is a key step towards well-designed IIT protocols that may help deliver a breakthrough in biological control of haplodiploid pests in the future.

1.14 Research scope and aims of the PhD thesis

In many insects in which parental care has not evolved, parental investment is predominantly hinged on the provisioning of eggs with the complete energy supply for development of offspring until they start feeding independently (McGinley et al. 1987). Egg size is a reliable predictor of energy content and parental investment (Macke et al. 2011; Clutton-Brock 1991) as it has been found that offspring hatching from larger eggs benefit from a higher fitness advantage (Parker & Begon 1986). Recent empirical studies suggested that haplodiploid species can adjust their sex allocation not only through increasing the relative number of eggs but also via investment of more resources into eggs of one sex over the other (Macke et al. 2011). This hypothesis has thus far only been tested for the haplodiploid spider mite *T. urticae,* which displays highly female-biased sex ratios (Macke et al. 2011). However, such sex allocation strategies remain to be tested in other haplodiploid species.

It is generally suggested that the there is a trade-off between fecundity and egg size (or offspring size) (e.g., McGinley et al. 1987; Winkler & Wallin 1987; Czesak & Fox 2002; Kolm et al. 2005). The theory behind this trade-off is based upon the assumption that the available resources to invest into offspring is limited. This means females can produce less larger offspring or more smaller offspring (Smith & Fretwell 1974) and females are selected to optimize return from investment into individual offspring produced (Smith & Fretwell 1974; Atkinson & Begon 1987; Kolm et al. 2005). The shape of this trade-off function can be influenced by various factors including parental traits like maternal size (Kindsvater & Alonzo 2014; Ford & Seigel 2006; Rollinson & Rowe 2015) or fitness benefits offered by male (Kempenaers & Sheldon 1997, Pischedda et al. 2010).

Pezothrips kellyanus has an extraordinary post-mating split sex ratio: some mated females produce female-biased offspring, whereas others are constrained to produce extremely male-biased brood (male-only in some cases), for yet unknown reasons (Nguyen 2015). Constrained sex allocation is a dead-end for endosymbionts as they will not be inherited by constrained females. Nguyen (2015) showed that *Cardinium* and *Wolbachia* can mitigate constrained sex allocation in this species with infected females more likely producing female-biased brood (Nguyen 2015). However, the paternal and/maternal factors and the mechanism that endosymbionts target to overcome constrained sex allocation in *P. kellyanus* is unknown. Therefore, *P. kellyanus* may be a good study model to investigate the mechanism of sex allocation and constraining factors affecting sex allocation in haplodiploid insects. So far studies in sex allocation of haplodiploids has focussed on hymenopterans while this study is one of the first for thrips. Moreover, this study system may provide an opportunity to examine the interactive role of reproductive parasites on evolution of sex allocation, sexual conflict and mating systems in haplodiploidy. Pursuant to this scope the specific aims and hypotheses of this thesis were:

I. Mechanism of sex allocation in P. kellyanus (Chapter 2):

- To determine whether females have the potential to influence sex allocation and offspring fitness through egg size adjustment.

This chapter determines whether fertilisation only occurs for eggs exceeding a certain size threshold value. Moreover, if females are capable to adjust the sex allocation via eggs size, then, in the context of sexual conflict, the role of male and mating on egg size is important to be addressed. Therefore, this chapter will investigate the effect of mating on resource allocation towards eggs.

Hypothesis: Theory suggests that when there is a fitness and size differences between male and female offspring, an efficient mechanisms of sex ratio control should broaden the scope for adaptive sex ratio adjustment (Pen & Weissing 2002), and this might also include the differential resource investment to embryos of different sexes at the egg stage. Finkler et al. (1998) suggested that the positive association between egg size and fitness is likely caused by resource investment. *Pezothrips kellyanus* shows a considerable sexual size dimorphism, with females being larger than those of males (Nguyen et al. 2016). Stillwell et al. (2010) suggested that, generally, sexual size differences are generated by the plasticity in growth rate, the development time and the physiological regulation of these differences (e.g. sex differences in regulation by the endocrine system). However, this asymmetry in size of different sexes might also be a consequence of an egg size-mediated fertilization. Therefore, it is hypothesized that in *P. kellyanus* the size of eggs of mated females developing into females is larger than those developing into males (small egg size is correlated with a reduced likelihood of fertilization). Moreover, given that paternal genes can only be inherited to daughters, it is likely that mating can induce higher resource investment towards egg size to increase fertilization success. This incremental effect may influence patterns of different biological trade-offs (e.g. egg size-number and reproduction-survival trade-offs).

II. Assessing the factors constraining sex allocation in P. kellyanus (Chapter 3):

- To determine whether extremely male-based offspring production in some mated females is due to fertilization failure due to adaptive egg size adjustment.

Hypothesis: the split sex ratio in *P. kellyanus* may be explained with the conditional sex allocation theory (Trivers & Willard 1973) in which females in better condition produce more female offspring, whereas females suffering lower resource arability are constrained to produce more male offspring. Therefore, if egg size is a contributing factor for fertilization success, offspring sex and fitness in *P. kellyanus*, then it is hypothesised that females of lower condition (that may be reflected in smaller body size) may fail in sufficient egg provisioning and fertilization. Alternatively, they may adaptively reduce resource allocation and fertilization success to avoid production of less viable females. On the other hand, females in better condition may be selected to produce larger eggs to augment fertilization success female production and as an evolutionary stable strategy to overcome access of male production in the population.

III. Unravelling the role of endosymbionts in egg size-mediated sex allocation in P. kellyanus (Chapter 4):

- To determine the role of the reproductive parasites *Cardinium* and *Wolbachia* in modifying patterns of sex allocation via maternal resource investment into egg size. Moreover, to identify the interactive effect of *Cardinium* and *Wolbachia* on offspring fitness both directly and indirectly through egg size change.

Hypothesis: given that the vertical transmission of both endosymbionts depends on female production in hosts, it is hypothesised that either or both endosymbionts can increase the resource allocation towards egg size to increase fertilization success and female brood production in *P. kellyanus.* Moreover, as the models of endosymbiont invasion in haplodiploid populations suggest that CI is not efficient for invasion of both endosymbionts (Vavre et al. 2000; Egas et al. 2002), it is hypothesized that manipulation of egg size-mediated sex allocation and offspring fitness may potentially be a complementary strategy to boost their prevalence in host populations.

CHAPTER II

Egg size-mediated sex allocation and mating-regulated reproductive investment in a haplodiploid thrips species

Female (left) and male (right) of *Pezothrips kellyanus* during copulation

Alihan Katlav, James M. Cook, Markus Riegler

Katlav, A., Cook, J. M., & Riegler, M. (2021). Egg size-mediated sex allocation and mating-regulated reproductive investment in a haplodiploid thrips species. *Functional Ecology*, *35*, 485-498. https://doi.org/10.1111/1365-2435.13724

Egg size-mediated sex allocation and mating-regulated reproductive investment in a haplodiploid thrips species

2.1 ABSTRACT

The partitioning of resources between male and female offspring is a fundamental aspect of the reproductive biology of animals. Sex allocation can be adjusted by controlling offspring sex ratio or by sex-specific resource allocation towards individual eggs. The latter is well-known in birds and reptiles, but less studied in other organisms, especially haplodiploids with females that can adjust offspring sex via fertilization control. Here, we investigated whether egg size can affect sex allocation in a haplodiploid thrips, *Pezothrips kellyanus*. First, we established that larger eggs are more likely to be fertilized and become females than smaller eggs, which remain unfertilized and become males. This size-dependent fertilization may be regulated by anatomical constraints that allow sperm access only to larger eggs. Furthermore, across both sexes, larger eggs developed into offspring with higher fitness, suggesting that egg size is a reliable proxy for resource allocation in this species. During the early phase of reproduction, mating increased investment by females towards egg size thereby facilitating fertilization success and increased female offspring survival. However, this came with a reduced life-time fecundity, reflecting that mating manipulates reproductive strategies in favour of increased daughter production. This may be in conflict with the optimal offspring sex ratio of females which is theoretically less female-biased than for males. We detected both egg-size mediated sex allocation and a mating effect on resource allocation under both laboratory and field conditions, suggesting that they are maintained by natural selection. We provide evidence for a finely-tuned egg-size dependent fertilization mechanism that mediates sex allocation strategies and is moderated by mating. Furthermore, our findings establish a new perspective about potential effects of sexual conflicts on egg size and sex allocation.

Keywords

life history; sexual selection; sexual conflict; offspring fitness; fertilization; sex ratio

2.2 INTRODUCTION

Sex allocation is a central concept in evolutionary biology and refers to the differential allocation of resources to male and female function. Whenever average fitness returns differ between sons and daughters, selection may favour parental sex biased investment (Charnov 1993). Parents may achieve this by varying the number of sons versus daughters (i.e. biased sex ratio) (Hardy 2002; West 2009) and this has been widely studied in various animals with different sex determination systems (Hamilton 1967; Komdeur & Pen 2002). However, sex allocation can also involve differential investment into female versus male offspring (Petrie et al. 2001; Magrath et al. 2004), including at the egg stage (sex-specific egg size) (Fox & Czesak 2000; Radder et al. 2009). Adjustment of egg size is a major mode of resource allocation, especially in animals without parental care; it provides offspring with a sufficient energy supply until they start feeding independently after hatching (Clutton-Brock 1991; Fox et al. 1999). Most studies of egg size and offspring sex have focussed on vertebrates (e.g. Cordero et al. 2000; Pike & Petrie 2003). For example, in some reptiles the amount of yolk allocation after fertilization (and thus, egg size) can influence the sex of embryo (Radder et al. 2009). However, only a few studies have assessed egg size as a factor in sex allocation studies of arthropods (Tammaru et al. 2010; Macke et al. 2011), despite substantial interest in the evolution of insect egg size and shape (Church et al. 2019).

Many arthropods are haplodiploid, i.e. diploid females develop from fertilized eggs and haploid males from unfertilized eggs. It has been widely accepted that this sex determination system facilitates the evolution of adaptive sex ratios, which enable females to adjust offspring sex by fertilization control (Charnov 1982; West & Sheldon 2002). More recently, egg size has been shown to affect offspring sex in the spider mite *Tetranychus urticae*, in which larger eggs are more likely to be fertilized and become females than smaller eggs that remain unfertilized and become males (Macke et al. 2011). Yet, the underlying mechanism for this size-dependent fertilization remains unresolved. In vertebrates, sex-specific egg size variation has been found in birds. In birds, females are the heterogametic sex with the potential ability to control the sex of eggs before fertilization by virtue of sex chromosome inheritance, and this may allow the evolution of sex-specific provisioning before fertilization (Oddie 1998; Cordero et al. 2000). However, in haplodiploid insects sex is determined upon fertilization, just before oviposition, and thus offspring sex cannot contribute to selective egg-provisioning decisions (Boudreaux 1963; Helle 1967; Chapman 1998). Consequently, how female haplodiploid insects can control sex allocation via egg size remains unknown.

Theory suggests that adaptive sex allocation in haplodiploids can cause sexual conflicts over offspring sex ratio (de la Filia, Bain & Ross 2015). In haplodiploidy, the optimal offspring sex ratio is less female-biased in females than males because females can pass on their genes to both daughters and sons, whereas males only pass their genes on to daughters (Gardner 2014; Shuker et al. 2009). This sexual conflict may select for male adaptations that increase daughter production after mating. For example, males may transfer seminal proteins that facilitate fertilization by increasing the sperm release from the spermatheca of females – an internal structure in females that stores sperm after mating (Shuker et al. 2009). In a spider mite species, this higher fertilization rate is achieved by increasing egg size after mating (Macke et al. 2012). However, change in egg size may be coupled with other manipulations that shift the partitioning of the overall available energy budget towards reproduction (Arnqvist & Rowe 2005). This may also include shifts between early and late reproduction, or between size and number of offspring (Stearns 1992; Eberhard 1996). Theory also suggests that reproductive efforts are traded-off with other life-history traits (Stearns 1992). For instance, longevity is reduced once females have mated (Harshman & Zera 2007), especially if mating increases maternal reproductive efforts (Kemp & Rutkowski 2004). Such trade-offs are generally studied by focusing on fecundity patterns (e.g. Li et al. 2015; Krueger et al. 2016). However, integration of egg size may provide a more comprehensive analysis of the effect of mating on reproductive investment patterns. Moreover, knowledge about sex allocation mechanisms may contribute to an improved assessment of resource investment costs and the trade-offs between reproduction and lifespan.

Study of sex and resource allocation in haplodiploid arthropods is heavily biased towards parasitic and social hymenopterans (Schneider & Vinuela 2007; Johnson et al. 2009), with some studies also on mites (Nagelkerke & Sabelis 1998; Macke et al. 2011; 2012a). Furthermore, few studies on haplodiploids have evaluated the impact of mating on females, beyond the consequences of unmated females being constrained to produce only sons (Godfray 1990; Macke et al. 2012; Li et al. 2015). There is a need for empirical studies targeting other haplodiploid taxa, to test the generality of the evolutionary models proposed to date. Thrips have evolved haplodiploidy independently from the Hymenoptera, so constitute an ideal parallel system for sex and resource allocation studies (Crespi 1992). Like in other haplodiploids, female thrips can control egg fertilization and offspring sex by regulating the release of sperm from the spermatheca (Heming 1970; Moritz 1988; Dallai et al. 1996). However, what factor governs sperm access to eggs remains unknown. Moreover, despite previous interest in the role of egg size in resource allocation in thrips (e.g. Kranz 2005), the effect of mating on sex and resource allocation has not been evaluated.

In this study, we investigated the role of egg size in the sex allocation of a haplodiploid species, Kelly's citrus thrips, *Pezothrips kellyanus* (Bagnall). First, we examined whether egg size is sex-specific by collecting egg size measurements and following the development until individual sex became apparent. Given that eggs in the majority of insects (including thrips) are fertilized just before oviposition (Chapman 1998; Heming 1970), we hypothesized that any sex-specific allocation by mothers would occur before fertilization and fertilization would then depend upon egg size. Therefore, the egg size distribution should be similar between mated and virgin females, and any egg-size sexual dimorphism would result from selective fertilization of larger eggs, leading to a smaller egg-size range for male embryos produced by mated than by virgin females (Figure 2.1).

Second, we tested whether mating affects a female's resource allocation strategy. We expected that egg size patterns would fall into one of the two hypothetical scenarios:

a) No effect of mating: egg size is similar in mated and virgin females, but male-destined eggs of mated females are smaller than eggs of virgins (Figure 2.1A).

b) An effect of mating: egg size is larger in mated than virgin females, but male-destined eggs of mated females are the same size as eggs of virgins (Figure 2.1B).

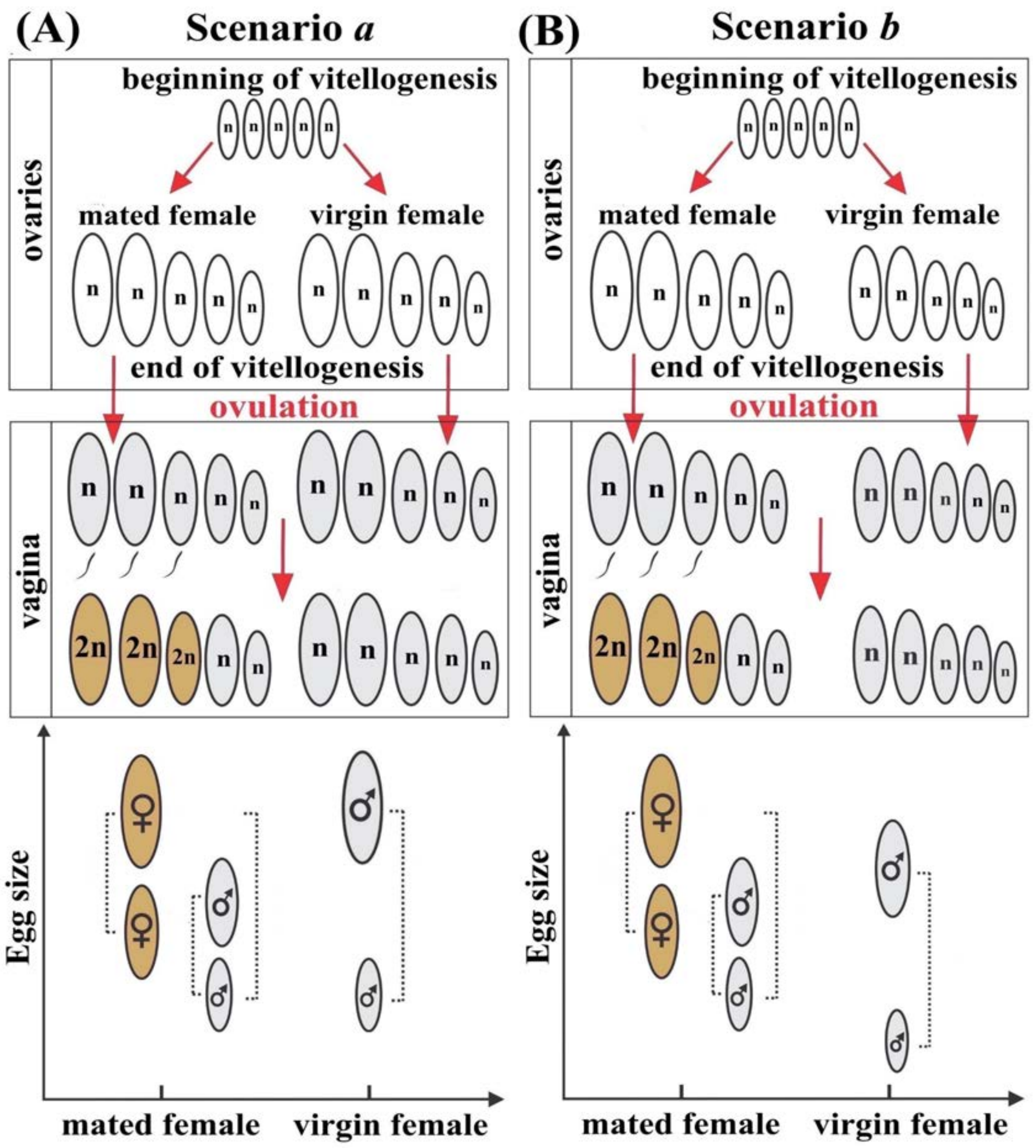


Figure 2.1 Sex-specific egg size in haplodiploids with selective fertilization of larger eggs and the effect of mating on egg size. At the time point of oviposition, equal egg size range between mated and virgin females indicates that eggs are randomly provisioned prior to fertilization and, then, larger eggs are fertilized selectively. (A) Scenario *a*: mating does not change the egg size between mated and virgin mothers. (B) Scenario *b*: mating increases the egg size in mated females as found for *P. kellyanus* (this study). Eggs of thrips are ellipsoidal. Dashed lines represent egg size ranges. Eggs before ovulation are presented in white. Unfertilized (n) and fertilized (2n) eggs after ovulation are presented in grey and gold.

Finally, upon detection of a mating effect on egg size, we assessed whether (1) increased egg size as a consequence of mating results in a higher reproductive investment, or, alternatively, is compensated with a reduced clutch size, (2) increased egg size is costly in terms of daily and/or life-time fecundity or other life-history traits of mothers, (3) egg size influences offspring fitness, and (4) egg size-related sex allocation and resource allocation patterns revealed in the laboratory assays are also seen under natural field conditions.

2.3 MATERIALS AND METHODS

2.3.1 Laboratory thrips population

A laboratory population of *P. kellyanus* was established with field-collected individuals from Kulnura, New South Wales, Australia, and maintained on lemons sprinkled with cumbungi pollen (*Typha* sp.). Individuals were maintained and experiments conducted at 20±1°C, 70±2% RH and 16:8 h light:dark. Australian populations of *P. kellyanus* are infected with one strain each of endosymbiotic *Cardinium* and *Wolbachia* bacteria at high prevalence (Nguyen et a. 2016). In this host, both strains cause independently cytoplasmic incompatibility while thelytokous parthenogenesis and male killing have not been observed (Nguyen et al. 2017). We used a laboratory population that was fixed for both bacteria and confirmed this by diagnostic PCR before conducting experiments (Nguyen et al. 2016). This was done not only to avoid potential confounding effects of CI, but also to best reflect their naturally high infection prevalence in the field. All experiments with laboratory individuals were conducted with evenly-aged cohorts reared at controlled densities [Supporting Information (see Appendix I)].

2.3.2 Egg collection from virgin and mated females (experiment 1)

Newly emerged females (1-2 days old) were isolated in small Petri-dishes with pollen and honey-water solution for six days to allow for the completion of their pre-oviposition period (see Appendix I). Then, females were either kept virgin or provided with ten virgin males (2-3 day old) for mating. Mating was observed and singly-mated females were isolated in individual Petri dishes. Subsequently, females were placed individually on an agarose plate to allow oviposition on day 7 (1st clutch) and day 10 (2nd clutch) for 24 hours each. In total, 672 eggs were collected (1st clutch: 172 and 194 eggs; 2nd clutch: 144 and 162 eggs from 19 virgin and 19 mated females, respectively). Females that did not lay eggs were excluded from the experiment. After day 10, all subsequent eggs produced by each female were counted daily until the females' death (life-time fecundity per female; Figure 2.2D). Agarose plates and food supply were renewed every second day (see Appendix I). In order to assess whether our mating protocol led to successful insemination, we confirmed the presence of motile sperm in the dissected spermathecae of 12 females that had been mated.

2.3.3 Egg size measurement

All eggs of the 1st and 2nd clutch were transferred to a concave microscope slide, covered with a coverslip, and photographed using a light microscope equipped with a camera (Zeiss Axio Scope A1, Germany). Egg length and width were measured using AxioVision v.4.8.2 with auto-scaling at 20x. Consistent with several other thrips species (Kranz 2005), eggs of *P. kellyanus* are prolate ellipsoids in the early stage. Thus, we calculated the size of each egg using the formula:

egg volume = $(4/3)\,\pi a^2 c$

where a is the equatorial radius (half egg width) and c is the polar radius (half egg length).

Then, eggs were transferred to microcentrifuge tubes for embryonic and larval development to the pupal stage, the earliest stage at which sex becomes readily recognisable (see Appendix I). Then, individuals were allowed to develop to adulthood. Interestingly, mated females of *P. kellyanus* have a bimodal brood sex ratio pattern, i.e. while most mated females generally produce female-biased broods, some mated females produce male-only broods (Nguyen et al. 2017). In our study, ~30% of mated females fell into the second category. The cause for this is yet unknown but for the purpose of this study we grouped mothers into three mother types and their offspring into four offspring types (Table 2.1).

Mother type	**Acronym**	**Offspring type**	**Acronym**
Virgin female with only male offspring	V	son of V	S_V
Mated female with only male offspring	M(♂)	son of M(♂)	$S_{M(♂)}$
Mated female with female and male offspring	M(♀+♂)	son of M(♀+♂) daughter of M(♀+♂)	$S_{M(♀+♂)}$ $D_{M(♀+♂)}$

Table 2.1 Mothers were categorised into three types according to their mating status and their offspring sex ratio, and offspring in four types, according to sex and mother type.

2.3.4 Testing egg size-mediated sex allocation model

To test whether there is a sex-specific resource allocation in *P. kellyanus*, and whether it is affected by mating, we compared the egg size between females and male offspring types, and between the mother types. First, we evaluated the effect of mating on egg size by comparing mated females with virgins. Then, we examined the egg size distribution and range variation (ERV: egg volume$_{max}$ – egg volume$_{min}$) per mother and compared these across mother types. We further confirmed the accuracy of ERV results by calculating the coefficient of egg size variation (CV$_{egg\ volume}$) within each mother and compared it across mother types. This measurement is obtained by standardizing the variance by the mean and is generally used to compare standard deviations among groups with different means (Sokal & Rohlf 1995).

The analysis of egg size across three mother types (irrespective of offspring type) and four offspring types where performed using a general linear mixed-effects model (GLMM), with “mother type” and “offspring type” as fixed factors, respectively, and “clutch order (clutch 1 and 2) nested within mother ID” as a random factor. The same analysis was performed on maximum and minimum egg size, ERV and $CV_{egg\ volume}$. Moreover, for M(♀+♂) we further evaluated the probability of an egg being female or male as a function of its size using a binary mixed logistic regression with “clutch order nested within mother ID” as a random factor.

2.3.5 Effects of mating on early reproductive investment and the trade-off between egg size and clutch size

We examined the effect of mating on the early reproductive investment (ERI) by comparing the sum of egg volumes of the 1st and 2nd clutch between virgin and mated mothers. The analysis was performed using a general linear model (GLM) with “mother type” as a fixed factor. We further assessed whether increase in egg size of the two examined clutches is traded-off with reduction in clutch size. For this we used a mixed linear regression and modelled the clutch size as a function of “average egg size” and “mother type” as fixed factors, and “clutch order nested within mother ID” as random factor. Moreover, mother forewing length was measured (see Appendix I) as a reliable proxy of “body size” (Nakao 1994) and incorporated to the analysis as a covariate. We excluded M(♂) from these analyses due to low sample size as only two M(♂) produced eggs in both examined clutches.

2.3.6 Effects of mating on mother fecundity patterns, survival rate and reproductive lifespan

To test whether mating influences the early reproductive investment and/or has other consequences on mother fitness, we examined maternal fitness traits including daily and life-time fecundity, longevity and reproductive lifespan (oviposition period) across mother types. The daily fecundity (reproductive rate) was calculated by dividing the life-time fecundity by reproductive lifespan. The fecundity data was analysed using a GLMM (Bonferroni-adjusted) with “mother type” as a fixed factor and “clutch-ID” as random factor. Mother survival rate and reproductive lifespan were analysed using Kaplan–Meier curves with log-rank and Tarone-Ware (chi square) tests ($p<0.05$), with the former considering the data homogeneously along the curve, while the latter placing more emphasis on variations of intermediate duration with approximately the same weight for early and late events.

2.3.7 Effects of egg size on offspring fitness

To test whether egg size is a suitable proxy of resource content, we assessed egg size effects on offspring fitness. For this, we scored survival probability (61% of all examined eggs developed into adults), development time to adult emergence and adult offspring body size. Upon eclosion, adults were placed in a microcentrifuge tube and frozen at -20 °C. Later, forewing length was measured as a proxy of adult body size (see Appendix I). The data were modelled as functions of egg size using a binary mixed logistic regression for survival probability with “mother type” as a fixed factor, and a mixed linear regression for development time and adult size with “offspring type” as a fixed factor, and “clutch order nested within mother ID” as a random factor.

2.3.8 Effect of egg size on neonate larval size (experiment 2)

We investigated the correlation between egg and neonate larval size. Since this involved destructive sampling, and the sex of neonate larvae cannot be easily determined, we used male larvae produced by virgin mothers. Seven-day old virgin females (n = 21) were provided with an oviposition plate for 24 hours. From each female 4 to 8 eggs (~50% of eggs) were randomly collected and measured. After egg hatch, neonate larvae (n = 86) were placed on ice for five seconds, photographed, and their body outline area (excluding legs) measured and correlated with egg size. Larval size was modelled as a function of egg size using mixed linear regression with “mother ID” as a random factor.

2.3.9 Egg size and sex allocation in field-collected females (experiment 3)

In order to test if sex and resource allocation patterns detected in the laboratory population also occur in nature, we conducted another experiment on adult females collected from a wild population in Kulnura. After collection of one clutch from these field females, egg size, offspring sex and survival, development time from egg to adult stage, and adult offspring size were recorded to evaluate egg size effects on sex allocation and offspring fitness. Females were categorized into M(♀+♂) or V/M(♂), i.e. mothers producing male-only offspring with unknown mating status. All data analyses were performed as explained for experiment 1.

2.3.10 Statistical analyses

The statistical analyses were performed using R (v. 3.5.1; R Core Team 2018) and SPSS (V.25, IBM Corporation). All data were tested for normality using Shapiro-Wilk test ($p > 0.05$) and q–q plots. For the data which violated normality assumptions log10 transformations were applied to stabilize the variances. GLM and GLMM were run using the package “*lme4*” and *“car”* to test the significance of the fixed factors and the estimation of the random factors for mixed-effect models. Upon finding significant effects of factors, pairwise comparisons were undertaken with Bonferroni post-hoc tests. Goodness of fit was assessed by residual plots which confirmed the appropriateness of linear models used.

2.4 RESULTS

2.4.1 Egg size differences among offspring and mother types

Offspring types significantly differed in the size of eggs they hatched from (Table 2.2), with $D_{M(♀+♂)}$ hatching from larger eggs than all other offspring types (Figure 2.2A). In M(♀+♂), in particular, eggs developing into females were 11% larger than those developing into males ($F_{1, 201} = 40.19$, $P < 0.0001$) (Figure 2.2A). The probability for eggs > 1.86×10^6 µm^3 (average size of female eggs) to be female was 94%, whereas it was below 37% for the eggs < 1.66×10^6 µm^3 (average size of male eggs) (Figure 2.2B; $F_{1, 201} = 51.44$, $P < 0.0001$). Despite synchronous onset of reproduction of virgin and mated females (see Appendix I), mother types differed significantly in egg size produced (Table 2.2), with M(♀+♂) producing the largest eggs (Figure 2.2C), while V and M(♂) produced eggs with similar size ($F_{1, 418} = 0.27$, $P = 0.60$) (Figure 2.2C).

2.4.2 Egg size-dependent fertilization and effect of mating on egg size range patterns

Despite a larger egg size in mother type M(♀+♂), its egg size distribution appeared similar to other mother types (Figure 2S1A). Although minimum and maximum egg size were significantly larger in M(♀+♂) than other mother types (Table 2.2; Figure 2S2A-B), egg size variation (ERV) was similar among all mother types (Table 2.2; Figure 2S2C). This similar size variation was confirmed when $CV_{\text{egg volume}}$ was compared among different mother types

(Table 2.2; Figure 2.3A). Interestingly, despite similar egg sizes among all male offspring types (Figure 2.2A), the distribution of egg size appeared narrower in $S_{M(♀+♂)}$ than in in other male offspring types (Figure 2S1B). Therefore, these results indicate that resource allocation towards eggs occurs randomly before fertilization (irrespective of offspring sex), and females produce a broad spectrum of egg sizes. The incremental effect of mating on egg size did not change this spectrum but rather the probability of fertilization increased for larger eggs (scenario *b*; Figure 2.1B).

2.4.3 Effect of mating on mother fecundity patterns

Female life-time fecundity was affected by mating, with V showing highest life-time fecundity (Table 2.2; Figure 2.2D). However, all mother types had similar daily fecundity (Table 2.2), suggesting that mating status did not affect oviposition rate. Similar results were obtained when the egg number of each of the first two clutches was compared across mother types (1st clutch: $F_{2,\,36} = 1.17$, $P = 0.32$; 2nd clutch: $F_{2,\,33} = 1.80$, $P = 0.182$).

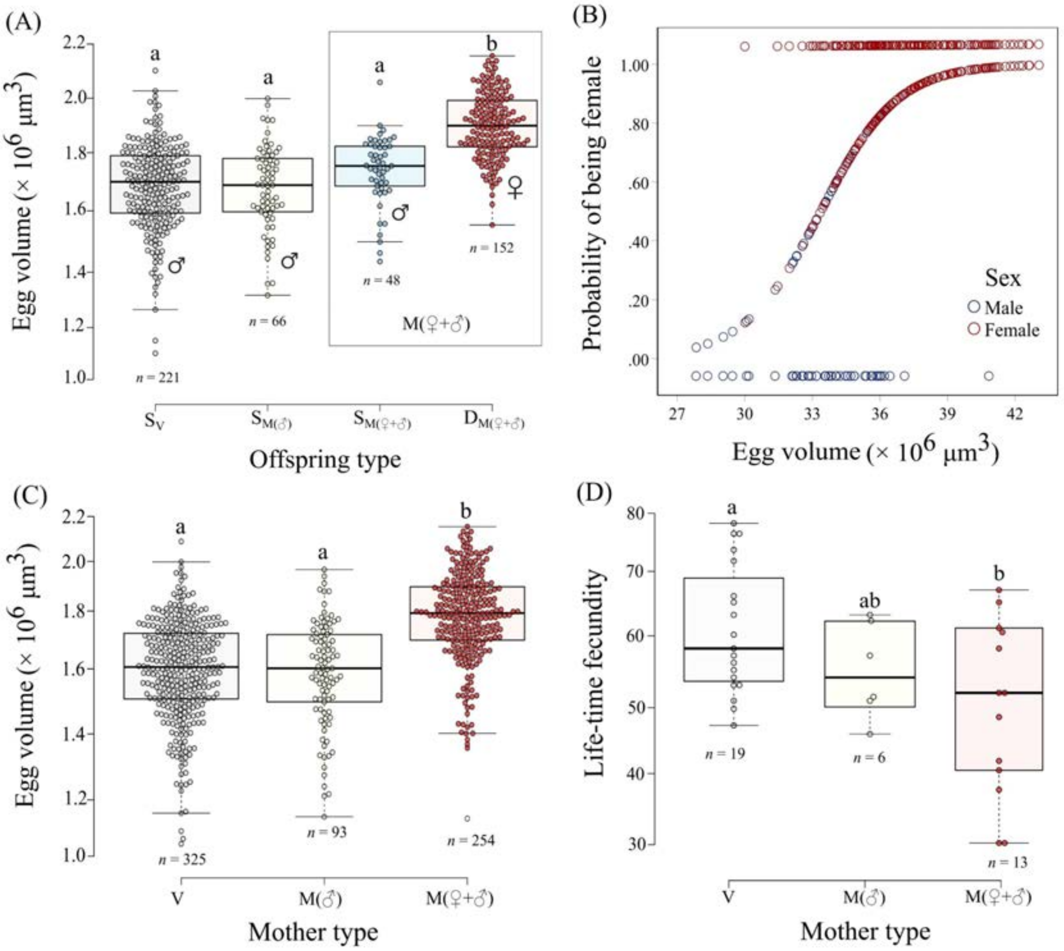


Figure 2.2 (A) Egg sizes of the four offspring types, represented as egg volumes in box plots with a bee-swarm dot plot overlay (error bars correspond to 95% confidence limits; n = number of eggs examined). The different letters indicate significant differences ($p < 0.05$; Bonferroni-corrected multiple comparisons. (B) Mixed logistic regression curve showing the probability of offspring sex versus egg size for M(♀+♂). The small circles on the curve correspond to predicted values while those on top/bottom are observed values. (C) Box plot of egg size of the three mother types (n = number eggs examined). (D) Box plot of life-time fecundity (total egg number) among the three mother types (n = number of mothers examined).

2.4.4 Effect of mating on early life-reproductive investment (ERI) and trade-off between egg size and clutch size

Consistent with the egg size results, M(♀+♂) had a larger ERI than V (Table 2.2; Figure 2.3B). Regression analysis revealed a trade-off between egg-size and clutch-size (Table 2.2; r^2 =

0.49), but the interaction between clutch size*mother type was insignificant, indicating a similar slope of the trade-off for M(♀+♂) and V (Table 2.2; Figure 2.3C). The model was a slightly better fit when mother size was incorporated as a covariate in the analysis (clutch size: $F_{1,\,61} = 11.36$, $P = 0.0013$, $r^2 = 0.56$), with a positive effect of mother size on egg size ($F_{1,\,61} = 10.25$, $P < 0.002$).

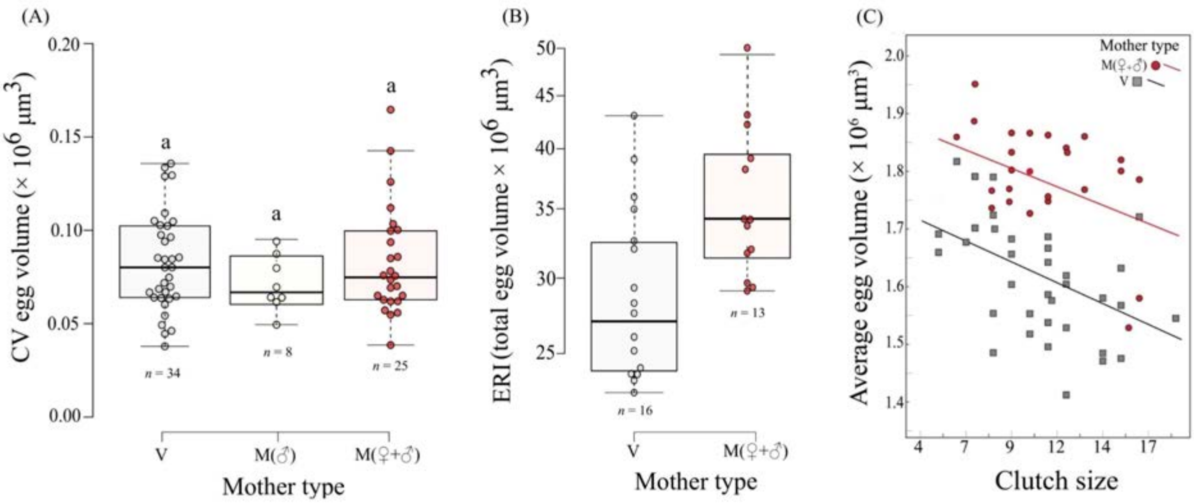


Figure 2.3 (A) Comparison of coefficient of variation of egg size ($CV_{\text{egg volume}}$) amongst mother types (n = number of mothers examined, with both clutches combined). (B) Comparison of early life reproductive investment (ERI; calculated as total egg volume of 1st and 2nd clutch) between individual M(♀+♂) and V (n = number of mothers examined). The different letters indicate significant differences ($p < 0.05$; Bonferroni-corrected multiple comparisons). (B) Trade-off between egg size and clutch size (1st and 2nd clutches) in M(♀+♂) and V. Lines represent predicted values and individual symbols represent individual data points. Box plots are represented as a bee swarm dot plot overlay.

Table 2.2 Results of the overall statistical analyses of mother type (or offspring types) on reproductive fitness traits of *Pezothrips kellyanus*. σ^2 and SD denote variance and standard deviation. *F* and *P* values show the effects of fixed factors (significance level $\alpha = 0.05$). Asterisks indicate statistically significant *P* value.

Reproductive trait/fitness	**df, N**	***F***	***P***
Egg volume (eggs with identified offspring sex)			
Offspring type	3, 486	36.89	**<0.0001**
Random effect (Mother ID/Clutch order)	$\sigma^2 = 0.34e\text{-}3$, SD = 0.018		
Residual	$\sigma^2 = 0.92e\text{-}3$, SD = 0.031		
Egg volume (all eggs produced)			
Mother type	2, 672	20.93	**<0.0001**
Random effect (Mother ID/Clutch order)	$\sigma^2 = 0.56e\text{-}3$, SD = 0.023		
Residual	$\sigma^2 = 1.44e\text{-}3$, SD = 0.038		
Minimum egg size			
Mother type	2, 69	15.67	**<0.0001**
Random effect (Mother ID/Clutch order)	$\sigma^2 = 0.68e\text{-}3$, SD = 0.082		
Residual	$\sigma^2 = 1.92e\text{-}3$, SD = 0.044		
Maximum egg size			
Mother type	2, 69	33.85	**<0.0001**
Random effect (Mother ID/Clutch order)	$\sigma^2 = 0.18e\text{-}3$, SD = 0.013		
Residual	$\sigma^2 = 0.57e\text{-}3$, SD = 0.024		
Egg-size range variation			
Mother type	2, 69	0.41	0.66
Random effect (Mother ID/Clutch order)	$\sigma^2 = 0.027$, SD = 0.051		
Residual	$\sigma^2 = 0.024$, SD = 0.156		
Coefficient of variation (CV) of egg size			
Mother type	2, 69	0.66	0.51
Random effect (Mother ID/Clutch order)	$\sigma^2 = 0.11e\text{-}3$, SD = 0.01		
Residual	$\sigma^2 = 0.019$, SD = 0.13		
Life-time fecundity			
Mother type	2, 38	4.307	**0.021**
Daily fecundity			
Mother type	2, 38	0.71	0.49
Early-life resource investment (ERI)			
Mother type [M(♀+♂) *vs* V]	1, 29	3.99	**0.008**
Egg size (trade-off with clutch size)			
Clutch size	1, 61	10.25	**0.002**
Mother type [M(♀+♂) *vs* V]	1, 61	55.69	**<0.0001**
Clutch size × Mother type	1, 61	1.02	0.31
Random effect (Mother ID/Clutch order)	$\sigma^2 = 0.92e\text{-}4$, SD = 0.009		
Residual	$\sigma^2 = 0.55e\text{-}3$, SD = 0.023		

2.4.5 Effect of mating on mother survival probability and reproductive lifespan

We detected no differences in survival probability among all mother types (Figure 2.4A; log-rank test, $x^2 = 0.74$, $df = 2$, $P = 0.68$). However, V mothers showed the longest reproductive lifespan (Figure 2.4B; log-rank test, $x^2 = 8.77$, $P = 0.012$; Tarone-Ware test, $x^2 = 9.68$, $P = 0.008$).

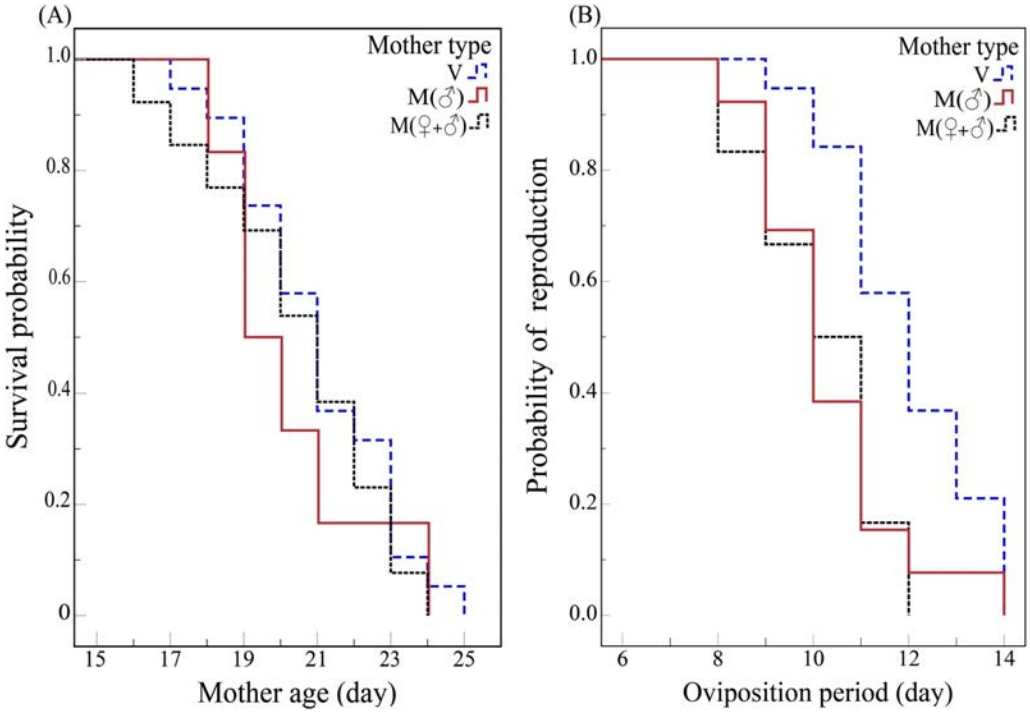


Figure 2.4 (A) Survival probability of three mother types across time. (B) Reproduction probabilities of mother types plotted against oviposition period.

2.4.6 Effect of egg size on offspring fitness

Survival to adulthood increased with egg size, and this was unaffected by mother type and the interaction between egg size*mother type (Table 2.3, Figure 2.5A), reflecting similar survival of male and female offspring. However, egg size did not influence development time to adulthood, although females reached adulthood 0.5-0.8 day later than males (Table 2.3).

Experiment 2 showed that neonate larval size increased with egg size (Table 2.3; Figure 2.5B) and experiment 1 showed that larger eggs developed to adults with longer forewings (Table

2.3; Figure 2.5C). Moreover, female offspring had 1.4× longer forewings than males, revealing a clear sexual size-dimorphism in *P. kellyanus* (Figure 2.6). However, we did not detect a significant interaction between egg size*offspring type (Table 2.3).

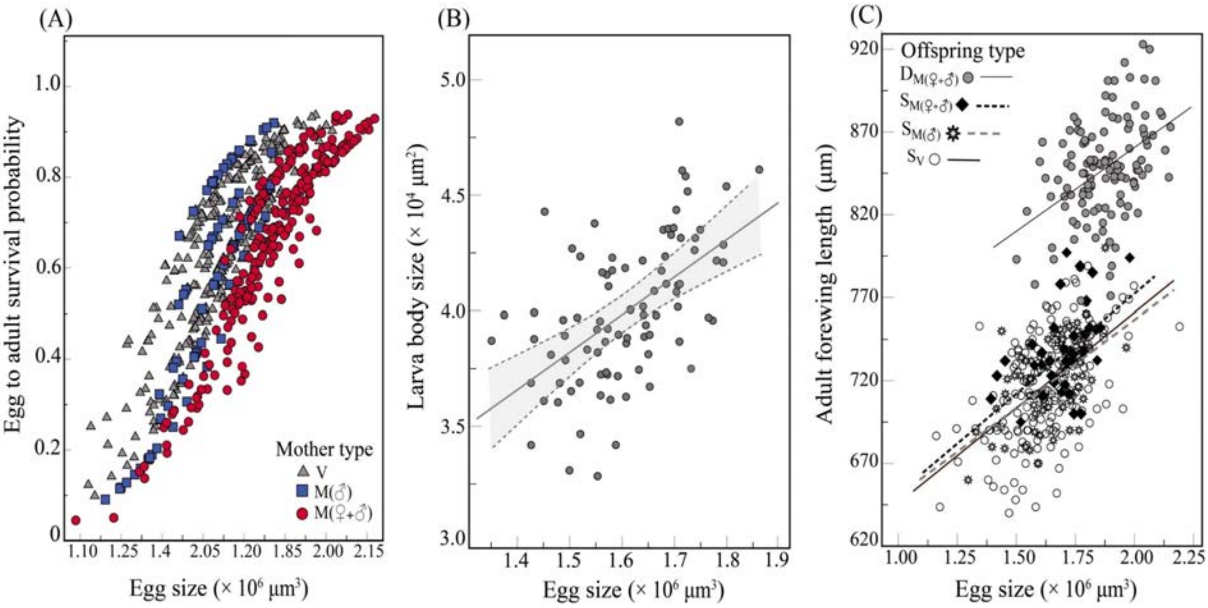


Figure 2.5 (A) Binary mixed logistic regression of the survival probability (egg to adult) and egg size, representing the best linear unbiased predictors with mother ID as a random covariate. (B) Mixed linear regression of larval size (body outline area) and egg size (volume); the grey area between dashed lines represents lower and upper 95% confidence limits for the linear predictor. (C) Mixed linear regression of forewing length with egg size; individual symbols correspond to observed individual data**.**

2.4.7 Sex and resource allocation in a field population

Overall, patterns of reproductive allocation revealed in the field population were very similar to those observed in the laboratory population (experiment 1). Field-collected M(♀+♂) produced larger eggs than V/M(♂) ($F_{1,\,170} = 8.87$, $P < 0.0001$), despite having similar forewing lengths ($F_{1,\,20} = 1.63$, $P = 0.21$). Furthermore, M(♀+♂) produced significantly larger female-destined eggs than male-destined eggs ($F_{1,\,91} = 41.69$, $P < 0.0001$). However, for the laboratory population, male-destined eggs were larger in M(♀+♂) than V/M(♂) from the field ($F_{1, 91} = 16.23$, $P < 0.001$). Furthermore, we substantiated the positive correlation between egg size and offspring fitness (for more details see Appendix I).

Table 2.3 General linear mixed models of egg size effect on offspring fitness traits (Gaussian error distribution with identity link function) in *Pezothrips kellyanus*. σ^2 and SD denote variance and standard deviation. *F* and *P* values show the effects of fixed factors (significance level $\alpha = 0.05$).

Biological trait/fitness	**df, N**	***F***	***P***
Survival probability to adulthood			
Egg size	1, 672	14.28	**< 0.001**
Mother type	2, 672	0.49	0.61
Egg size × Offspring type	2, 672	0.009	0.92
Random effect (Mother ID/Clutch order)	$\sigma^2 = 0.002$, SD = 0.045		
Residual	$\sigma^2 = 0.23$, SD = 0.48		
Development time			
Egg size	1, 429	0.33	0.56
Offspring type	3, 429	83.2	**< 0.0001**
Egg size × Offspring type	3, 429	0.85	0.46
Random effect (Mother ID/Clutch order)	$\sigma^2 = 1.09e-06$, SD = 0.001		
Residual	$\sigma^2 = 0.019$, SD = 0.13		
Neonate larva body size (offspring of V mother)			
Egg size	1, 86	21.28	**< 0.0001**
Random effect (Mother order)	$\sigma^2 = 0.30e-3$, SD = 0.017		
Residual	$\sigma^2 = 0.63e-3$, SD = 0.025		
Adult size (adult forewing length)			
Egg size	1, 416	117.87	**< 0.0001**
Offspring type	3, 416	173.02	**< 0.0001**
Egg size × Offspring type	3, 416	0.11	0.95
Random effect (Mother ID/Clutch order)	$\sigma^2 = 1.226e-05$, SD = 0.029		
Residual	$\sigma^2 = 2.270e-04$, SD = 0.015		

2.5 DISCUSSION

Our key finding is that in the haplodiploid thrips *P. kellyanus* the probability of an egg being fertilized, and therefore becoming female, increases with egg size. Egg size-mediated fertilization also occurs in a spider mite species (Macke et al. 2011), belonging to another arthropod class which has independently evolved haplodiploidy. A similar mechanism might be responsible for the egg-size sexual dimorphism found in some predatory wasps (O'Neill 1985; Budrienė et al. 2013). Therefore, size-dependent fertilization may have arisen independently in several haplodiploid arthropod lineages. The importance of egg size for fertilization success is also known for some diplodiploid animal species. For example, in free-spawning marine invertebrates larger eggs have a higher chance of collision with sperm (Deaker et al. 2019). Likewise, egg size-dependent mechanism may also apply in some diplodiploid insects where smaller eggs remain unfertilized, do not hatch and then act as trophic eggs for hatched siblings of the same clutch (Kudo et al. 2006). In haplodiploidy, however, unfertilized eggs still develop into males, and size-dependent fertilization of eggs may drive an adaptive control over egg provisioning that allows females to adjust their sex allocation in response to environmental cues (Charnov 1982).

Another important finding of our study is that mating increases the early-life reproductive investment of females, since mated females, M(♀+♂), produced larger eggs than virgins, albeit without a change in clutch size. However, we detected a trade-off between egg and clutch size with a surprisingly similar pattern in M(♀+♂) and V. Therefore, the incremental effect of mating on early-life reproductive investment is apparently exerted via directional selection that only acts on egg size but not number. Despite the larger egg size of mated females, they showed similar variation in egg size to virgin females (Figure 2.1B). This may highlight that egg size variation exists prior to mating, and mating increases egg size in general. However, sons of mated females had a lower egg-size range than those of virgin females. Therefore, sex-specific egg size in *P. kellyanus* results from selective fertilization of larger eggs, which had already been provisioned during vitellogenesis. This argument is consistent with earlier studies showing that egg fertilization in many insects occurs just before oviposition when vitellogenesis is already completed and without any opportunity for postzygotic provisioning (Heming 1970 1995; Chapman 1998). However, it contrasts with the sex-specific egg provisioning observed in birds. In birds, maternal inheritance of either the female or male sex chromosome determines the sex of the ova before fertilization and subsequently could allow for biased resource investment towards one offspring sex before fertilization (Cordero et al. 2000).

2.5.1 Different fertilization mechanisms in thrips and mites

Dissection of mated females (Figure 2.6B) confirmed storage of motile sperm in their spermathecae. In thrips, the spermatheca opens to a fertilization chamber through a narrow spermathecal duct, which is equipped with contractor muscles that may control sperm release into the fertilization chamber (Heming 1970; Dallai et al. 1996). After ovulation, an egg passes the opening of the spermathecal duct, and stimulates the contractor muscles that then allow access of sperm to the egg (Moritz 1988; Heming 1995). Such a mechanosensory mechanism of egg fertilization occurs across a wide range of insects (Chapman 1998). In this context, our findings suggest that larger eggs are more likely to activate this mechanism than smaller eggs, probably by imposing higher mechanosensory stimulation on spermathecal muscles (Figure 2.6C). In comparison, in studied mite species, sperm flows from the spermatheca into the ovariole cavity where fertilization of oocytes occurs earlier, during vitellogenesis (Boudreaux 1963; Helle 1967). Nevertheless, despite clear evidence that the sex-specific egg-size difference commences before fertilization in the spider mite *T. urticae* (Macke et al. 2011), the mechanism underlying fertilization of larger eggs in this species remains unresolved. Macke et al. (2011) postulated spatial segregation of large and small oocytes in the ovary as a likely mechanism that may facilitate exposure of larger oocytes to more sperm.

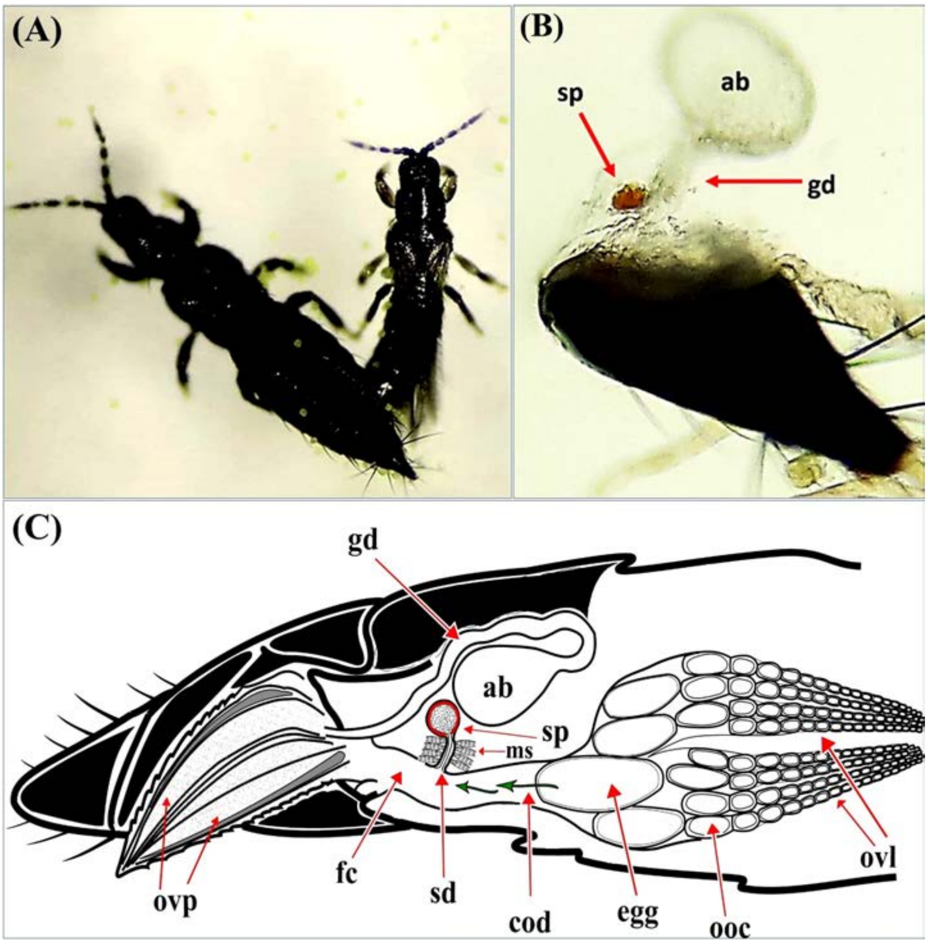


Figure 2.6 (A) Sexual size dimorphism of female (left) and male (right) *Pezothrips kellyanus*, displayed as a copulating pair. (B) Dissected posterior segments of a female's abdomen with the brown spermatheca (sp) and transparent apical bulb (ab) of the accessory gland, opening to the base of the ovipositor via the gland duct (gd). (C) Mechanosensory mechanism of fertilization with larger eggs being more likely to stimulate spermathecal muscles and receive sperm. Lateral view of the female genitalia with ovariole (ovl), oocyte (ooc), common oviduct (cod), fertilization chamber or vagina (fc), spermathecal duct (sd), spermathecal muscles (ms) and ovipositor (ovp).

2.5.2 The possible (nonexclusive) explanations for the evolution of increased early-life resource investment after mating

The synchronous onset of reproduction in both mated and virgin mothers dismisses the possibility that larger egg size in M(♀+♂) is due to an earlier sexual maturity and more advanced oocyte development after mating. Rather, the larger egg size suggests that mating stimulates increased egg provisioning as demonstrated for a spider mite species (Macke et al. 2012). However, unlike in the spider mite species where mating gradually increases the egg size in subsequent clutches, in *P. kellyanus* this occurs immediately for early-life reproduction. Similar patterns have been found in an orb-web spider where females lay larger eggs in heavier sacs early in life (Ameri et al. 2019). This may be a consequence of a higher metabolic efficacy in younger females (Rose 1991) or may arise from selective pressures that favour increased fertilization success as sperm is more abundant and viable after mating in early adulthood (Stearns 1992; King 2000). The latter is less likely for a polyandrous species, like *P. kellyanus* (personal observations), because re-mating may replenish sperm. More importantly, in haplodiploids increased fertilization for daughter production may be in conflict with a mother's optimal offspring sex ratio which is, theoretically, less female-biased than for males (Shuker et al. 2009). This pattern may arise from a sexual conflict that favours males to induce production of more daughters (Shuker et al. 2009). However, this male effect might also be overridden by sexual selection acting on the male to monopolize more of a female's reproductive budget for his sperm and leave less for his rivals in subsequent matings (Arnqvist & Nilsson 2000). Such a strategy may also minimise the risk of sperm competition with ejaculates of subsequent males (Parker 1970). Males may achieve this higher fertilization by transferring accessory gland compounds that accelerate vitellogenesis (Gillott 2003) or by stimulating female feeding behaviour that escalates her reproductive output (Perry 2011).

Mating, however, does not always lead to fertilization success (Greenway et al. 2015). For example, in some parasitoid wasps, fertilization failure has been attributed to lower male quality (Henter 2004). In other parasitoid wasps, fertilization success increases with maternal size (Santolamazza Carbone et al. 2007). It is possible that maternal effects that influence egg size (Fox at al. 1997) contribute to this fertilization success. In our study, the potential role of maternal effects in fertilization success is probably reflected in M(♂), i.e. in the mated *P. kellyanus* females that failed to produce daughters despite successful insemination. These females evidently produced smaller eggs than M(♀+♂). This bimodal reproductive pattern may also derive from a sexual conflict that cause a female counter-adaptation against the male's evolutionary interest in daughter production. Alternatively, this pattern may have evolved under kin selection (Bourke & Chan 1994; Smith et al. 2019) which entails higher resource allocation in unconstrained females [M(♀+♂)] as an altruistic strategy to alleviate the cost of constrained sex allocation in M(♂) and counterbalance the excess of male production (Godfray 1990).

Finally, higher resource allocation towards female in M(♀+♂) may also be associated with the presence of *Cardinium* and *Wolbachia*. These endosymbiotic bacteria are maternally inherited, and, increasing their host's daughter production is a strategy for their transmission success (Werren 1997). This is generally achieved by inducing reproductive manipulations (Werren 1997), as the CI observed in *P. kellyanus* (Nguyen et al. 2017). It has previously been suggested that in haplodiploids these bacteria can boost fertilization success to increase daughter production (Egas et al. 2002).

2.5.3 Mating effects on early-life resource investment and life-history trait trade-off

Mechanisms that partition the energy budget towards offspring production can influence the evolution of parental life history traits (Stearns 1992). In *P. kellyanus* increased early-life resource investment in M(♀+♂) was linked with a lower life-time fecundity. However, this pattern was not due to a higher reproductive rate in virgin females, but instead to lower resource investment in eggs, which allowed them to extend their reproductive lifespan. This may be a strategy to save resources for potential daughter production after mating later in life (Macke et al. 2012; Gottlieb et al. 2014). Although mating is generally assumed to be costly for females in terms of lifespan and reproductive output (Arnqvist & Nilsson 2000), the exact effect of mating is quite variable among different species and not easily predicted (Fedorka & Mousseau 2002). Had we not examined the egg size and early-life resource investment, we would have erroneously concluded that M(♀+♂) suffered a reproductive cost in terms of lower life-time fecundity relative to virgins. Nevertheless, studies of temporal egg size variation are needed to reveal a clearer picture of the overall reproductive fitness (Ameri et al. 2019) and mating effects on associated trade-offs.

2.5.4 Egg size variation is due to a differential nutrient allocation

Egg size is regarded as a reliable indicator of maternal investment in offspring fitness (Fox 1997; Fox & Czesak 2000; Krist 2011). In *P. kellyanus*, larger egg size increased offspring survival to adulthood. However, similar female and male survival probability, despite developing from different egg sizes, may suggest sex-specific costs for juvenile development, and that females require more resources than males to achieve the same life stage (Weatherhead & Teather 1994). Thus, egg-size mediated fertilization may also have evolved to reduce developmental mortality in the costlier sex, as previously shown in birds (von Engelhardt et al. 2006). Similar adaptations are known for solitary bees with parental provisioning in brood cells (outside of the egg), with more resource provided to female than male brood cells (Radmacher & Strohm 2010). Another example is female parasitoid wasps that lay female-destined eggs in larger hosts due to a sex-specific host-size effect on off-spring fitness (Ueno 1999).

We further showed that the larval size in *P. kellyanus* increased with egg size and this fitness effect persisted until adulthood without change in development time, indicating a direct effect of maternal resource allocation on adult offspring body size, a reliable proxy of energy budget and fitness (Clutton-Brock 1988; Fox & Czesak 2000). Therefore, a positive effect of egg size on offspring body size and survival suggests that egg size variation is not simply due to variable chorion thickness and/or water content, but a differential nutrient allocation that affects fitness. Finally, the link between maternal investment and offspring body size together with the sex-specific survival and growth rates may provide a possible foundation for the evolution of sexual size dimorphism (Figure 2.6A) suggested in several studies (e.g. Tammaru et al. 2010; Teder 2014).

2.5.5 Egg size-mediated sex allocation is maintained by natural selection

The size-dependent fertilization of eggs and increased resource allocation after mating revealed in the laboratory population were also found in the field population from where the laboratory population originated. This suggests that these sex and resource allocation patterns are also expressed under natural environmental conditions and therefore maintained by natural selection. Finally, similar positive effects of egg size on offspring fitness were revealed for the field-collected individuals and this corroborates the fact that egg size is a reliable proxy for resource allocation.

2.6 FUTURE DIRECTIONS

The egg size-mediated fertilization model provides a new platform for further testing of several sex and resource allocation theories and their constraints. The next logical steps will be to assess whether egg size is influenced by maternal (Fox et al. 1999) and/or paternal factors (Shuker et al. 2006), and whether these are of environmental, genetic and/or epigenetic nature. It will also be important to disentangle the interactive role of female and male effects on egg-size adjustment. Addressing this in the context of sexual conflicts may reveal whether females respond differently to male traits, or males have evolved adaptions to directly influence maternal investment (Crean & Bonduriansky 2014). Furthermore, it will be important to test the effect of maternally-inherited endosymbionts on egg size, resource and sex allocation. It is expected that such effects should form part of the endosymbiont's strategies to increase their prevalence in the host populations and this could also lead to a host-endosymbiont arms race over sex and resource allocation (Werren 2011).

2.7 APPENDIX I

2.7.1 Supplementary material and methods

2.7.1.1 Laboratory thrips population

Thrips collection and rearing

For the establishment of *Pezothrips kellyanus* (Bangall) (Thysanoptera: Thripidae) laboratory populations, thrips were collected from lemon flowers, in a citrus orchard in Kulnura, Central Coast, New South Wales (NSW), Australia, on 12th of January 2017. This was not an ideal time for collecting large numbers of thrips due to the end of the summer flowering period. In overall 14 females and only one male was collected from over 10 different trees. The species identity of individuals was confirmed using the description by pervious literature (Webster Cooper, & Mound 2006). All thrips individuals were kept in a small container with one fresh lemon for one generation. Fresh pollen of *Typha sp.* (cumbungi) was sprinkled on the lemon as an additional food source every second day to increase fertility. This thrips population was kept in a controlled environmental chamber at 20±2°C, 70±2% RH and 16:8 h (L:D) photoperiod. The next generation (F1) consisted of a small number of females (N<20) and larger number of males (60<N). Thrips were supplied with nine fresh lemons at the beginning of each generation. The sex ratio of F3 was still highly male-biased (~80%), possibly due to cytoplasmic incompatibility (CI) caused by variable infections with *Cardinium* and *Wolbachia* (Nguyen et al. 2017). Therefore, it was decided to establish offspring lines from individual females as isofemale lines with homogenous endosymbiont infection status [co-infected (C^+W^+)], in order to overcome any potential issues with CI.

Haplotyping and endosymbiont screening

DNA extraction from 20 F3 individual thrips was conducted using GenElute™ Mammalian Genomic DNA Miniprep Kit. Standard PCR techniques were used to verify endosymbiont infection status using the *wsp* gene (primer pair 81F-691R) for *Wolbachia* and the 16S rRNA gene (primer pair CLOf1-CLOr1) for *Cardinium* (Nguyen et al. 2016). The *Wolbachia* strain and the *Cardinium* strain of this population have previously been characterised, and their gene sequences were identical with the ones from other Australian populations (Nguyen et al. 2016). The primer pair LCO1490 and HCO2198 was also employed to amplify COI (Folmer et al. 1994) to test DNA quality and subsequent haplotyping. Following the direct sequencing and BLAST search (NCBI, Bethesda, Maryland, USA) we characterised the haplotype of the population as H7, consistent with a previous study (Nguyen et al. 2016).

Isofemale lines for establishment of fixed endosymbiont infections

Following the bacterial PCR screening, eight (40%) and five (25%) individuals were uninfected and co-infected (C^+W^+) (with weak PCR signals), respectively. However, seven (35%) individuals were C^+W^+ (with strong PCR signals). This indicated that the female founders from the field had been polymorphic in infection status confirming earlier findings by Nguyen et al. (2016) for this population. Thus, another 20 F3 females were randomly selected and kept in isolation in separate small containers with a single fresh lemon and pollen. These females were kept in containers for a period of ten days to ensure sufficient production of offspring. Subsequently, the females were removed from the containers to test for their infection status with PCR. Six of the 20 females were co-infected. Of these six only four produced female-biased offspring, and two females produced only male offspring. All offspring of the four co-infected females (N>60) were mixed together in a small container supplied with fresh lemon and pollen. This established the co-infected line (C^+W^+). This line was also provided with ~20 more males from seven uninfected isofemale lines in order to ensure successful mating, obtain more female offspring in the next generation (F4) and higher diversity of genetic background. This line was then maintained in bigger containers for nine more generations before the start of the experiment. Each generation ten female offspring were randomly sampled to check for infection status with PCR. All samples of each generation were found co-infected, indicating fixation of the infection and high transmission efficiency for *Cardinium* and *Wolbachia*.

2.7.1.2 Supporting information on the experiments

For this study, three experiments were undertaken. Experiment 1 and 2 were undertaken with individuals of the laboratory line. Experiment 1 was the largest and is described in detail below. Experiment 2 was a small experiment to assess the larval development of male offspring of virgin females. Experiment 3 was performed to assess sex and resource allocation patterns in field-collected individuals.

Experiment 1

Establishment of virgin and mated thrips for

In order to obtain mated and virgin females under standardised conditions, 100 females were randomly sampled from the laboratory population and placed in cohorts of 20 females on individual similar-sized fresh high-quality lemons, one each in five round plastic containers (700 ml; Sistema, New Zealand) with a round filter paper on the bottom. The females were allowed to lay eggs for 24 hours and were then removed. After 6-7 days, a total of about 250 larvae emerged. To ensure equal larval density we restricted the number of larvae to 40 per lemon and discarded the rest. The thrips were kept in the containers supplied with *Typha sp.* pollen ad libitum every second day, until they reached the pupal stage when visual differentiation between sexes became apparent (Nguyen et al. 2017). To ensure the virginity of adults, female and male pupae were separated, placed into small Petri dishes (diameter 30 mm; height 15 mm) with moistened filter paper and kept until adult emergence. Newly-emerged females (1-2 days old) were randomly selected and either retained as virgin or individually transferred to a copulation chamber, a small Petri dish supplied with 10 randomly selected virgin males (2-3 days old). The mating behaviour was observed and recorded (duration range = 1'20″-2'30″). Then, the female was removed from the chamber to avoid re-mating; thus, all mated females were singly-mated. All virgin and mated females were kept individually in small Petri dishes with their ID being recorded.

Comparison of oviposition onset in virgin and mated females and determination of optimal time for egging

In order to determine the effect of mating status on the onset of oviposition, we compared the pre-oviposition period between virgin and mated females. For this purpose, 42 females (22 virgin and 20 mated females, after observation of copulation) were individually transferred to oviposition chambers topped with an agarose plate (0.6% w/v) (Agarose I, AMRESCO®, USA), sealed with parafilm and supplied with *Typha* sp. pollen ad libitum (one sprinkle) and a piece of absorbent cotton wool soaked with honey solution (50% w/v). Females were monitored daily, and agarose plates and food supply were renewed every second day until first oviposition occurred.

We did not detect any influence of mating status on the time of onset of oviposition: the mean pre-oviposition periods were 6.41 ± 0.32 days for virgins and 6.20 ± 0.38 days for mated females (*t*-test: $t = 0.42$, $df = 40$, $P = 0.67$). Within six-seven days more than 80% of mated and virgin females had started reproduction. Therefore, we concluded that this would be an ideal time for the females to be exposed to an oviposition plate for the first time so that eggs from at least 80% of females can be obtained on the same day.

Rearing from egg to adult emergence

After egg size measurement, each egg was promptly transferred to a small agarose gel block contained within the lid of a 0.2 mL microcentrifuge tube. This embryonic development chamber protected the embryo from desiccation and was prepared by pouring 30 µL agarose (0.6% w/v) into the interior cavity of the tube's lid. Once eggs hatched, each individual larva was separately transferred to a larval chamber, a 1.5 mL microcentrifuge tubes with the interior side of its lid covered by a fresh lemon leaf disc (0.8 cm diameter) sprinkled with *Typha sp.* pollen (ad libitum). Larval chambers were supplied with fresh leaf discs and pollen every three days.

Offspring adult size

Overall 61% of all eggs reached adulthood. This included adult male offspring ($N_{(\text{clutch 1})} = 107$; $N_{(\text{clutch 2})} = 96$) of virgins (V), adult male ($N_{(\text{clutch 1})} = 25$; $N_{(\text{clutch 2})} = 12$) and female offspring ($N_{(\text{clutch 1})} = 61$; $N_{(\text{clutch 2})} = 56$) of mated mothers [M(♀+♂)], and adult male offspring ($N_{(\text{clutch 1})} = 56$; $N_{(\text{clutch 2})} = 17$) of mated mothers with only male offspring [M(♂)]. Offspring individuals were placed in a microcentrifuge tube and frozen at -20 °C. Then, forewing length was measured as a proxy of body size (Nakao 1994). For this purpose, the right forewing was spread on a glass slide with a fine brush under a binocular Leica DMRB microscope, photographs were taken with Leica Quantimet 500c (Cambridge, UK) and analysed using Image-Pro 6.0 software (TIANGE, Ltd, USA).

Egg size and sex allocation in field-collected females (experiment 3)

This experiment was conducted to test whether the sex-specific resource allocation towards egg size can also be detected in a field population. Thirty-five thrips females of unknown mating history were randomly sampled from Kulnura (14 December 2017), the site from where the laboratory population was originally established. Individuals were collected from 15 different trees with a minimum distance of 20 metres between trees. Two days after collection, thrips were allowed to lay eggs for 24 h and were subsequently placed in the freezer for later forewing measurement. Females were also screened for *Cardinium* and *Wolbachia* by diagnostic PCR. Only 21 females produced eggs, including one female that was *Cardinium*-only infected with only male offspring. This female was excluded to standardize the infection status across all experiments. All other females were infected with both *Cardinium* and *Wolbachia*. From each female, 5 to 12 eggs were randomly collected (n = 172) for size measurement. These eggs constituted about 70 to 75 % of the produced eggs. Offspring sex, survival to adulthood, development time from egg to adult stage and adult forewing length were recorded to evaluate the effect of egg size on offspring fitness. Four-teen females that produced at least one (or more) female and male offspring were categorised as M(♀+♂). Seven females produced only male offspring and they were categorised as constrained female V/M(♂) because we were not able to discriminate whether they were virgin [V mothers] or mated females with only male offspring [M(♂) mothers]. We did not test the effect of egg size on female life time and daily fecundity and other traits including longevity and reproductive lifespan because we did not have information about female age, mating history and the number of eggs produced before the experiment.

2.7.2 Supplementary statistical analyses

Experiment 1: to check whether data from the 1st and 2nd oviposition day can be pooled we conducted a general linear model, repeated measure ANOVA on eggs size of each mother with oviposition day (= clutch order) as within-subject factor and "mother type" as between-subject factor across the mothers with two egg clutches. This allowed us to investigate whether there were time-dependent changes in egg size between the 1st and 2nd clutch. As only two M(♂) produced eggs on both days we excluded this mother type from our comparison. Our result showed that the clutch order did not significantly influence egg size (clutch order: $F_{1, 29} = 4.02$, $P = 0.06$; clutch order*mother type: $F_{1, 29} = 1.09$, $P = 0.31$). We further tested the effect of clutch order on clutch-specific sex ratio in M(♀+♂) using GLMMs with "clutch order" as fixed factor and "mother ID" as random factor. We also showed that clutch order did not significantly influence clutch-specific sex ratio ($F_{1, 26} = 2.18$, $P = 0.16$). Therefore, the egg size data of the 1st and 2nd clutch were pooled. However, we still incorporated the "clutch order" as random effect (nested with mother ID or alone depending on dependant variables) in a GLMM analysis to partition the variances in egg size attributable to clutch order.

2.7.3 Supplementary results

2.6.7.1 Results of the experiment 3

Sex-specific egg size in a field population

Size analysis of the eggs laid by field-collected mothers confirmed sex-specific resource allocation toward egg size as revealed in the laboratory, with M(♀+♂) producing significantly larger eggs than V/M(♂) ($F_{1, 170} = 8.87$, $P < 0.0001$). For mothers, the forewing length was not different between V/M(♂) and M(♀+♂) ($F_{1, 20} = 1.63$, $P = 0.21$). However, unlike in experiment 1, the clutch size (only one was collected for field individuals) was marginally larger in V/M(♂) than in M(♀+♂) ($F_{1, 20} = 4.56$, $P = 0.048$), which may be due to the unknown age and mating history of field-collected females.

Probability of egg being female in M(♀+♂) mother

Consistent with the laboratory population, a logistic regression model demonstrated that the possibility of an egg to develop into a female is highly positively correlated with egg size ($F_{1, 90} = 32.37$, $P < 0.0001$).

Egg size and offspring fitness effects in a field population

For offspring of field-collected females, we confirmed a significant effect of egg size on survival to adulthood ($F_{1, 169} = 4.98$, $P = 0.026$). Moreover, consistent with the laboratory population this was not significantly influenced by mother type ($F_{1, 20} = 2.37$, $P = 0.12$) and egg size*mother type ($F_{1, 169} = 1.90$, $P = 0.16$). Furthermore, mixed linear regression analysis confirmed no significant influence of egg size on larval development time in all offspring types (egg size: $F_{1, 431} = 0.44$, $P = 0.65$; egg size*offspring type: $F_{1, 119} = 0.15$, $P = 0.88$). Finally, we also detected an effect of egg size on adult forewing length as found for the laboratory population (egg size: $F_{1, 119} = 4.27$, $P < 0.001$; offspring type $F_{1, 119} = 2.31$, $P = 0.023$; egg size*offspring type $F_{1, 119} = 3.64$, $P < 0.001$). Considering the significant effect of offspring type on adult forewing length we examined the effect of egg size on adult size separately for each offspring type: female, $F_{1, 63} = 18.17$, $P < 0.0001$, $r^2 = 0.21$; male from M(♀+♂), $F_{1, 13} = 3.45$, $P = 0.08$, $r^2 = 0.17$; male from V/M(♂), $F_{1, 43} = 6.18$, $P = 0.017$, $r^2 = 0.13$). The marginally insignificant relationship between egg size and offspring forewing length in males of M(♀+♂) could be due to the smaller sample size.

2.7.4 Supplementary figures: *Figures 2S1-2S2*

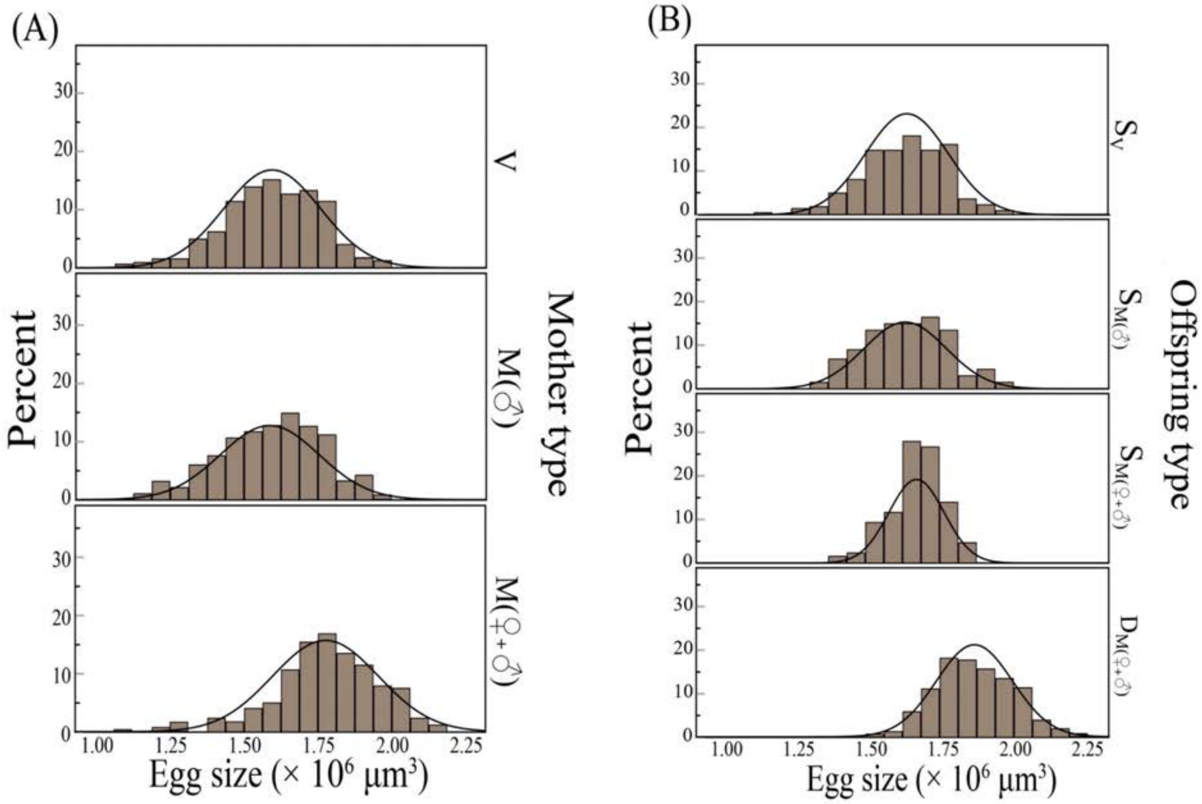


Figure 2S.1 (A) Size (volume) distribution of all eggs produced by different mother types (all measured eggs included regardless of survival): M(♀+♂) n = 254, mean = 1.78 × 10^6 µm^3, SD (standard deviation) = 0.180 × 10^6 µm^3; M(♂) n = 93, mean = 1.58 × 10^6 µm^3, SD = 0.167 × 10^6 µm^3; and V n = 325, mean = 1.59×10^6 µm^3, SD = 0.164 × 10^6 µm^3. (B) Egg size (volume) distribution of different offspring types (data for the offspring that survived to the pupal stage when sex becomes): $D_{M(♀+♂)}$ n = 152, mean = 1.86 × 10^6 µm^3, SD = 0.103 × 10^6 µm^3; $S_{M(♀+♂)}$ n = 48, mean = 1.67 × 10^6 µm^3, SD = 0.185 × 10^6 µm^3; $S_{M(♂)}$ n = 66, mean = 1.67 × 10^6 µm^3, SD = 0.138 × 10^6 µm^3; and S_V n = 221, mean = 1.66 × 10^6 µm^3, SD = 0.139 × 10^6 µm^3.

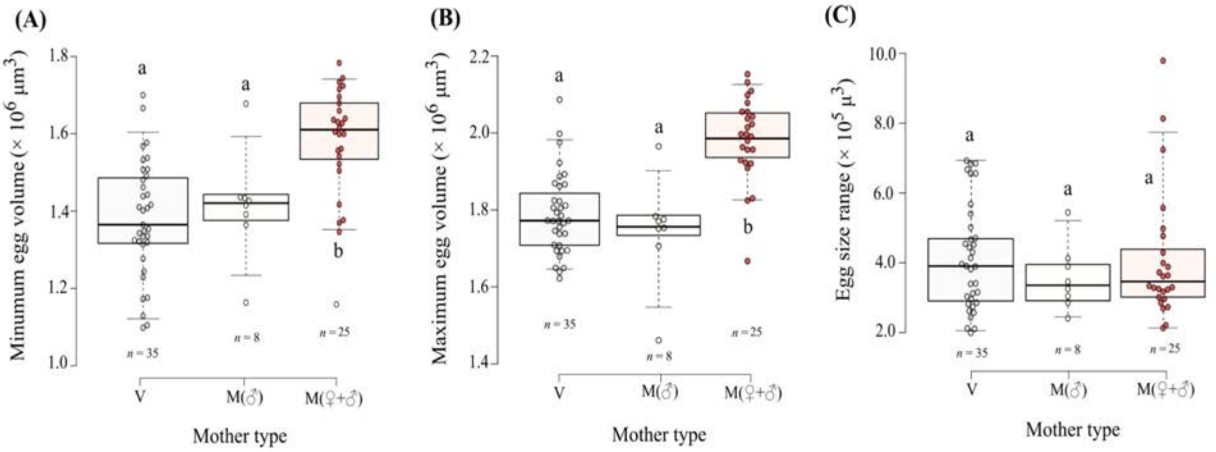


Figure 2S.2 (**A)** Minimum egg size across different mother types. (B) Maximum egg size across different mother types. (C) Comparison of egg size range (egg size$_{max}$ – egg size$_{min}$) across different mother types; mean values; error bars correspond to 95% confidence limits (n = number of mothers examined, with both clutches combined). The different letters indicate significant differences ($p < 0.05$; Bonferroni-corrected multiple comparisons). Box plots are given with a bee-swarm dot plot overlay.

CHAPTER III

Constrained sex allocation after mating in a haplodiploid thrips species depends on maternal condition

Dissected spermatheca of *Pezothrips kellaynus* female after mating.

Alihan Katlav[1], Duong T. Nguyen[2], James M. Cook[1], Markus Riegler[1]

1 Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, New South Wales 2751, Australia, **2** Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Private Bag 4008, Narellan, New South Wales 2567, Australia

Katlav, A., Nguyen, D. T., Cook, J. M., & Riegler, M. (2021). Constrained sex allocation after mating in a haplodiploid thrips species depends on maternal condition. *Evolution. 75*, 1525-1536. https://doi.org/10.1111/evo.14217

Constrained sex allocation after mating in a haplodiploid thrips species depends on maternal condition

3.1 ABSTRACT

In females of haplodiploid animals, female production requires fertilisation, whereas male production does not. Therefore, haplodiploid species can display extraordinary sex ratios. Constrained sex allocation occurs when a female cannot produce daughters. This can be due to virginity but may also occur after mating due to insemination failure, selfish genetic elements or physiological constraints. Here, we investigated the mechanism underlying constrained sex allocation in *Pezothrips kellyanus*. In this species some mated females produce highly female-biased broods, yet, for unknown reasons, others produce extremely male-biased broods. Using crossing experiments controlled for maternally inherited endosymbionts we confirmed that constrained females were successfully inseminated. Furthermore, male-biased offspring production was not paternally inherited, ruling out paternal sex ratio elements previously identified as sex ratio distorters in some parasitoid wasps. Next, we excluded mating time and paternal fitness effects (male size) on sex allocation. However, we found that constrained sex allocation only occurred in small females producing smaller eggs than large females producing larger eggs and female-biased broods. Consequently, the bimodal sex allocation pattern correlates with maternal condition, and may have arisen (adaptively or non-adaptively) in association with an egg size-mediated fertilization mechanism recently detected in some haplodiploids, with egg size positively affecting fertilization success.

Keywords: Split sex ratio**,** selfish genetic element, sex ratio distorter, fertilization success, body size, egg size, conditional sex allocation

3.2 INTRODUCTION

Sex allocation is an important aspect of animal reproduction and involves parental control over production of female and male offspring (Charnov 1982; West 2009). Due to its strong and direct influence on fitness, sex allocation is shaped by strong natural selection (West 2009). In addition, as a binary variable that can be easily scored, sex ratios lend themselves to the development and testing of adaptive models. Despite the ubiquity of strong selection on sex ratios, the response to selection depends partly on the control mechanisms available to parents (West and Sheldon 2002). Understanding these control mechanisms are pivotal for deciphering adaptive value of sex ratio variations among females (West 2009), and may help elucidate the evolutionary forces that shape mating systems, sex ratio dynamics and population structure (Helms et al. 2005). Of particular interest to sex allocation research is haplodiploidy, a sex determination system that has evolved in almost 15% of invertebrate species (Normark 2003). In this system, males are haploid and develop from unfertilized eggs, whereas females are diploid and develop from fertilized eggs. Therefore, haplodiploidy enables females to control offspring sex allocation via fertilization control (Charnov 1982), and allows the optimisation of population sex ratios in response to local conditions (King 1987; West and Sheldon 2002). Females can achieve this by storing sperm acquired during copulation in the spermatheca and subsequent control of sperm access to eggs (Hamilton 1967; Godfray 1994).

Control over sex allocation, however, is not always achieved, as in some situations females are unable to produce female offspring (Godfray 1990). Generally referred to as constrained sex allocation, this phenomenon is mainly associated with virginity, and can occur in 1-25 % of females of a population (Kranz et al. 2000), with more pronounced examples of up to 50% virginity in some parasitoid wasp species (Hardy et al. 1998; Godfray and Hardy 1990). If some females fail to mate, they may still reproduce, but produce only sons (Grafen 1986). This excess male production by unmated females can then lead to the selection of unconstrained (mated) females that produce more daughters (Godfray and Hardy 1993). Such a bimodal reproduction pattern (also known as split sex ratio) has been well studied in hymenopterans (e.g. Ode et al. 1997; West et al. 1998; Metzger and Bernstein 2008; Rautiala et al. 2018), but less extensively so in other haplodiploid taxa (e.g. Higgins and Myers 1992; Kranz et al. 2000). However, for many species the factors causing constrained sex allocation remain elusive.

Most studies of split sex ratios have either addressed pre-copulation constraints like limited mating opportunities in parasitoid wasp species (e.g. Guertin et al. 1996; Ode et al. 1997) or examined the selection processes driven by worker-biased population structures and relatedness asymmetries in eusocial species (e.g. Aron et al. 1999; Chan et al. 1999; Gardner et al. 2012). However, more recent studies have shown that constrained sex allocation can also occur in females that have already mated. This can be attributed to insemination failure, limited sperm quantity and/or quality (King 2000; Bovin 2012; Chirault et al. 2019), male age (Damiens et al. 2003), male body size (Henter 2004) and genetic factors (Khanh et al. 2005). For example, in the parasitoid wasp *Uscana semifumipennis*, females mated with smaller males produce more male-biased sex ratios, presumably due to their lower quantity or quality of sperm (Henter 2004). In some species, males repeatedly mate even when sperm-depleted, leading to infertility of some mated females (King 2000). This is costlier for mononandrous females that become unreceptive after a first mating despite a lack of sperm in their spermathecae, a situation also known as pseudo-virginity (van den Assem 1970).

Another important source of constrained sex allocation in haplodiploids is selfish genetic elements (SGE) (Stouthamer et al. 2002). Common examples in many arthropod lineages involve maternally inherited endosymbiotic bacteria and, less frequently, paternal sex ratio (PSR) elements as described in a few parasitoid wasp species (Hyemenoptera). These SGEs bias host reproduction towards the offspring sex that favours their own transmission to subsequent host generations (Werren et al. 1988; Stouthamer et al. 2002; Wedell and Price 2015). For example, in some haplodiploids the maternally inherited bacterial endosymbionts *Wolbachia* and *Cardinium* can cause cytoplasmic incompatibility (CI) that results in mortality or male development of fertilised embryos in uninfected females mated with infected males (Werren and Stouthamer 2003; White et al. 2011). This indirectly increases the reproductive fitness of infected females, because they are capable of daughter production and sex ratio control in matings with both infected and uninfected males (Weeks et al. 2001; Hurst and Frost 2015). In contrast, PSR elements are paternally inherited and are often supernumerary B chromosomes (Dobson and Tanouye 1996; Hunter et al. 1993), but may also be transposable elements or heritable viral infections (Hunter et al. 1993). Males bearing a PSR element (hereafter PSR males) produce functional sperm that fertilize eggs but the PSR element subsequently eliminates paternal chromosomes (except the PSR element itself), converting female-destined embryos to PSR males (Werren 1991). However, the existence of PSR elements has never been explored beyond three genera of parasitoid wasps: *Encarsia*, *Nasonia* and *Trichogramma* (Dobson and Tanouye 1996; Hunter et al. 1993; Stouthamer et al. 2001; Werren 2011).

Another constraining factor of sex allocation is maternal fitness (Trivers and Willard 1973; Charnov 1982). A key component of maternal fitness is body size (Visser 1994; Seidelmann et al. 2010). An effect of maternal body size on sex allocation has been demonstrated in solitary bees, where smaller mothers are constrained to invest in male offspring (the smaller and therefore less costly sex); and this indirectly increases the fitness return from daughters and, therefore, selects larger females to invest more into the costlier production of female offspring (Seidelmann et al. 2010). This was also shown in some parasitoid wasps in which smaller females produce male-biased broods because they are less likely to find larger hosts required for daughter production (Heinz 1996; Santolamazza-Carbone et al. 2007). However, the mechanism that causes smaller females to produce male-biased offspring remains unknown.

Recent studies have shown that in some haplodiploids, mothers can control sex allocation by adjusting egg size: larger eggs are more likely to be fertilized and become female than smaller eggs that remain unfertilized and become males (Macke et al. 2011; Katlav et al. 2021). This mechanism may have an adaptive explanation because female offspring need more maternal resources than males (Katlav et al. 2021). Consequently, larger mothers with more resources can produce larger eggs and more daughters compared to smaller mothers (Macke et al. 2011), an analogous yet opposite pattern to the classic examples in mammals (Trivers and Willard 1973). However, it remains to be tested whether such maternal effects can also explain the constrained sex allocation in mated females of haplodiploid species more widely. Addressing this in haplodiploid taxa, other than Hymenoptera, can help elucidate the generality of maternal effects on sex allocation and whether constrained sex allocation is an evolutionary adaptive strategy resulting from fitness constraints limiting the reproductive effort.

Given that haplodiploidy has independently evolved in different arthropod taxa (Otto and Jarne 2001), study of sex allocation in other orders than the more commonly studied Hymenoptera may help test the generality of the underlying mechanisms and factors that constrain sex allocation. Thrips (Thysanoptera) are alternative attractive models for haplodiploidy (Crespi 1992), including for sex allocation studies, yet not many sex allocation studies have been performed on thrips. In this study, we examined evidence for constrained sex allocation in

Kelly’s citrus thrips, *Pezothrips kellyanus* (Thripidae). This species has a bimodal sex ratio pattern, with some females being constrained to produce male-biased broods (M-broods), while unconstrained females produce female-biased broods (F-broods) (Nguyen 2015; Katlav et al. 2021; Chapter 2). In addition, in this species, plasticity in maternal investment at the egg stage not only determines offspring body size but also drives an egg size-mediated fertilization mechanism, with a higher chance of fertilization of larger than smaller eggs (Katlav et al. 2021). This raises the question whether constrained sex allocation depends on the maternal condition via the egg size. However, alternative reasons for constrained sex ratio patterns linked to other factors, such as insemination failure, paternal SGEs or paternal fitness must also be considered (Fig. 3.1).

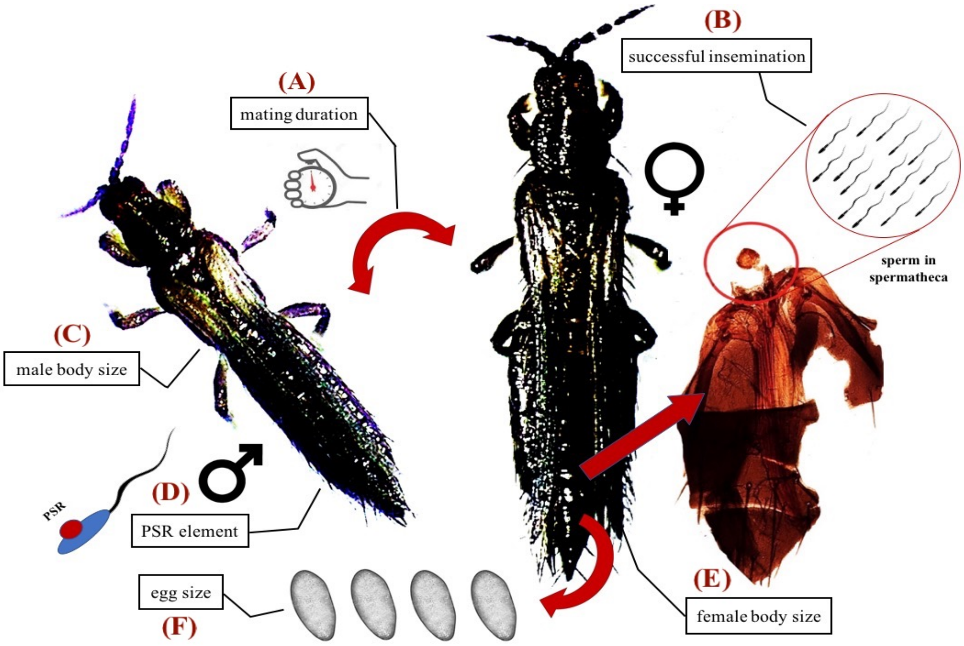


Figure 3.1 Potential factors that can be responsible for constrained sex allocation (production of M-broods) in haplodiploid species can be mating-associated, paternal or maternal: (A) mating duration; (B) insemination success (presence of motile sperm in the dissected spermathecae of mated females); (C) male body size (forewing length) as important proxy of paternal fitness constraining sex allocation; (D) paternal sex ratio (PSR) elements; (E) female body size (forewing length); (F) egg size as constraining factor for fertilization success because of an egg size-mediated fertilization mechanism.

Here, we first conducted crossing experiments and tested whether constrained sex allocation in females (M-brood production) is due to unsuccessful insemination. We then assessed if it is associated with paternal effects. For this, we examined if the constrained sex allocation (M-brood production) is paternally inherited, and, therefore, potentially due to a PSR factor. Then we evaluated whether male body size and mating duration have an effect. Finally, we tested if constrained sex allocation depends on the maternal condition and operates via the egg size-mediated fertilization mechanism recently demonstrated in this species (Katlav et al. 2021; Chapter 2).

3.3 METHODS

3.3.1 Thrips maintenance

A laboratory population of *P. kellyanus* (Figure 3S1) was established using field-collected individuals from Kulnura, New South Wales, Australia in 2017. Since then it has been maintained on organic lemons (following the protocol described in Chapter 2) for 15 generations. This laboratory population was fixed (100% infected) with one strain each of the two bacterial endosymbionts *Cardinium* and *Wolbachia*, which also occur at high prevalence in Australian field populations (Nguyen et al. 2016). While these bacteria have very high maternal transmission rates (>90%) in *P. kellyanus*, they can independently induce cytoplasmic incompatibility (CI) in matings between infected males and uninfected females, resulting in male-only offspring (Nguyen et al. 2017). Therefore, to avoid CI effects, and to best represent the field situation, the coinfection status of the experimental population was verified by diagnostic PCR (Nguyen et al. 2016) before each experiment, using 20 randomly selected individuals of each sex, and subsequently, for all insects used in crossing experiments. For each experiment, we collected offspring of same-aged parental females and males reared at the same larval density (see Appendix I).

3.3.2 Mating effectiveness and dissection of spermathecae (experiment 1)

Twenty five newly emerged virgin females were randomly selected from same-aged parents. Twenty were individually mated once with one randomly selected male in a sealed Petri dish, while five remained virgin (Supplementary Methods). After mating they were isolated in oviposition chambers consisting of covered agarose gel plates (0.6% w/v) and supplied with one sprinkle of *Typha* sp. pollen and a piece of absorbent cotton wool soaked with honey solution (50% w/v; Figure 3S2A-B). Females were monitored daily until a first egg clutch was produced. Soon after oviposition females were removed, immobilized on ice for 10 seconds, their spermatheca dissected and transferred to a concave microscope slide with a drop of Ringer's solution and topped with a coverslip. The maximum diameter of each spermatheca was measured in the uncrushed state to subsequently compare between constrained and unconstrained mothers; this was done under a 100X 1.25 oil-immersion objective of a phase-contrast light microscope (Zeiss Axio Scope A1, Germany). Then the spermatheca was crushed and examined for the presence of motile spermatozoa. The spermathecae of the five virgin females were included as control. Once eggs hatched on the agarose gel plates (after 6-7 days), all larvae of each mother were carefully transferred to one lemon using a fine brush and maintained in a small container for development (Chapter 2). Upon emergence of adults, the sex ratio was recorded.

3.3.3 Test for the presence of a PSR element (experiment 2A)

To test for presence of a PSR factor, we conducted a crossing experiment that ran over two generations. For the first generation, a set of newly emerged F_0 females (1 to 2 days old) was kept as virgins (n = 16) and others were singly mated (n = 26) with non-sibling F_0 virgin males (2 to 3 days old). Virgin and singly mated females were individually transferred to small containers. Each container had a lemon on which cattail pollen (*Typha* sp.) was sprinkled every second day and each female was allowed to oviposit on the lemon for 10 days (Figure 3S2C-D; Figure 3S4). Upon emergence of all adult offspring (F_1), the offspring sex ratio was recorded for each mother. Given that PSR elements are paternally inherited, we hypothesized that (1) virgin females should only produce non-PSR males, and subsequent crosses between these hypothetically non-PSR males and females should result in F-broods; (2) male offspring of constrained females are hypothetically PSR males and subsequent crosses between them and females should result in M-broods; (3) if this is so, unconstrained females producing F-broods should have mated with non-PSR males (Figure 3.2). Males with an offspring sex ratio of 80-100% were categorized as potential PSR males to cover a possible imperfect transmission of the potential PSR element to fertilized eggs (Hunter et al. 1993; Werren & Stouthamer 2003).

In the second generation (F_1) of the experiment, we established eight families. Each family comprised eight randomly sampled sibling virgin females (to control for any female genetic effects): four females were individually mated with sibling males of a virgin mother (to control for any male genetic effects), and the other four females were individually mated with sibling males of a constrained mother (Table 3.1). After mating, males were removed and singly mated females were allowed to oviposit individually on a lemon for 10 days as described above, and their offspring sex ratio was recorded upon emergence of all adults.

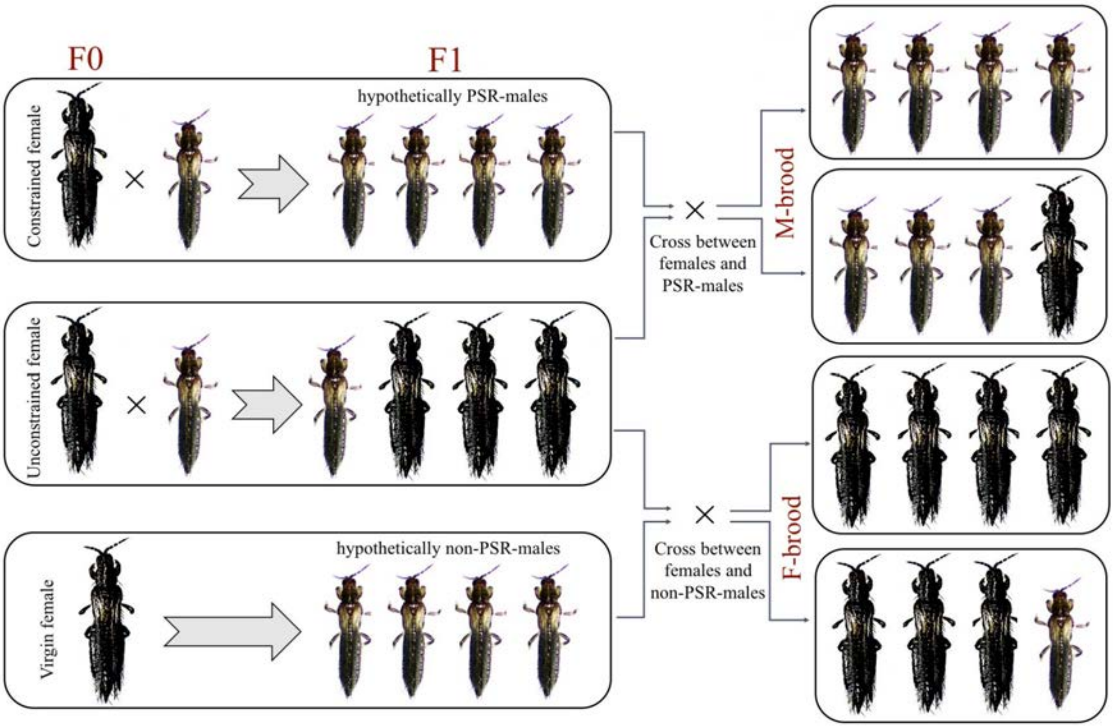


Figure 3.2 PSR crossing experiments over two successive generations for evaluation of inheritance patterns of M-brood production in *Pezothrips kellyanus*. F_0 mothers are either singly mated or kept virgin. Under the scenario of PSR-regulated constrained sex allocation, it is hypothesised that F_1 males produced by constrained mated mothers are PSR-males and subsequent crosses between them and F_1 females (produced by unconstrained females) should result in male-biased/male-only (M-brood) production; whereas F_1 males produced by virgin mothers are non-PSR-males and subsequent crosses between them and F_1 females should result in female-biased/female-only (F-brood) production.

3.3.4 Mating time and male size measurements (experiment 2B)

As part of the PSR experiment, the mating of each individual pair was monitored under a stereo-microscope and the duration was recorded using a stopwatch. After mating, males were individually transferred to 0.5 ml microcentrifuge tubes and stored at -20 °C for subsequent measurement of the average length of both forewings (excluding fringes) of each male as a reliable proxy of body size (Nakao 1993). For this, we removed and spread the forewings on a glass slide topped with a coverslip under a binocular Leica DMRB microscope, photographs were taken with a Leica Quantimet 500c camera (Cambridge, UK) and the length was measured using Image-Pro 6.0 software (TIANGE, Ltd, USA). Subsequently, we evaluated the probability of constrained sex allocation (M-brood production) as a function of average male wing size and mating time using a mixed logistic regression with “generation order” as a random factor.

3.3.5 Maternal fitness assay (experiment 3)

Katlav et al. (2021) have previously found that female fitness influences egg size and therefore fertilization success (Chapter 2). Consequently, female fitness could also affect constrained sex allocation. Therefore, we examined if egg size of a female before mating can predict the offspring sex ratio after mating. To test this, 60 newly emerged females (1 to 2 days old) were isolated in small Petri dishes (25 mm diameter) for five days with food supply renewed every second day to ensure sufficient egg load and induce simultaneous oviposition in examined females (Chapter 2). Then, females were allowed to oviposit on plates with agarose gels (0.6% w/v) for 24 h. A total of 45 females simultaneously laid eggs within this time. Four to six eggs per female (~40% of total eggs per female on that day) were randomly selected for size measurement on the day of oviposition. Egg volume was calculated following the method described by Katlav et al. (2021) (Chapter 2). Subsequently, each female was individually mated with a randomly selected virgin male in a copulation chamber. Mating behaviour was observed and, soon after, females were individually transferred to a lemon and allowed to oviposit for 10 days. Upon emergence, the adult offspring number and sex ratio was recorded for each mother. Mothers were assigned to have either M-broods or F-broods and subjected to the measurement of both forewings and the average length was calculated as a proxy of body size. We evaluated the probability of constrained sex allocation (M-brood production) of mated females as a function of the average egg size using a logistic regression model with the total offspring number incorporated as a covariate. The same analysis was conducted with mother average forewing length used as a covariate to examine if egg size-mediated constrained sex allocation is a function of maternal size. Finally, to account for variances, we analysed the egg size between the mother types and incorporated “mother ID” as a random factor.

All analyses were executed in R 3.5.1 (R Core Team 2018). For mixed logistic regression analyses, we used a generalized linear model (*glmer* function) with (“family = binary”; α = 0.05) in the “*lme4*” package. For analysis of variances we conducted a GLM one-tailed F test (*lmer* function) using the “*lme4*” and “*car*” packages.

3.4 RESULTS

3.4.1 Mated constrained females are successfully inseminated

The dissection of spermathecae (Figure 3S3) revealed motile sperm in all mated females (n= 20), but not in virgin females (n= 5). Nine mated females produced F-broods in the first clutch (sex ratio 0-9%), and 7 of these produced only female offspring. In contrast, 11 mated females produced M-broods (sex ratio 87-100%), among which 9 produced only male offspring despite having received abundant motile sperm. These constrained females had a marginally smaller spermatheca (diameter ± SD: 144.1 ± 10.72 μm in constrained *vs* 159 ± 20.53 μm in unconstrained females; $F_{1,20}$ = 3.43, *P*= 0.051, one-tailed test).

3.4.2 M-brood production is not paternally inherited

To investigate the inheritance of M-brood production we assessed the offspring sex ratio of lineages over two consecutive generations. In the first generation, 13 of 16 virgin and 22 of 26 mated females produced offspring. All 13 virgin mothers produced only male offspring. However, among the mated mothers, nine produced M-broods (constrained mothers) and the rest produced F-broods (unconstrained mothers). In the second generation, approximately 63% of females mated with males of constrained mothers produced F-broods and about 40% of females mated with males produced by virgin mothers produced M-broods (Table 3.1; Figure 3.3). Therefore, M-brood production was not consistent with a PSR-regulated non-Mendelian inheritance pattern. Furthermore, the prevalence of M-brood production was not different between matings that involved males produced by virgin and unconstrained mothers.

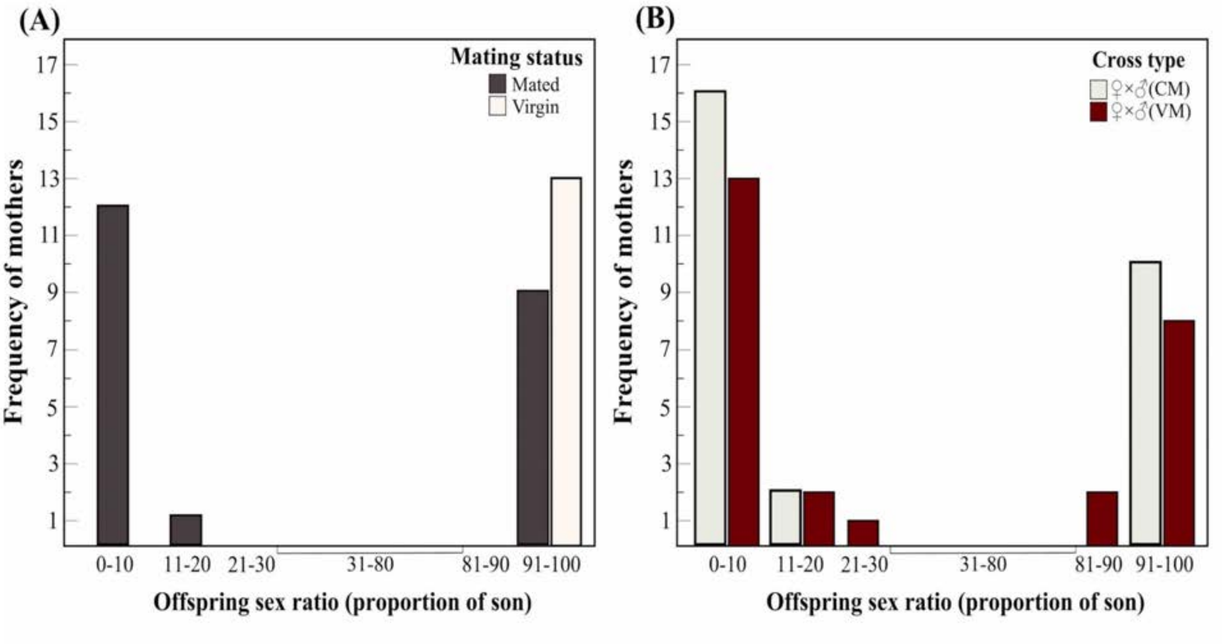


Figure 3.3 Offspring sex ratios in PSR crossing experiments. (A) Offspring sex ratios of F_0 virgin (n = 13) and mated (n = 22) females. (B) Offspring sex ratios of F_1 females mated with males of constrained mated mothers [♀×♂(CM)] (n = 28) and F_1 females mated with males of virgin mothers [♀×♂(VM)] (n = 26).

Table 3.1 Offspring sex ratio presented as % of male offspring of the total offspring, as produced by different families of the second generation (F_1) of the PSR test. Each family comprised eight crosses (♀×♂): eight randomly selected sisters (F), four mated with sib sons of a constrained mated mother (CM) and the other four mated with sib sons of a virgin mother (VM). Dashes denote unavailable sex ratio data in crosses where the female died prior to offspring production.

NO.	♀×♂	Fam. 1	Fam. 2	Fam. 3	Fam. 4	Fam. 5	Fam. 6	Fam. 7	Fam. 8
1	F × CM	5.9	100	0.0	100	5.6	97.7	-	100
2	F × CM	100	8.8	-	0.0	100	0.0	1.5	14.3
3	F × CM	7.1	0.0	2.1	100	92.6	12.5	7.7	3.3
4	F × CM	0.0	-	100	93.9	0.0	-	0.0	-
5	F × VM	0.0	98.1	0.0	87.5	18.6	21.7	94.4	10.5
6	F × VM	0.0	0.0	2.3	-	100	2.3	100	84.3
7	F × VM	-	-	94.4	0.0	0.0	0.0	9.1	5.3
8	F × VM	100	0.0	1.9	100	92.3	-	100	0.0

3.4.3 Sex allocation is not constrained by mating duration and male size

We examined the effects of mating duration and male size on sex allocation across the 1st and 2nd generation of the PSR experiment. Neither mating duration ($F_{1,90} = 2.06$, $P = 0.15$) nor male size ($F_{1,88} = 0.80$, $P = 0.37$) affected the probability of M-brood production. The results were the same when analysis was conducted on the 2nd generation separately with the inclusion of "family ID" as a random factor (mating duration, $F_{1,64} = 3.02$, $P = 0.082$; male size, $F_{1,62} = 0.003$, $P = 0.95$).

3.4.4 Constrained sex allocation is associated with maternal condition

We examined if constrained sex allocation after mating is attributable to maternal effects such as body size and resource allocation towards eggs. We found that females with smaller average egg size were more likely to produce M-broods ($r^2 = 0.41$; $p = 0.0005$; Figure 3.4a). Furthermore, prior to mating, unconstrained females produced larger eggs than constrained females ($F_{1,225} = 12.25$; $p < 0.001$; Figures 3.5 and 3S5). This was irrespective of the total offspring number, which was similar in constrained and unconstrained females ($F_{1,46} = 0.2047$; $p = 0.651$). The model effect size of the model was slightly higher when "mother forewing" was included as a covariate in the analysis of sex allocation; however, the effect of egg size was reduced although remained still significant ($r^2 = 0.44$; $p = 0.012$). This is due to the positive correlation between maternal body size and egg size ($r^2 = 0.47$; $p < 0.001$), suggesting that constrained sex allocation is a function of maternal body size ($r^2 = 0.31$; $p < 0.001$; Figure 3.4b).

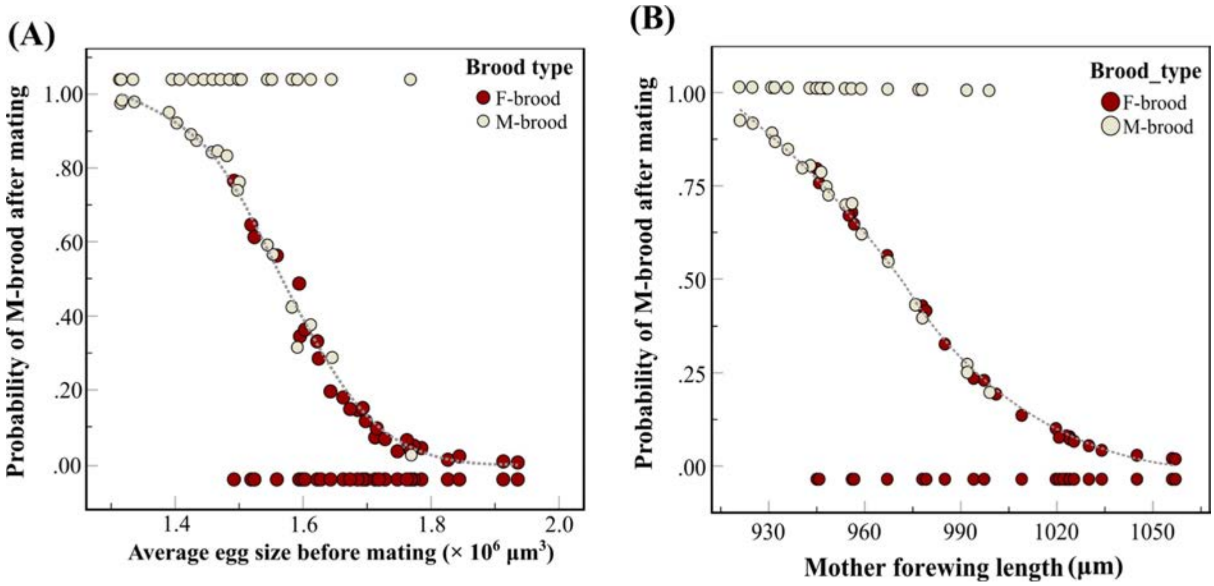


Figure 3.4 Correlation between maternal fitness effects and probability of constrained sex allocation after mating (A) Mixed logistic regression of average egg size before mating and the probability of constrained sex allocation (M-brood production) after mating. (B) Mixed logistic regression between maternal body size (forewing length) and the probability of constrained sex allocation (M-brood production) after mating. The circles on the curve correspond to predicted values and those on top/bottom to observed values.

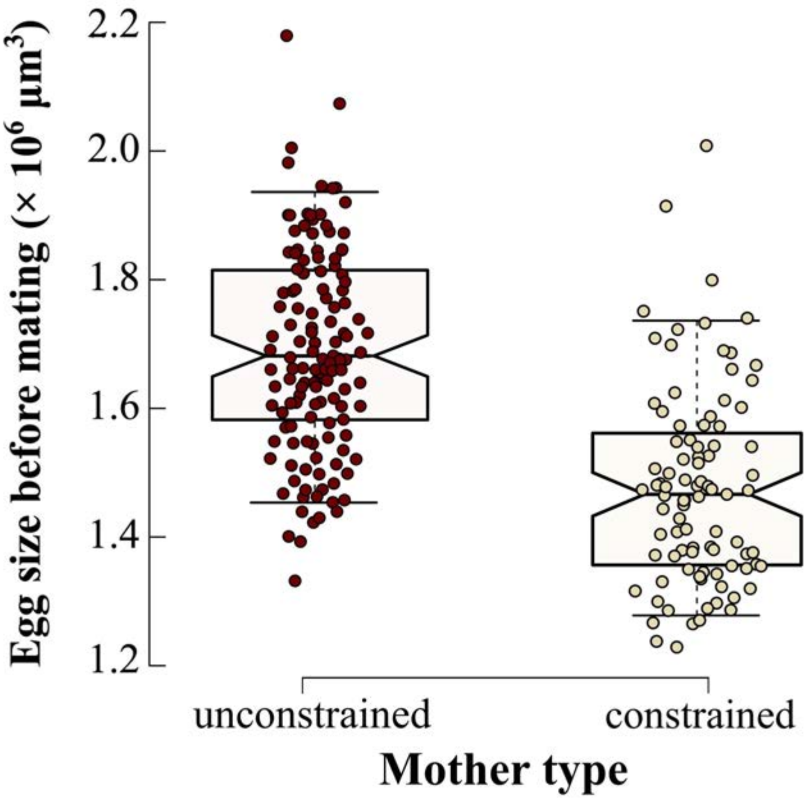


Figure 3.5 Egg size difference before mating between constrained and unconstrained mothers, represented as egg volumes in box plots with a jittered dot-plot overlay (error bars correspond to 95% confidence limits).

3.5 DISCUSSION

Our study of *P. kellyanus* revealed patterns of constrained sex allocation whereby not all mating events secured female production. A similar phenomenon is also observed in birds and mammals where post-conception mortality of one sex may constrain mothers in sex ratio adjustment, and selection may favour compensatory adaptations by females such as sex-biased abortion or sex-biased infanticide to mitigate such biases (Frank 1990). However underlying mechanisms and/or factors constraining sex allocation have only been investigated in relatively few haplodiploid arthropod species. We tested whether this constrained sex allocation in mated females is due to paternal or maternal factors (Figure 3.1) previously demonstrated to be responsible for constrained sex allocation in some hymenopterans (Godfray 1990; Henter 2004). First, we found that copulation leads to effective sperm transfer. Then, our experiments found no evidence for paternal effects such as PSR and male size and mating time, which might affect sperm transfer. However, we found that constrained sex allocation is related to maternal condition (body size) which influences egg size – a trait that is important for fertilization success in this species (Katlav et al. 2021; Chapter 2). Larger females produce larger eggs, and larger eggs have a greater chance of being fertilised. Consequently, larger females produce F-broods and smaller females M-broods. This result can be interpreted under both non-adaptive and adaptive scenarios: 1) constrained sex allocation could be associated with a lower energy budget available to smaller females for egg provisioning, and, thus, could constitute a non-adaptive by-product of female condition; 2) alternatively, it may be an outcome of female adaptive control over egg size, but not number, in response to their condition, because smaller females produce many smaller eggs rather than fewer larger eggs, suggesting that constrained females are selected to produce many males rather than few females. Furthermore, the strongly bimodal pattern of sex allocation across individuals to either produce F-broods or M-broods suggests that there are trade-offs at population levels that prevent the manifestation of more gradual variation patterns in sex ratio bias across individuals.

3.5.1 Neither mating nor paternal effects on constrained sex allocation in *P. kellyanus*

The dissection of spermathecae revealed motile sperm in all constrained mothers, even those producing male-only offspring. Therefore, constrained sex allocation appears not to be due to insemination failure. However, we did not quantify ejaculate size and quality. Several studies of other arthropod taxa have shown that males may vary in sperm quality (Hunter & Birkhead 2002). Moreover, males may differ in the sperm accessory compounds that influence ejaculate size, sperm motility and viability (Gillot 2003; Katvala et al. 2008). If variation in sperm ejaculate size and quality was responsible for the constrained sex allocation, then we would have expected to see a more gradual variation of sex ratios rather than the extremely male-biased (male-only in some cases) production. Sometimes failure in fertilization may result from blockage of the spermathecal duct due to the ejaculates of several mating events (see Godfray 1990). Moreover, some studies have shown that males can become sperm depleted over time and may lose sperm quality due to ageing (Godfray 1990; Liu et al. 2020). However, these cases do not apply to our study given that all the males used in our crossing experiments were young, virgin and only mated once with virgin females.

Body size is generally a reliable proxy of male reproductive fitness in terms of pre-copulatory and post-copulatory mating success (Simmons et al. 1996). Larger males can inseminate more females than smaller ones (Parker & Simmons 1994) and may also have higher fertilization rates by transferring more sperm in a shorter mating event (Simmons et al. 2001; Bangham 2002). However, such patterns are quite variable across different species. For example, in some damselflies correlation between body size and mating duration (and ensuing fertilization success) is frequency–dependent (Wong Muñoz et al. 2013). In some hemipterans, smaller males tend to mate longer during which they transfer the same ejaculate size as larger males do in a shorter period, leading to a similar overall reproductive success among differently sized males (Danielsson 2001). We found that constrained sex allocation in *P. kellyanus* was independent of male body size. Moreover, mating duration was not influenced by male body size, and did not affect offspring sex ratio. Therefore, our results provide no evidence for such paternal factors affecting sex allocation.

3.5.2 Constrained sex allocation after mating in *P. kellyanus* is not due to PSR elements

In some species, males may pass on SGEs that can cause sex ratio distortion (Stouthamer et al. 2002). The most extreme examples in haplodiploids are paternally inherited PSR elements that can convert a fertilized embryo into a haploid male due to paternal genome elimination (Werren and Stouthamer 2003). However, we found that M-brood production in *P. kellyanus* was not consistent among females that were mated with males of M-broods. More than half of these females were still able to produce F-broods, which is inconsistent with the model of M-brood lineages that produce PSR males (Figure 3.2). This sex ratio pattern, however, would not immediately exclude involvement of PSR because it is not always fully expressed, as shown in *Encarsia pergandiella* (now *Encarsia marthae* Gebiola et al. 2017) in which almost 40% of females mated with potential PSR males still produced female-biased offspring (Hunter et al. 1993). The most likely explanation for this is either leaky inheritance of the PSR factor or counter adaptations of the host genome against this SGE (Hurst et al. 1996). This may be achieved by a female’s decision to lay some or all of her eggs unfertilized to reduce the spread of a PSR element in the population, because haploid males do not inherit paternal traits. However, in our study ~40% of females mated with males from virgin mothers also produced M-broods. This contradicts expected PSR patterns that should only be seen in lineages deriving from PSR fathers due to the paternal inheritance of PSR (Figure 3.2).

3.5.3 Maternal factors affect constrained sex allocation

Maternal condition may influence adaptive sex allocation (Charnov 1982), and can often cause sex ratio skews in populations (Nager et al. 1999; Bosch 2008). We showed that constrained sex allocation in *P. kellyanus* depends on maternal body size, a common proxy for fitness (Fox & Mousseau 1998). As predicted by the sex allocation theory of Trivers and Willard (1971), smaller females may suffer from fitness deficits that constrain their reproductive output.

Smaller body size may limit the available energy budget for egg provisioning (Seidelmann et al. 2010). A similar correlation between maternal size and resource investment is known for some solitary bees, in which smaller females have limited cell provisioning ability, and thus shift their sex allocation towards sons, because males require fewer resources for development (Bosch and Vicens 2006; Seidelmann et al. 2010). In *P. kellyanus*, lower fitness of constrained mothers is reflected in their smaller body size, which limits egg provisioning. This effect might be exacerbated by a size-scaling constraint in the reproductive anatomy of the female (Rönn et al. 2011) including smaller ovaries (Berrigan 1991) and spermatheca (present study). These factors may also limit the access of sperm to smaller eggs, as egg size in *P. kellyanus* determines fertilization success (Katlav et al. 2021; Chapter 2).

In a previous study it was found that constrained mothers produced a similar number of eggs and adult offspring to unconstrained mothers (Katlav et al. 2021; Chapter 2). This may imply that M-brood production in constrained females is not due to immature mortality of daughters, biasing the secondary sex ratio towards males, but due to a lower fertilization rate (primary sex ratio). However, variation in mother size and egg size cannot alone justify the strong bimodal pattern of M and F brood production. An alternative reproductive strategy for constrained mothers could be to produce fewer eggs without loss of egg size, but we found no effect of mother size on egg number, while there was an effect on egg size. This may also suggest an adaptive maternal strategy in which females adjust their offspring sex ratio in response to their energy budget. Hence, changes in egg size may also be a consequence of the adaptive decision to avoid unnecessary resource investment (Carranza 2002). Mothers should invest sufficient resources into eggs to ensure not only fertilization but also survival of fertilized eggs (Katlav et al. 2021; Chapter 2). Therefore, fertilization of small eggs by constrained mothers could waste resources because it would lead to the failed development of daughters, whereas small eggs can support successful development of sons. Although production of unfertilized eggs is not preferable, it is still advantageous over complete reproductive failure (as in diplodiploid species). This is because in haplodiploids, females generally receive an immediate fitness increment via offspring production, even if it is less favourable excessive male production (Godfray 1990).

3.5.4 Evolution of post-mating constrained sex allocation

There may be a sexual conflict over offspring sex ratios in haplodiploids because mothers pass on their genes to sons and daughters, but fathers only transmit their genes to daughters (Shuker et al. 2009). Therefore, selection is expected to favour male traits that enhance the fertilization rate (Hawkes 1992). One profound effect of mating is that it triggers higher resource allocation towards individual eggs to ensure fertilization and survival of females (Katlav et al. 2021; Chapter 2; Macke et al. 2012). On the other hand, constrained sex allocation by mated females may be a consequence of the sexual conflict over sex allocation that drives a female to act antagonistically against male attempts to increase daughter production. This is well demonstrated in the lower resource allocation of constrained *P. kellyanus* females even after mating compared to unconstrained females (Katlav et al. 2021; Chapter 2). This may suggest that the incremental effect of mating on maternal investment is activated only in larger females, which enjoy more resources for egg provisioning as reflected in their egg sizes even prior to mating.

Alternatively, the bimodal sex ratio pattern in *P. kellyanus* may have evolved as a cooperative strategy to help balance the population-wide sex ratio by female individuals adaptively embarking on either reproductive mode depending on the fitness cost of female production for them. Therefore, this may rather ameliorate the sexual conflict over the optimal sex ratio, analogous to split sex ratios in some ants (Bourke & Chan 1994). This pattern is also similar to the split sex ratio occurring in some halictid bees, with social nests that are female-biased and solitary nests that are male-biased (Smith et al. 2019).

According to the equal sex ratio theory, commonly attributed to Fisher (1930), yet originally conceptualised by Carl Düsing (1884), in a panmictic population, natural selection would favour the evolution of equal investment towards male and female offspring (also see Edwards 1998). In our study, under whatever (non-mutually exclusive) selection pressures suggested above, M-brood production by constrained *P. kellyanus* females may be an evolutionary stable strategy to incline population sex ratio towards a balanced status near the Fisherian equilibrium (Fisher 1930). Such a split sex ratio may reduce the probability of sib-mating as both sexes require dispersing from their natal patch to find a mate (Crespi 1993). Under this scenario, the average relatedness between mothers and their diploid daughters is reduced. Therefore, it is likely that the extremely female-biased production by unconstrained females has arisen in response to the excess male production by constrained females (Godfrey 1990). Alternatively, constrained sex allocation might be an adaptive population-level response to female-biased offspring sex ratios of unconstrained females. The Fisherian theory of sex ratios predicts that an equilibrium sex ratio should be reflective of equal investment in males and females. As we demonstrated that larger female eggs are more costly, the equilibrium primary sex ratio in this species may be male biased. Furthermore, these different strategies might have evolved together. This remains to be further verified by studying the sex ratios of field populations.

3.6 CONCLUSIONS AND FUTURE DIRECTIONS

Our study demonstrates the importance of maternal fitness for sex allocation in a haplodiploid thrips. We found that female body size can constrain the egg size-mediated fertilization process recently established as a mechanism of sex allocation in haplodiploidy (Macke et al. 2011;

Katlav et al. 2021). Smaller *P. kellyanus* females were constrained to produce smaller eggs which remained unfertilized after mating and resulted in a male-biased offspring production. This sex allocation pattern could be an adaptive maternal strategy, if not only an outcome fitness constraint, in which a female may adjust her offspring sex ratio in response to her available energy budget; yet this remains to be confirmed by further experiments.

Our findings further raise the question of why constrained females mate at all if they are destined to produce only sons. One possible reason is to avoid the cost of injury or harassment by males at courtship (den Hollander and Gwynne 2009), another possible reason is a cryptic fitness benefit of mating (Head et al. 2005). Currently, our findings are based on singly mated females, so the effects of multiple mating on sex allocation still need to be tested. In some species, the ejaculate of a first mating is not sufficient to switch on the fertilization of eggs in a female (Lederhouse 1981). However, this might not be the case for *P. kellyanus* given that in our study single mating warranted successful insemination and fertilization in unconstrained females. Moreover, constrained sex allocation may be a driving factor in the evolution of assortative mating (Crespi 1989a), given that constrained females may not be a promising partner for a male to pass on his genes. It will be important to test whether males in *P. kellyanus* prefer females that are larger (Crespi 1989a). Moreover, constrained sex allocation may drive evolution of sexual selection favouring male factors that boost mating success with unconstrained females (Andersson and Simmons 2006). Therefore, follow-up studies on the mating biology of *P. kellyanus* should examine whether male preference interacts with female phenotypic variation in a way to mitigate constrained sex allocation. Finally, how constrained sex allocation interacts with abiotic (e.g. temperature) and biotic (e.g. endosymbionts, population density, diet and host plants) factors remains to be tested in future studies.

3.7 APPENDIX II

3.7.1 Supporting information

3.7.1.1 Supplementary methods: Thrips laboratory population establishment and maintenance

A laboratory population of *Pezothrips kellyanus* was established using individuals collected from lemon flowers in a citrus orchard, Kulnura, New South Wales, Australia in mid-January 2017. This laboratory population was fixed (100% infected) with one strain each of the bacterial endosymbionts *Cardinium* (*c*Pkel1) and *Wolbachia* (*w*Kelly), which also occur at high prevalence in Australian field populations (Nguyen et al. 2016; Stouthamer et al. 2019). The stock culture was obtained by mixing offspring of females that were positive for both endosymbionts, and this resulted in a population fixed (100%) for both endosymbionts (Katlav et al. 2021). Prior to the experiments, this population (Figure 3S1) was maintained for 15 generations at 20±2°C, 70±2% RH and 16:8 h (L:D) photoperiod in plastic containers (22cm L × 20cm W × 10cm H) containing vermiculite as a pupation substrate on the bottom and 9 to 11 organic lemons on which fresh pollen of *Typha sp.* was sprinkled as an additional food source every second day (Nguyen et al. 2017).

3.7.1.2 Establishment of same-aged parental females and males

We randomly sampled 150 females from the stock population and transferred them as six separate cohorts of 25 females each on individual similar-sized fresh high-quality lemons placed in a small plastic container (700mL; diameter 10cm; height 11.5cm) with a small filter paper on the bottom. Females were removed after they had laid eggs for 24 hours. After emergence of larvae we restricted the number of larvae to 40 to 45 per lemon to ensure equal larval density. The thrips larvae were kept on the individual lemons supplied with ad libitum

Typha sp. pollen every second day until pupation when the different sexes are readily recognizable (Katlav et al. 2021). From each cohort, female and male pupae were separated and placed into small Petri dishes (diameter 30 mm; height 15 mm) with moistened filter paper and kept until adult emergence. Newly-emerged females (1 to 2 days old) were randomly selected and either retained as virgins or individually transferred to a copulation chamber, a small Petri dish (25 mm diameter) supplied with one randomly selected virgin male each (2 to 3 days old). The mating event was monitored and the duration was recorded. Virgin and mated females were kept individually in small Petri dishes labelled with unique ID numbers.

3.7.1.3 PCR diagnosis for verification of endosymbiont infection

To avoid the confounding effect of cytoplasmic incompatibility induced by *Cardinium* and *Wolbachia* (Nguyen et al. 2017), and to best represent the field situation, the co-infection status of the experimental population was verified by diagnostic PCR before each experiment using 20 randomly selected individuals of each sex, and subsequently, for all examined individuals in crossing experiments. DNA extraction was conducted using a 5% Chelex solution (10 mM Tris-HCl; pH 8.0) following the method explained by Kobayashi et al. (2013). Standard PCR techniques (Nguyen et al. 2016) were used to test whether each thrips individual was infected with *Cardinium* and/or *Wolbachia* using *Cardinium*-specific and *Wolbachia*-specific primers (Table S1). The DNA quality of the individuals was tested by amplifying COI (Table S1).

3.7.2 Supplementary table

Table 3S1 Primer sequences used in this study for PCR assays.

Primer name	Primer sequence (5’ -3’)	Target gene	Reference
81F	TGGTCCAATAAGTGATGAAGAAAC	*wsp*	Zhou et al. 1998
691R	AAAAATTAAACGCTACTCCA	*Wolbachia*	
Wsp for	TGGTCCAATAAGTGATGAAGAAACTAGCTA	*wsp*	Jeyaprakash and Hoy 2000
Wsp rev	AAAAATTAAACGCTACTCCAGCTTCTGCAC	*Wolbachia*	
CLOf1	GGAACCTTACCTGGGCTAGAATGTATT	*16S rDNA*	Duron et al. 2008
CLOr1	GCCACTGTCTTCAAGCTCTACCAAC	*Cardinium*	
LCO1490	GGTCAACAAATCATAAAGATATTGG	*COI*	Folmer et al. 1994
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA		

3.7.3 Supplementary figures: *Figures 3S1-3S5*

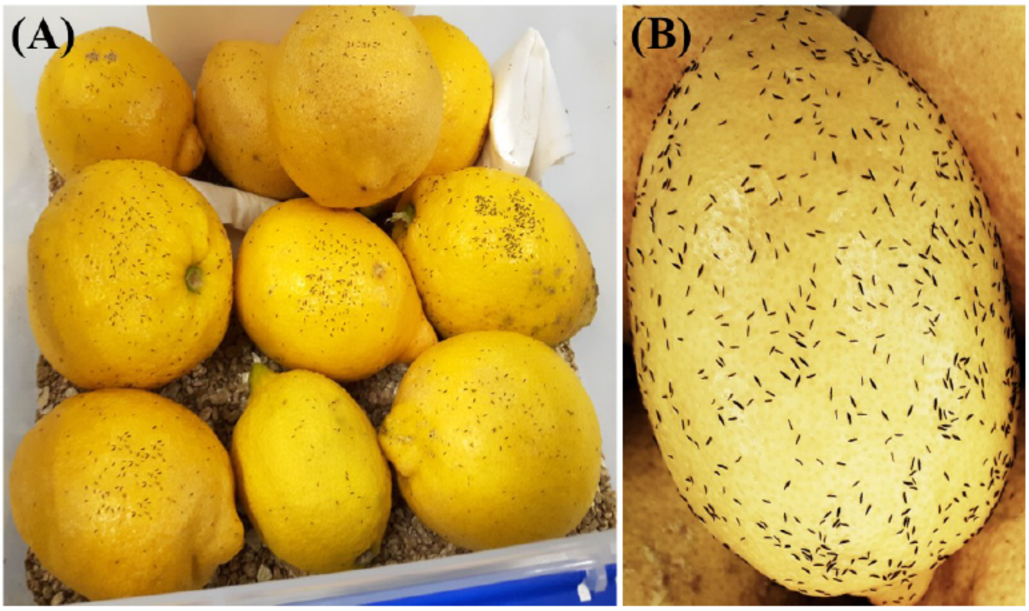


Figure 3S.1 Stock laboratory population of *Pezothrips kellyanus* reared on organic lemons. A) Lemons in larger rearing box. B) Thrips congregating on a fresh lemon.

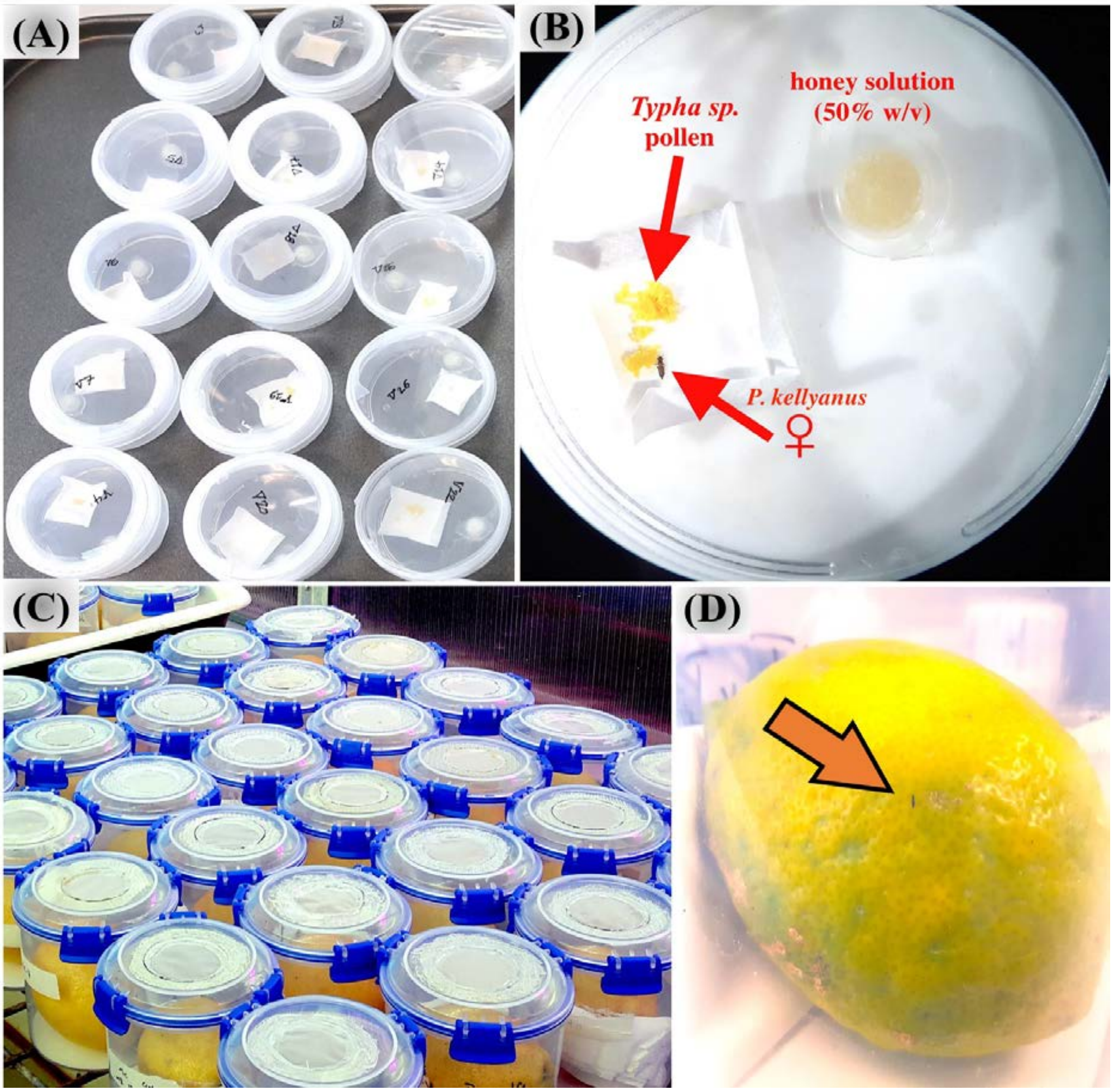


Figure 3S.2 Overview of oviposition chambers and small lemon containers used in the crossing experiments. A) Oviposition chambers (diameter 35 mm; height 10 mm) tapped with agarose plates (0.6 % w/v; Agarose I, AMRESCO, USA) on top. B) Oviposition chamber supplied with absorbent cotton wool soaked with *Apis mellifera* honey diluted in distilled water (50% w/v) and cumbungi pollen (*Typha* sp.). C) Small containers with single organic lemons on which individual females were allowed to oviposit for ten days. D) Individual female in a small container containing a single lemon in larger view.

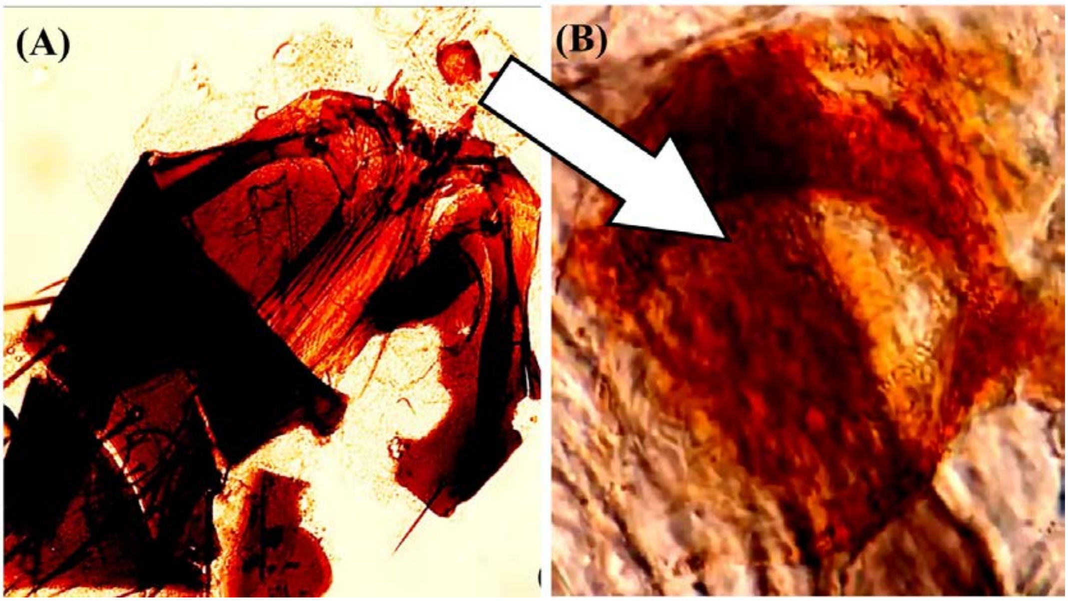


Figure 3S.3 Abdominal dissection of a mated female of *Pezothrips kellyanus*. A) The red and ball-like spermatheca is situated near the posterior region of the abdomen next to the base of ovipositor valves. (B) Squashed spermatheca with spermatozoa inside.

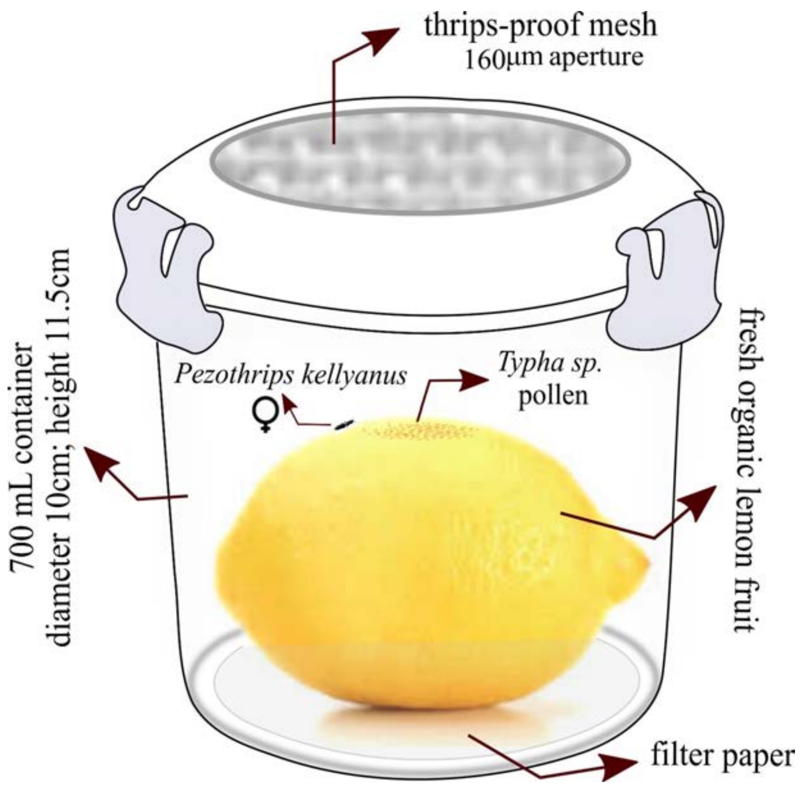


Figure 3S.4 Schematic view of a small container harbouring an individual *Pezothrips kellyanus* female which was allowed to oviposit on an organic lemon for ten days for offspring sex ratio assessment.

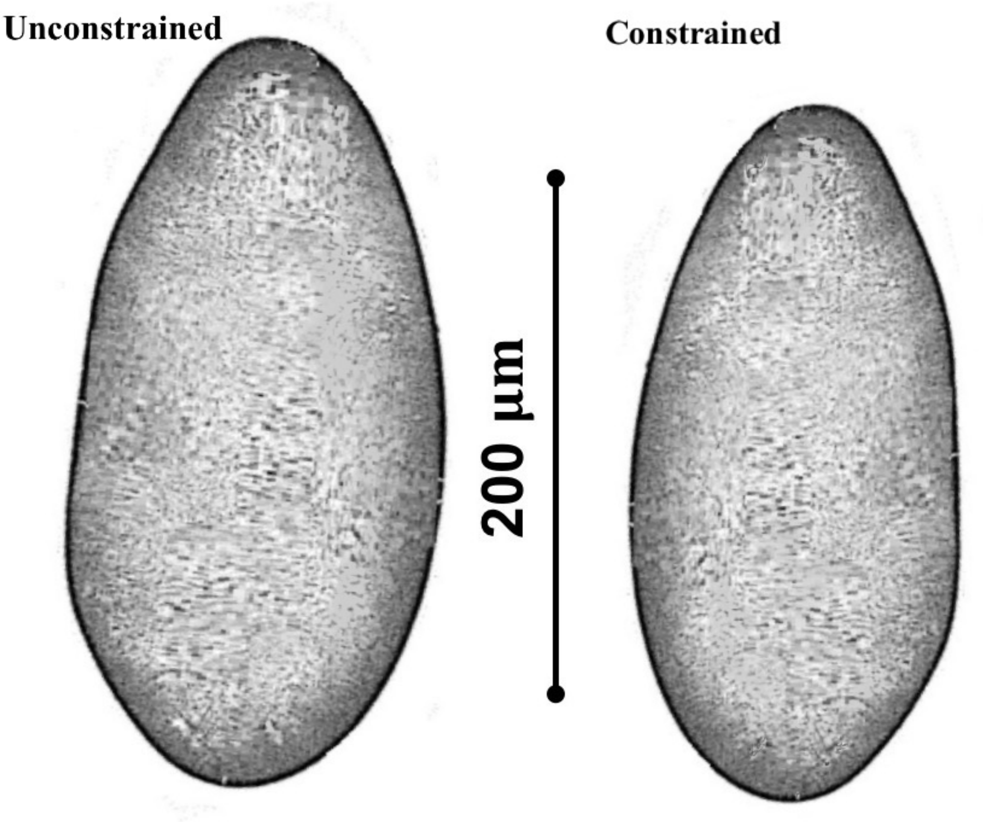
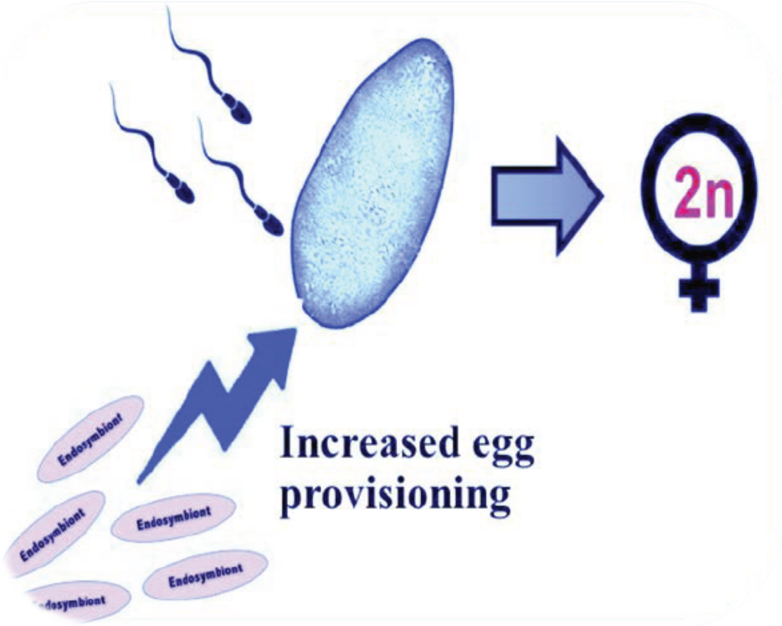


Figure 3S.5 Size comparison between eggs of unconstrained and constrained mothers. Left: an egg of a mother while still virgin, but producing female-biased brood after mating (unconstrained mother). Right: an egg of a mother while still virgin, but producing male-biased brood after mating (constrained mother).

CHAPTER IV

Endosymbionts affect sex allocation and fitness through egg-size manipulation in a haplodiploid host species



Endosymbionts can increase egg provisioning to enhance egg size-mediated fertilization rate in *Pezothrips. kellyanus*

Endosymbionts affect sex allocation and fitness through egg-size manipulation in a haplodiploid host species

4.1 ABSTRACT

Maternally inherited endosymbionts are ubiquitous among arthropods and engaged in a broad range of interactions with their hosts. The bacteria *Cardinium* and *Wolbachia* are the most dominant endosymbionts that spread in host populations by increasing female production in hosts either through reproductive manipulations or by improving overall host fitness. However, not much is known if they can also highjack a host's sex allocation system other than manipulating sex ratios post-zygotically. In haplodiploid host species females can adjust their offspring sex ratio by fertilization control, and endosymbionts may gain an opportunity to increase the proportion of diploid daughters relative to haploid sons by favouring higher fertilization rates. It has recently been established that sex allocation in haplodiploids can be mediated by egg size, with fertilisation of larger eggs being more likely than of smaller ones. Here we investigated whether *Cardinium* and *Wolbachia* can manipulate sex allocation in the haplodiploid thrips *Pezothrips kellyanus* by affecting egg size and therefore regulating egg provisioning. In this host species, most mated females produce female-biased broods, while some mated females are constrained to produce extremely male-biased broods. We found females harbouring *Cardinium* produced larger eggs than *Wolbachia*-coinfected females, which again produced larger eggs than uninfected females. This ensured higher fertilisation success and, therefore, resulted in a higher female bias in their broods when compared to other infection types. Beyond this, we demonstrated complex host fitness interactions between

Cardinium and *Wolbachia*, with indirect (via change in egg size) and direct impacts on offspring fitness in terms of survival rate, development time, and adult size and longevity. Overall, we found that *Cardinium* conferred fitness advantages whereas *Wolbachia* seemed to act antagonistically. These results suggest endosymbiont-specific adaptations to their different modes of reproductive manipulations and may also explain the previously reported higher prevalence of *Cardinium* than *Wolbachia* in *P. kellyanus* field populations.

Keywords

*Cardinium***,** *Wolbachia*, sex ratio distorter, fertilization success, invasion biology, egg size, fitness, cytoplasmic incompatibility

4.2 INTRODUCTION

Maternally inherited endosymbionts are pervasive among arthropods and play pivotal roles in host biology, population dynamics and evolution (Werren 1997; Duron et al. 2008; Hilgenboecker et al. 2008). Several also manipulate host reproduction and behaviour, as well as the evolution of mating systems (Charlat et al. 2003; Moran 2006; Tregenza & Wedell 2000; Engelstädter & Hurst 2009). *Wolbachia* and *Cardinium* bacteria are the most widespread endosymbionts and occur in 50% and 13% of all arthropod species, respectively (Weinert et al. 2015). The bacteria influence host fitness and life-history traits in variable fashions depending on both their own and host genotypes, as well as ecological and environmental factors (Zug & Hammerstein 2015). This has been well demonstrated for *Wolbachia*, which can have deleterious (or parasitic), indifferent or beneficial host effects (interactions). In the case of parasitism, the bacteria have evolved a variety of strategies to ensure their spread in the host population while their detrimental effects can persist (Fine 1975; Dedeine et al. 2003).

Since *Wolbachia* and *Cardinium* are vertically transmitted within host eggs, but not sperm, their spread across host populations is facilitated by favouring reproduction of infected over uninfected females (Turelli 1994; Werren 1997; Zchori Fein & Perlman 2004). This is generally achieved by manipulation of host reproduction (Stouthamer et al. 1999; Werren et al. 2008). Cytoplasmic incompatibility (CI) is the most common reproductive manipulation induced by both *Wolbachia* (e.g. Hoffmann et al. 1986; Bourtzis et al. 1996) and *Cardinium* (e.g. Hunter et al. 2003; Gebiola et al. 2016). In its simplest way, CI occurs when eggs of an uninfected female are fertilized with sperm of an infected male (Werren 1997), and leads to (i) increased mortality of all embryos in diplodiploid hosts (Werren et al. 2008), or (ii) increased mortality of female (fertilized, diploid) embryos (FM-CI) and/or haploidization of fertilized embryos and development of male offspring (MD-CI) in haplodiploid hosts (Bordenstein et al. 2003; Vavre et al. 2003). Consequently, infected females have a fitness advantage over uninfected females, because they can produce offspring (including female offspring) upon mating with both uninfected and infected males. The reproductive advantage of the infected female hosts, in turn, ensures greater endosymbiont prevalence in the next host generation, and, therefore, CI can be considered a drive mechanism for these endosymbionts to colonise host populations.

Models incorporating CI strength, endosymbiont transmission efficiency and host fitness effects suggest that CI is a less efficient drive mechanism in haplodiploids than in diplodiploids because of the production of uninfected males by incompatible matings (Egas et al. 2002). Therefore, a higher proportion of infected hosts (higher infection threshold level) than in diplodiploid hosts is required for an endosymbiont to reach a stable equilibrium at which infection prevalence is maintained (Vavre et al. 2000; Egas et al. 2002). The highest infection threshold level for reaching a stable equilibrium is expected for MD-CI because a high production of uninfected sons as a consequence of CI reduces the likelihood of incompatible mating events and frequency of infected females. Therefore, endosymbionts may have evolved other compensatory strategies to increase fitness of their hosts. For example, in some diplodiploid hosts *Wolbachia* can confer resistance to some RNA viral infections (Hedges et al. 2008); in the haplodiploid mite *Metaseiulus occidentalis*, *Cardinium* is associated with increased reproductive life span and/or fecundity (Weeks & Stouthamer 2004).

Another adaptive strategy of endosymbionts is to bias the host sex allocation towards females rather than males in compatible matings, as males are a dead end for endosymbiont inheritance. Some studies have shown that *Wolbachia* in diplodiploid hosts can manipulate the primary sex ratio towards females by manipulating sex chromosome inheritance (Kern et al. 2015; Kageyama et al. 2017), and in haplodiploid hosts by increasing the egg fertilization rate (Werren & O'Neill 1997; Egas et al. 2002). For example, *Wolbachia*-infected females produce more female offspring than uninfected females in the spider mite *Tetranychus urticae* (Vala et al. 2003) and the parasitoid wasps *Leptopilina heterotoma* (Vavre et al. 2000) and *Nasonia vitripennis* (Bordenstein & Werren 2000). In the whitefly *Bemisia tabaci*, *Rickettsia* promotes host finess as well as higher female production that promote its spread in the host population (Himler et al. 2011). Similarly, two bacteriocyte associated endosymbionts, *Hamiltonella* and *Arsenophonus*, have been found to cause female-biased sex ratios by increasing fertilization in whiteflies (Shan et al. 2019; Wang et al. 2020). This suggests that increasing the fertilization rate may have independently evolved as a compensation strategy in diverse maternally inherited endosymbionts, albeit the underlying mechanisms remain unknown.

Females in haplodiploid species can control their offspring sex ratio by regulating sperm access to eggs (Charnov 1982), with fertilisation resulting in the development of females while males develop from unfertilized eggs (Normark 2003). It has been shown that females allocate female function to larger eggs where fitness increments associated with size have greater reproductive value to the mother (Macke et al. 2011; Katlav et al. 2021; Chapter 2). Given that female eggs typically require more nutrients to ensure fertilization success and juvenile survival (Giron & Casas 2003), it has been hypothesised that endosymbionts may manipulate host sex allocation by influencing resource allocation towards eggs in such a way that it ensures higher fertilization.

Kelly's citrus thrips, *Pezothrips kellyanus*, is an excellent model to investigate the evolution of mating and reproductive patterns in haplodiploid insects in response to endosymbiont infections. This species has an egg size-mediated fertilization mechanism, i.e. larger eggs are more likely to be fertilized, and develop as females; moreover, larger eggs also develop into fitter offspring (Katlav et al. 2021; Chapter 2). Furthermore, maternal condition influences reproductive strategy, as smaller females retain all/most of their eggs unfertilized, producing less costly sons, despite harbouring viable spermatozoa in their spermatheca (Chapter 3). This results in a bimodal pattern with larger mated females producing strongly female-biased broods (F-brood), and smaller females producing strongly male-biased broods (M-brood) (Chapter 3). Beyond this, individuals of *P. kellyanus* are naturally co-infected with both *Wolbachia* and *Cardinium* endosymbionts (Nguyen et al. 2016). These bacteria appear to increase the egg fertilisation rate in *P. kellyanus*, since mated females infected with both bacteria are more likely than uninfected females to produce F-broods (Nguyen 2015). Consequently, we hypothesise that at least one of these two endosymbionts increases host fitness by causing females to produce larger eggs.

Elucidating the interactions of common endosymbiont co-infections on host fitness can help us to better understand how infections spread in host populations (Jaenike et al. 2010). Previous field studies of *P. kellyanus* have shown a lower prevalence of *Wolbachia* than *Cardinium* in Australia from where this species originates, and the complete absence of *Wolbachia* in invasive ranges of this species in New Zealand and the Mediterranean region (Nguyen et al. 2016). However, lower prevalence or absence of *Wolbachia* in wild populations is surprising, given 100% maternal transmission was observed in the laboratory, coupled with independent, but more invasion-efficient, CI for *Wolbachia* (FM-CI) than *Cardinium* (MD-CI) (Nguyen et al. 2017). These results raised the question whether *Wolbachia* has any negative host fitness effects that have undermined its invasion biology, and conversely, whether *Cardinium* has any positive host fitness effects that have helped its maintenance.

To address this, we investigated how the maternally inherited endosymbionts highjack the sex allocation system in this haplodiploid host species. We tested whether *Wolbachia* and *Cardinium* affect thrips fertilization success by increasing paternal or maternal fitness. Moreover, we analysed the interactions between these endosymbionts in manipulating host reproductive (mating duration, sperm transfer success, egg size and number, and fertilization success) and other fitness traits such as survival rate, offspring size, development time and longevity. Understanding these interactions may help disentangle the endosymbionts' mechanism in manipulation of hosts sex allocation and explain the endosymbiont-specific infection dynamics of *Cardinium* and *Wolbachia* in *P. kellyanus* populations.

4.3 MATERIALS AND METHODS

4.3.1 Establishment of laboratory lines with different infections

A laboratory population of *P. kellyanus* infected with both *Cardinium* and *Wolbachia* (I_{CW}) (Figure 4S1a) was established from insects collected in Kulnura, New South Wales in 2017 (Katlav et al. 2021; Chapter 2). Uninfected (U) and *Cardinium*-only (I_C) lines were then obtained by rifampicin (Figure 4S1b) and tetracycline hydrochloride treatments (Figure 4S2b), respectively, following the protocol of Nguyen et al. (2017). Our attempts to obtain a line with

Wolbachia-only infection (I_W) using the same protocol were unsuccessful, so we also fed adults with 5% w/v solutions of either streptomycin sulfate or chloramphenicol (>98%) (Sigma-Aldrich) for 48 h, but again without success (Supplementary Methods).

To homogenise the genetic background among the three lines, newly-emerged virgin females of I_{CW} and I_C (~100 virgins each) were introgressed with U males (almost 200 males each) for two successive generations. The three lines were propagated for seven more generations on organic lemons sprinkled with *Typha sp*. pollen under controlled conditions (20±1 °C, 70±2% RH and 16:8 h light:dark). Two generations before the experiment, population densities and sex ratios were controlled at the beginning of each generation [N= ~300; 1:1 (♂:♀)] by removal of excess individuals. The infection status of each line was verified just before the experiment using 20 randomly selected individuals per line and, subsequently, for all individuals included in the fitness assays at the end of the experiment via diagnostic PCR as described in Nguyen et al. (2016). All tested individuals showed the expected infection status (Supplementary Methods; Table 4S1). Sequencing of a 654 bp COI fragment of several randomly selected females of lab populations identified the same haplotype (H7) for all infection lines (Nguyen et al. 2016).

4.3.2 Establishment of experimental cohorts

To ensure a sufficient number of replicates and manageable workload, the experiment was established with three cohorts over three consecutive days. This further enabled us to ensure similar environmental and physiological conditions in experimental females of the G2 where the fitness assays commenced (Figure 4.1). From each cohort, we picked equally aged females and males for fitness assays described below, and following the same procedure described in Katlav et al. (2021) (Chapter 2; also see Supplementary Methods, Appendix I). These individuals were all reared at the same larval density (~80 individuals per container).

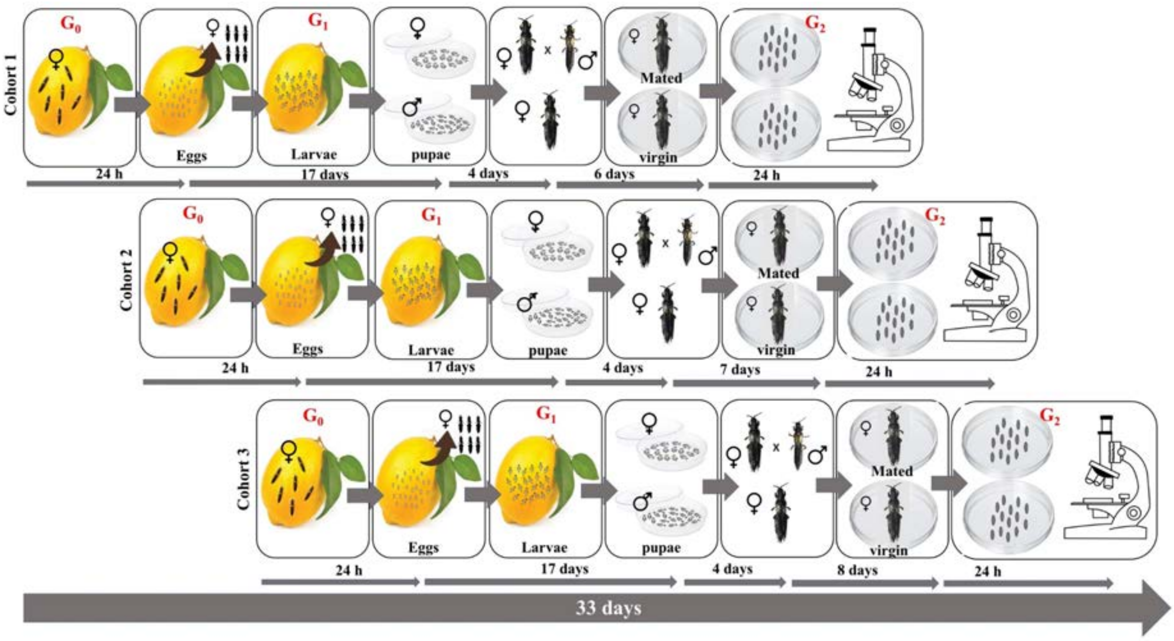


Figure 4.1 Overview of the establishment of the three experimental cohorts for fitness assays. Each experimental cohort originated from females (G_0 females), with three cohorts, each comprising all three lines with different infection types. Each subsequent cohort was established one day after the previous one. G_0 females were allowed to lay eggs for 24 hours on a fresh lemon. Offspring (G_1) was reared until the pupal stage. Upon pupation males and females were separated in different Petri dishes. Newly emerged G_1 adult females of cohorts 1, 2, and 3 were kept virgin or mated with a virgin males. Females were then isolated individually in Petri dishes without any oviposition opportunities for 6, 7, and 8 days, in the cohorts 1, 2 and 3 respectively. Then an agarose gel block was introduced for oviposition. The fitness assays where commenced at G2 (beginning with egg size measurements). The arrows represent the time between different steps. For better visualization, different life stages of the thrips are depicted larger than their actual size.

4.3.3 Fitness assay

For each line a set of 1-2 day-old females (Table 4.1) were randomly selected and kept virgin, while others were individually transferred to a copulation chamber (Nguyen et al. 2017) with ten 2-3 day-old virgin males each. All females were mated once with males of the same infection type (U×U, I_C×I_C, I_{CW}×I_{CW}). Copulation was observed and its duration recorded for each female. Although the lab populations can be assumed to be highly inbred, we controlled for parental relatedness by pairing males and females produced by different mothers (Supplementary Methods; Figure 4S2). After copulation, each mated female was immediately removed from the copulation chamber to avoid further matings and the mated male was preserved in a 1.5 ml microcentrifuge tube and frozen at -20 °C for subsequent size measurement and infection screening.

To induce simultaneous oviposition, females were isolated individually in small Petri dishes for at least six days to ensure they all were ready to oviposit. The average pre-oviposition period of *P. kellyanus* is six days independent of mating status (Katlav et al. 2021; Chapter 2) and infection status (Supplementary Methods). During this period, females were supplied with ad libitum *Typha* sp. pollen and honey-water solution (50% w/v), renewed every second day. Subsequently, females of each cohort were allowed to oviposit on an agarose plate for 24 h as described in Katlav et al. (2021) (Chapter 2). However, to ensure feasibility of fitness assay workloads, females of each subsequent cohort were allowed to lay eggs with one day delay compared to the previous cohort (Figure 4.1). Females tested for each line were categorized into three types depending on their mating status and offspring sex ratio: (1) virgin, V; (2) mated producing M-brood, M(♂); (3) mated producing F-brood, M(♀). Accordingly, the offspring were grouped into four categories depending on their sex [daughter (D) or son (S)] and mother type (Table 4.1).

4.3.4 Sperm detection in spermathecae

Ten newly emerged virgin females were randomly selected from each line and individually transferred to a copulation chamber. Each female was accompanied with ten virgin males (2-3 days old) of the same line. Mating was observed and then mated females were isolated individually in small Petri dishes for at least five days without the opportunity to oviposit to ensure sufficient egg load. On day six females were isolated in small boxes containing an organic lemon sprinkled with *Typha sp.* pollen for oviposition for 24 h. The lemons were incubated until emergence of adult offspring and the offspring sex ratio was evaluated. After oviposition females were removed, immobilized on ice for 10 s and their spermathecae dissected and transferred to concave microscope slides with a drop of Ringer's solution and topped with a coverslip to verify the presence of motile sperm using phase-contrast light microscope under a 100X/1.25 oil-immersion objective (Zeiss Axio Scope A1, Germany).

4.3.5 Effect of endosymbionts on egg size and fecundity in early reproductive effort

All the egg size and fecundity analyses were on the first clutch (one day of oviposition) and therefore involved the early reproductive effort. The fecundity (clutch size) and egg size of the first clutch of 183 females (Table 4.1) were scored. Five to eight eggs per female (~50% of total eggs per female) were randomly sampled. Egg size was measured on the oviposition day and egg volume calculated following the formula used by Katlav et al. (2021) (Chapter 2):

$$\text{egg volume} = (4/3)\ \pi a^2 c$$

where a is the half egg width (equatorial radius) and c is the half egg length (polar radius). Subsequently the egg size was compared among all three lines (see Appendix III). For comparison of the early reproductive effort among mother types, all egg size measurements were incorporated in the analysis regardless of offspring sex and survival to adulthood.

4.3.6 Endosymbionts and egg-size mediated sex allocation

After size measurement, each egg was immediately placed on an agarose gel plug (0.6% w/v) in the lid of a 0.5 ml microcentrifuge tube until it hatched (Katlav et al. 2021, Supplementary Methods; Figure 4S3a). Once hatched, neonate larvae were individually transferred to a 1.5 ml microcentrifuge tube supplied with a lemon leaf disk (0.8 cm diameter) and ad libitum *Typha* sp. pollen, both renewed every second day (Supplementary Methods; Figure 4S3b). Individual offspring was monitored daily until adulthood. Offspring sex was determined at the pupal stage, based on a distinctive sexual size dimorphism (Katlav et al. 2021; Chapter 2). Only individuals that reached at least the pupal stage (and therefore could be confidently sexed) were included in the egg size comparisons. All virgins produced only male offspring, confirming arrhenotoky in *P. kellyanus* (Nguyen et al. 2017). The two mated mother types [M(♂) and M(♀)] were distinguished according to their offspring sex ratio. The proportion of M(♂) and M(♀), as well as the population-level sex ratio of mated mothers (i.e. sex ratio of all mated females of the same infection type combined), were compared among the three infection lines. Subsequently, we investigated if these sex ratio parameters correlated with egg size and whether the endosymbionts influence egg size, and, thereby fertilization success and sex allocation.

4.3.7 Effect of endosymbionts on offspring fitness traits

Larval development and survival from egg to adulthood were recorded daily. After emergence, adults were transferred into other 1.5 ml microcentrifuge tubes with ad libitum pollen supplied every second day, but without honey water (dehydration stress) and a lemon leaf disk, and monitored daily for survival. Upon adult death, fore-wing length (a reliable proxy of offspring size in thrips - Nakao 1993) was measured, following the procedure of Katlav et al. (2021) (Chapter 2).

We examined the effect of infection type on offspring fitness traits among all offspring types. We also incorporated the effect of offspring egg size into the fitness traits analysis to calculate the net effect of endosymbiont on the examined traits. Finally, we examined the effect of infection type on offspring sex ratio in M(♀), with the offspring survival rate being incorporated in in the model to reveal the net endosymbiont effect on sex allocation.

4.3.8 Comparison of egg size and fecundity between co-infected and naturally uninfected mothers in early reproductive effort

The fitness assays with the antibiotics-treated line (U) was conducted eight generations post antibiotic treatment which allowed a reasonably sufficient period for recovery from antibiotic treatment (Douglas 2009; Li et al. 2017). However, in order to ensure that lower egg-size resource allocation is not an outcome of potential long term effects of microbiome disruption with antibiotic treatment, another experiment was conducted on naturally uninfected line (NU). Eggs were collected from same-aged mated and virgin I_{CW} (N=13) and naturally uninfected (NU; N=16) females. The NU line was established by combining two naturally uninfected isofemale lines from the same field population as laboratory lines (Kulnura, NSW, Australia) (see Supplementary Methods), and, therefore, it is fair to assume they are of the same gene pool. From each mother, three eggs were randomly selected and measured (n= 87 eggs). The clutch size and average egg volume for both virgin and mated females were compared between the two infection lines.

4.3.9 Statistical analyses

All statistical analyses were performed using RStudio (V.1.2.5001) and SPSS (V.25, IBM Corporation, USA). Prior to analysis, the distribution of variables was checked for normality using the Shapiro–Wilk test, and the homogeneity of variance using Levene’s test ($P<0.05$). Data violating normality and homogeneity assumptions were log10 or square-root-transformed. A chi-square test (Goodness-of-Fit) was used to examine the effect of infection on fertilization success. Moreover, to test for the same effect on 1st clutch sex ratio for each infection line (population-wide sex ratio), a Kruskal-Wallis test was used. Analysis of egg volume and number among different treatments were performed using a general linear model (three-way ANOVA) using “lme4” package with three fixed factors (infection type, mother type/offspring type, experimental cohort) and mother ID as a random factor to control for the variation between individual mothers. The same analysis was conducted for the offspring fitness data (development time, juvenile survival rate, adult fore-wing length and longevity) with the same fixed and random factors. Moreover, mother forewing and egg size (depending on the response variable) were incorporated in the model as a covariate (ANCOVA) to determine the net effects of fixed factors. The interactions of fixed factors were evaluated. Following a significant effect of factors or their interactions, additional post-hoc tests for multiple comparisons were conducted with Tukey’s HSD test ($p< 0.05$ level of significance) to determine which level differed within a fixed factor. A three-way ANOVA table for the treatments structure was constructed (Table 4.3; Table 4S1). There were no effect of cohorts and no interactions with other factors, and, therefore, the figures represent the combined data of all experimental cohorts.

4.4 RESULTS

4.4.1 Effect of endosymbiont on fertilization success and sex allocation

We showed that *P. kellyanus* represents a bimodal pattern of offspring sex ratio. Some mated mothers produced M-broods, while others produced F-broods. This indicates variable fertilisation rates across mothers. Comparison of the sex ratio among the three infection types revealed an endosymbiont effect because the proportion of female producing F-broods was higher in infected females. Further comparison between I_C and I_{CW} mated females revealed that this effect is due to *Cardinium* and not *Wolbachia*. *Cardinium*-infected mated females had a high proportion of M(♀), which is seen in the offspring sex ratios of $I_C \times I_C$ and $I_{CW} \times I_{CW}$ pairs when compared with U×U pairs (Table 2; Figure 4.2a). However, there was no evidence for an effect of *Wolbachia,* since the proportion of M(♂) in $I_{CW} \times I_{CW}$ did not differ significantly from that in $I_C \times I_C$ (Table 4.2; Figure 4.2a). Further analysis of the pooled sex ratio data (sex ratio at a population-level sex ratio) revealed 22% and 25% higher female-biased population sex ratio in $I_{CW} \times I_{CW}$ (~39%) and $I_C \times I_C$ (~36%) when compared with U×U (~61%) (Table 4.2).

4.4.2 Effect of endosymbiont on offspring sex ratio of M(♀)

Infection type had a significant effect on offspring sex ratio of M(♀) (Table 4.3). Comparison of I_C and U pairs indicates that *Cardinium* increased female production in M(♀). However, this effect was reduced by *Wolbachia* in I_{CW} pairs, for which female production in M(♀) was not significantly different from U pairs (Figure 4.2b). This difference in offspring sex ratio of differently infected M(♀) was unaffected by egg size (Table 4.3).

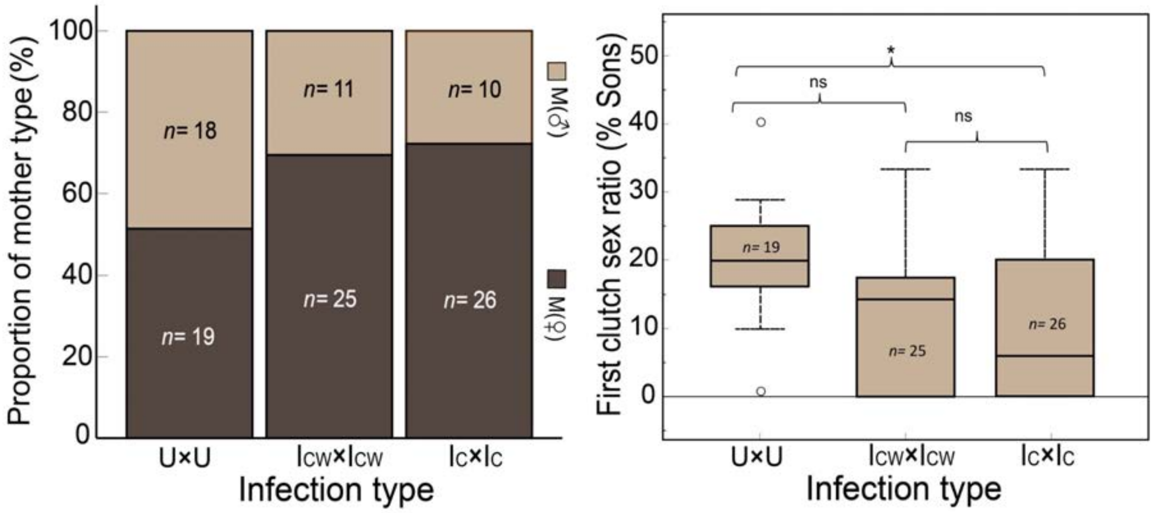


Figure 4.2 (a) Proportion of mated females with female-only or highly female-biased broods [M(♀)] and those with male-only or highly male-biased broods [M(♂)] among different infection types. (b) Sex ratio of mated females [M(♀)] with different infection types (two-way ANOVA followed by Tukey-test for multiple comparisons; data of all experimental cohorts pooled). *$p < 0.05$, ns = not statistically significant; n = number females examined (data of all experimental cohorts pooled).

4.4.3 Endosymbiont affects female size, but not copulation time and sperm transfer

Copulation time was similar among the three infection types and the two mated mother types (Figure 4.3a) indicating that copulation time does not drive the differences in fertilisation success. We also did not detect an effect of mother size or father size on copulation time (Table 4S2). However, we detected an effect of mother size, but not father size on sex allocation (i.e. mother types differ in body size) (Table 4S2): M(♀) were larger than M(♂) across all infection types (Figure 4.3b). Moreover, infected females were larger than uninfected females (Figure 4.3b), but this effect was not seen for fathers (Figure 4.3c). These patterns were consistent across all experimental cohorts (Table 4.3). Following dissection of spermatheca of mated females, motile sperm was detected in all individuals irrespective of infection type and offspring sex ratio. Therefore, the higher proportion of M(♂) among U pairs is unlikely to be due to a failure of sperm transfer during copulation.

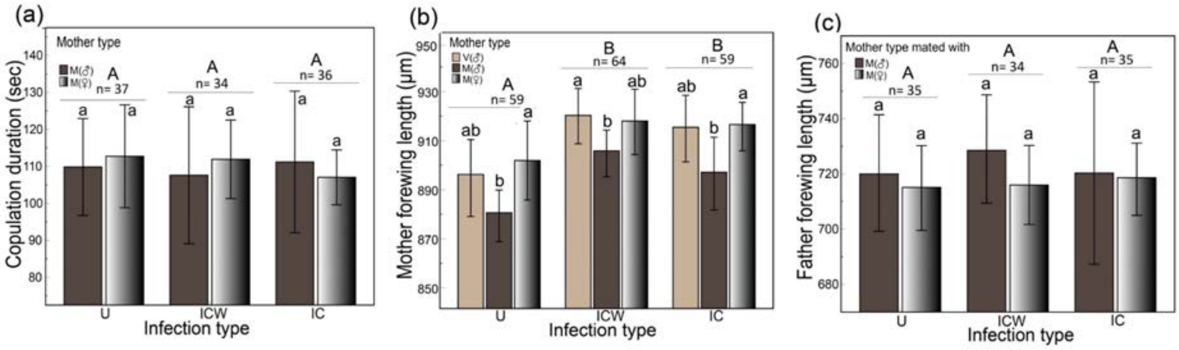


Figure 4.3 (a) Mean ± SE mother forewing length across different infection and mother types (n = number of mothers examined). (b) Mean ± SE father forewing length across different infection types and mated with different mother types [M(♀) and M(♂)] of the same infection type (n = number of fathers examined). (c) Mean ± SE copulation time (seconds) across different infection types and mother types (n = number of copulation cases examined). Different lowercase letters above the bars denote statistical differences between mother types within the corresponding infection type ($p < 0.05$). Different uppercase letters above the lines denote statistical differences between infection types under all mother types ($p < 0.05$; three-way ANOVA followed by Tukey test for multiple comparison).

Table 4.1 Mother and offspring types of *Pezothrips kellyanus* according to infection type, mating status, offspring sex ratio. Based on this differentiation there are three mother types and four offspring types for each of the three infection types (N = number of tested mothers; data of all cohorts were pooled).

	Mother types									
Infection type	Virgin			Mated producing male-only brood			Mated producing female-only/biased brood			
		Mother symbol	Offspring symbol		Mother symbol	Offspring symbol		Mother symbol	Offspring symbol	
	N	**V**	**S_V**	**N**	**M(♂)**	**$S_{M(♂)}$**	**N**	**M(♀)**	**$S_{M(♀)}$**	**$D_{M(♀)}$**
U	23	**V_U**	S_{VU}	18	$M(♂)_U$	$S_{M(♂)U}$	19	$M(♀)_U$	$S_{M(♀)U}$	$D_{M(♀)U}$
I_{CW}	28	**V_{CW}**	S_{VCW}	11	$M(♂)_{CW}$	$S_{M(♂)CW}$	25	$M(♀)_{CW}$	$S_{M(♀)CW}$	$D_{M(♀)CW}$
I_C	23	**V_C**	S_{VC}	10	$M(♂)_C$	$S_{M(♂C}$	26	$M(♀)_C$	$S_{M(♀)C}$	$D_{M(♀)C}$

Table 4.2 Proportions of M(♀) and M(♂) mothers across different infection types. Chi-Square (Goodness-of-Fit) was used to test whether infection influenced the fertilization success. Kruskal-Wallis test was used to test whether infection influenced significantly the sex ratio at a population level.

♀×♂	**Cohort 1**			**Cohort 2**			**Cohort 3**			**All cohorts combined**				**Population sex ratio + SE**	
	N	**M(♀)**	**M(♂)**	**N**	**M(♀)**	**M(♂)**	**N**	**M(♀)**	**M(♂)**	**N**	**M(♀)**	**M(♂)**	**Chi-square**	**Kruskal-Wallis test**	χ^2(*df*=2) = 7.894, ***p* = 0.019**
U×U	13	8 (61.5%)	5 (38.5%)	13	5 (38.5%)	8 (61.5%)	11	6 (54.5%)	5 (45.5%)	37	19 (51.3%)	18 (48.7%)	χ^2(*df*=1)=0.027, *p*=0.869	U×U	61.02 ± 6.852[b]
I_{CW}×I_{CW}	11	8 (72.7%)	3 (27.3%)	12	8 (66.7%)	4 (33.3%)	13	9 (69.2%)	4 (30.8%)	36	25 (69.4%)	11 (30.6%)	χ^2(*df*=1)=5.44, ***p*=0.020**	I_{CW}×I_{CW}	39.03 ± 6.95[a]
I_C×I_C	12	9 (75%)	3 (25%)	12	9 (75%)	3 (25%)	12	8 (66.7%)	4 (33.3%)	36	26 (72.2%)	10 (27.8%)	χ^2(*df*=1)=7.11, ***p*=0.008**	I_C×I_C	36.17 ± 6.54[a]

4.4.4 Effect of endosymbionts on egg size and fecundity

Egg volume among mother types. Mother type significantly affected the egg size: M(♀) produced larger eggs than V (suggesting a mating effect on egg size) and M(♂) (suggesting an egg size effect on fertilization success). These egg size patterns were consistent across the three infection types and experimental cohorts (Table 4.3). In addition, egg size increased with endosymbiont infection (Figure 4.4a): eggs of I_C females were, on average, 11% larger than those of U females, and 7.5% larger than those of I_{CW} females. These results indicate a positive effect of *Cardinium* on egg size, which is reduced by *Wolbachia* in females with both endosymbionts (Figure 4.4a). There was no significant effect of experimental cohort on egg size nor any significant interactions between factors (Table 4.3). Incorporation of mother forewing as a covariate in the model did not remove the effect of endosymbiont on average egg size (Table 4S2), suggesting that egg size is not only a function of mother size (Chapter 3), but also directly influenced by endosymbiont infection. There were no significant differences in egg size range variation across mother and infection types (Figure 4S4a; Table 4S2), suggesting consistent effects of mating and endosymbionts. These consistent effects are also evident in the analysis of minimum and maximum egg size, with mating and infection status affecting both parameters, albeit the maximum egg size difference between I_{CW} and I_C pairs was marginally insignificant (Figure 4S4b-c; Table 4S2).

Egg volume among offspring types. Eggs showed sexual size dimorphism, irrespective of infection type (Sex × Infection type: $F_{2, 440}= 0.54$; $p = 0.578$), as in M(♀) larger eggs developed into female offspring and smaller eggs into male offspring ($F_{1, 440}= 81.76$; $p < 0.001$). In addition, egg size was very similar among all male offspring types ($F_{2, 606} = 3.34$; $p = 0.071$) and independent of infection type (Male offspring type × Infection type: $F_{2, 606} = 0.015$; $p = 0.998$) (Figure 4.4b). Moreover, infection types affected egg size, with larger eggs being produced by I_C females than by females of other infection types, and eggs of I_{CW} females being larger than eggs of U females (Figure 4.4b; Table 4.3).

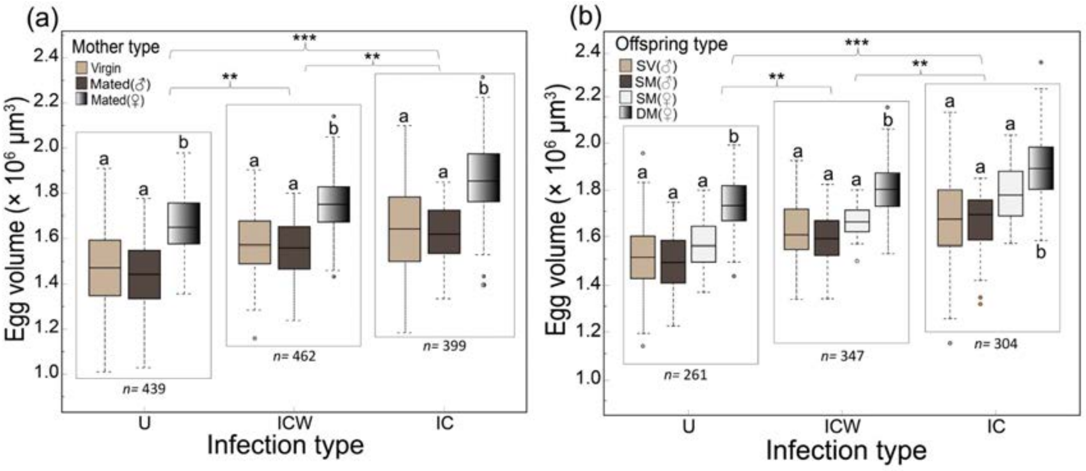


Figure 4.4 (a) Mean ± SE egg volume across different infection and mother types. (b) Mean ± SE egg volume across different infection and offspring types. Different lowercase letters above the bars denote statistical differences between mother and offspring types within the corresponding infection type ($p < 0.05$). Asterisks denote statistical difference levels between infection types and mother types (**$p < 0.01$; ***$p < 0.001$; three-way ANOVA followed by Tukey test for multiple comparisons; n = number eggs examined).

Egg number (clutch size). Across all mother types, the clutch size was significantly greater in U females ($F_{2, 181}$= 23.50; p = 0.013). However, this was not consistent across all experimental cohorts ($F_{4, 181}$ = 2.88; p = 0.024), with the third cohort showing no significant clutch size difference among infection lines ($F_{2, 62}$ = 1.56; p = 0.219) (Figure 4.5a). When the average egg size was included as a covariate in the model, there was no effect of infection type on the clutch size (Table 4.3), indicating that the endosymbiont effect on egg size is traded-off with clutch size. Moreover, we found that, independent of infection type ($F_{2, 183}$= 1.56; p = 0.289), clutch size and average egg size were negatively correlated ($F_{1, 183}$= 11.13; p = 0.0011, r^2 = 0.11) with a similar slope (Figure 4.5b). This confirms that the egg size and number trade-off in the clutch does not depend on infection type (Average egg size × Infection type: $F_{2, 183}$= 0.28; p = 0.75).

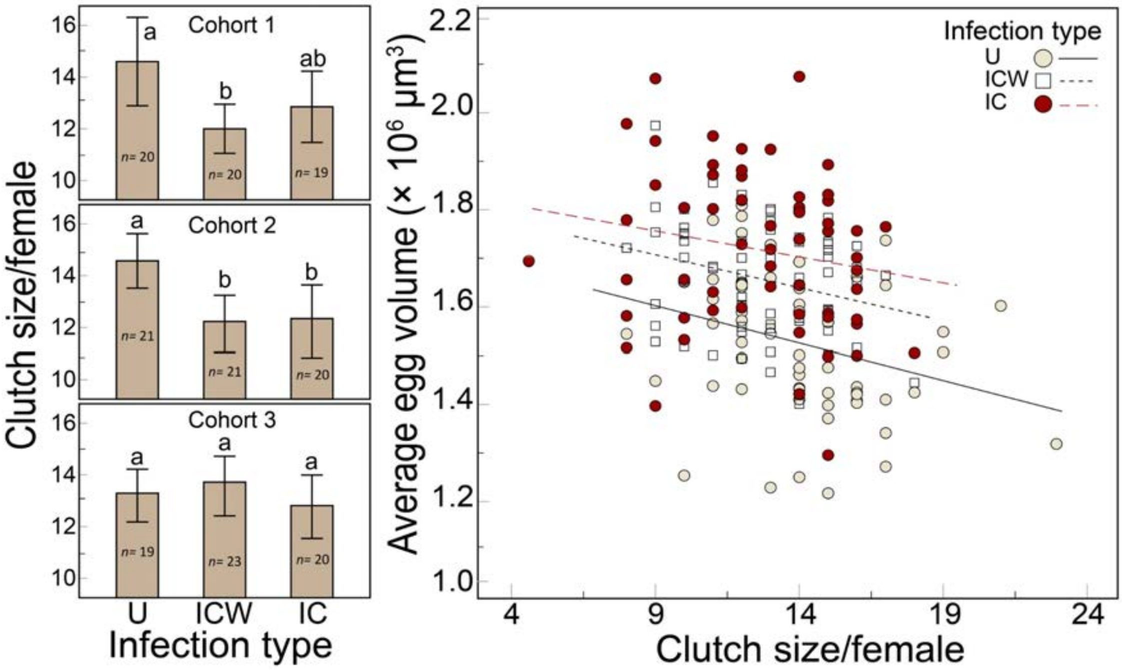


Figure 4.5 (a) Mean clutch size (numbers of eggs) ± SE across three cohorts with different infection types (n = number females examined). Different lowercase letters above the bars denote statistical differences between infection types within the corresponding experimental cohort ($p < 0.05$; three-way ANOVA followed by Tukey test for multiple comparison; data of both mated mother types pooled in the graph). (b) Egg size and number trade-off in the 1st clutch for different infection types (data of all mother types pooled in the model). Lines represent mean predicted values and individual symbols represent individual data points.

4.4.5 Effect of endosymbiont on offspring fitness traits

Offspring development time. Cardinium did not affect development time compared to uninfected individuals; however, co-infection with *Wolbachia* significantly extended development time (Figure 4.6a). Moreover, across all infection types, female offspring developed slower than male offspring (Figure 4.6a). Because of a significant interaction between infection type and experimental cohort (Table 4.3), we examined the endosymbiont effect in each experimental cohort separately. We found that the difference developmental time between I_{CW} and I was marginally insignificant in the second experimental cohort ($F_{1,222}= 3.78$; $p = 0.053$).

Offspring survival rate. Compared to U, survival from egg to adulthood was by 15% higher in I_C, independent of mother type. Co-infection with *Wolbachia* in mated I_{CW} females did not appear to modify this (Figure 4.6b). Moreover, incorporation of average egg size in the model as a covariate did not mask this endosymbiont effect, suggesting that the direct effect of endosymbionts on offspring survivorship is overridden by its effect on egg size (Table 4.3).

Offspring adult size. The effect of *Cardinium* on egg size persisted until adulthood in all mother types, with larger eggs in I_C developing to larger adults (across all offspring types) when compared to uninfected mothers. Similarly, the negative effect of *Wolbachia* on the egg size was also still evident in adults, with adult I_{CW} offspring being significantly smaller than adult I_C offspring (Figure 4.6c). When egg size was included as a covariate, the effect of endosymbiont remained significant (Table 4.3), suggesting that the effect of endosymbionts extends beyond egg size differences.

Adult offspring longevity under dehydration stress. Irrespective of infection type, female offspring lived on average 0.7 day longer than male offspring under dehydration stress. *Cardinium* increased offspring adult longevity by nearly one day, but this fitness effect did not significantly change in individuals co-infected with *Wolbachia* (Figure 4.6d). This endosymbiont effect was uniform across all offspring types. However, offspring in the first cohort lived ~ 0.37 day longer than those of the two subsequent cohorts. Incorporation of adult size as a covariate to the analysis revealed that this fitness effect is partly, if not completely, an endosymbiont effect on adult size (Table 4.3).

Table 4.3 Results of the statistical analyses of fitness traits, with all factors and experimental cohorts analysed together using three-way ANOVA or ANCOVA with *F* and *p* values showing the effects of the fixed factors and covariate, if applicable.

Biological characteristic/factor	**df, N**	***F***	***p***
Egg volume (among mother types)			
Infection type	2, 1300	34.56	**<0.001**
Mother type	2, 1300	76.18	**<0.001**
Cohort	2, 1300	0.68	0.503
Infection type × Mother type	4, 1300	0.75	0.556
Infection type × Cohort	4, 1300	0.49	0.739
Mother type × Cohort	4, 1300	1.47	0.214
Infection type × Mother type × Cohort	8, 1300	1.11	0.36
Egg volume (among offspring types)			
Infection type	2, 1046	31.63	**<0.001**
Offspring type	3, 1046	74.76	**<0.001**
Cohort	2, 1046	0.11	0.894
Infection type × Offspring type	6, 1046	0.79	0.577
Infection type × Cohort	4, 1046	1.02	0.397
Offspring type × Cohort	6, 1046	0.64	0.694
Infection type × Offspring type × Cohort	12, 1046	0.96	0.483
Egg number (1st clutch size)			
Infection type	2, 181	1.06	0.347
Mother type	2, 181	0.24	0.774
Cohort	2, 181	0.47	0.626
Infection type × Mother type	4, 181	0.68	0.601
Infection type × Cohort	4, 181	2.49	**0.045**
Mother type × Cohort	4, 181	1.14	0.338
Infection type × Mother type × Cohort	8, 181	0.56	0.811
Covariate (average egg size)	1, 181	5.57	**0.019**
Offspring sex ratio of M(♀) mother			
Infection type	2, 70	2.79	**0.031**
Cohort	2, 70	0.96	0.381
Infection type × Cohort	4, 70	0.92	0.456
Covariate 1 (average egg size)	1, 70	0.11	0.732
Offspring development time (day)			
Infection type	2, 912	36.61	**<0.001**
Offspring type	3, 912	68.13	**<0.001**
Cohort	2, 912	1.18	0.310
Infection type × Offspring type	6, 912	1.91	0.081
Infection type × Cohort	4, 912	2.61	**0.038**
Offspring type × Cohort	6, 912	2.22	0.052
Infection type × Mother type × Cohort	12, 912	0.78	0.667
Covariate (average egg size)	1, 912	1.15	0.283
Juvenile offspring survival rate			
Infection type	2, 183	11.11	**<0.001**
Mother type	2, 183	2.06	0.131
Cohort	2, 183	0.41	0.66
Infection type × Mother type	4, 183	0.59	0.67
Infection type × Cohort	4, 183	0.22	0.928
Mother type × Cohort	4, 183	0.96	0.430
Infection type × Offspring type × Cohort	8, 183	0.90	0.52
Covariate (average egg size)	1, 183	26.73	**<0.001**
Adult offspring size (forewing length)			
Infection type	2, 894	5.19	**<0.01**
Offspring type	3, 894	1361.28	**<0.001**

Cohort	2, 894	1.56	0.211
Infection type × Offspring type	6, 894	1.27	0.261
Infection type × Cohort	4, 894	1.73	0.145
Offspring type × Cohort	6, 894	0.48	0.817
Infection type × Offspring type × Cohort	12, 894	0.87	0.816
Covariate (egg size)	1, 894	270.41	**<0.001**
Adult offspring longevity under stress (day)			
Infection type	2, 894	54.66	**<0.001**
Offspring type	3, 894	4.34	**0.005**
Cohort	2, 894	14.93	**<0.01**
Infection type × Offspring type	6, 894	0.79	0.580
Infection type × Cohort	4, 894	0.51	0.728
Offspring type × Cohort	6, 894	0.62	0.712
Infection type × Offspring type × Cohort	12, 894	0.56	0.877
Covariate (adult size)	1, 894	44.56	**<0.001**

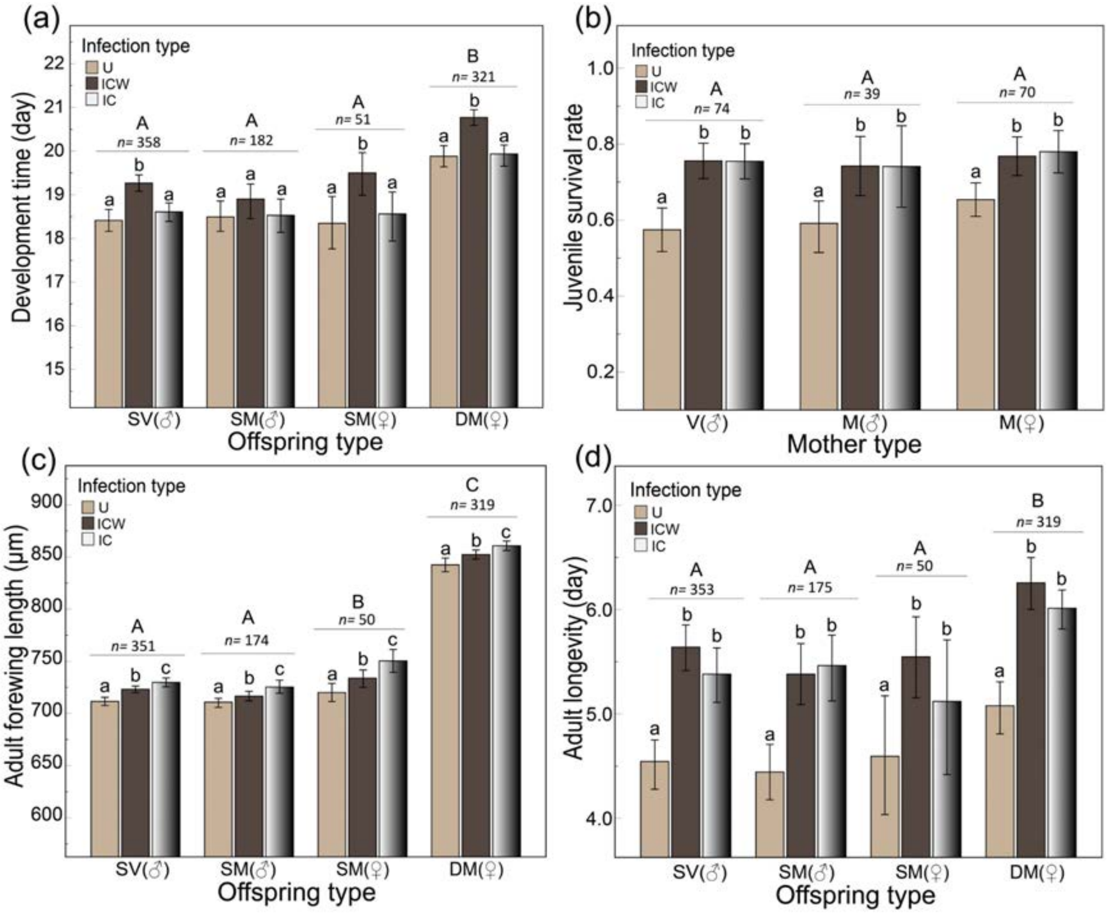


Figure 4.6 Offspring fitness in response to different infection types. (a) Mean offspring development time (days) ± SE. (b) Mean juvenile offspring survival rate ± SE. (c) Mean adult offspring forewing length ± SE. (d) Mean adult offspring longevity (day) ± SE (under dehydration stress) across different infection types and offspring types. Different lowercase letters above the bars denote statistical differences between infection types ($p < 0.05$) in each offspring type. Different uppercase letters above the lines denote statistical differences between offspring types under all infection type treatments ($p < 0.05$; three-way ANOVA followed by Tukey test for multiple comparison; n = number females examined).

4.4.6 Comparison of egg size and fecundity between I_{CW} and NU females in early reproductive effort

To independently confirm the lower reproductive effort of uninfected individuals without the previous exposure of the U line to antibiotics, we compared the egg size and fecundity between I_{CW} and NU virgin and mated females. Consistent with the previous experiment, NU females produced significantly smaller eggs than I_{CW} females (Infection type: $F_{1, 87}$= 29.46; $p < 0.001$). Mating also significantly increased egg size, irrespective of infection type (Mating status: $F_{1, 87}$= 18.09; $p < 0.001$; Mating status × Infection type: $F_{1, 183}$= 1.93; $p = 0.177$) (Figure 4.7a). However, the effect of endosymbionts on fecundity was not detectable (Infection type: $F_{1, 39}$ = 3.74; $p = 0.064$), probably due to a smaller sample size; although we confirmed that mating did not affect clutch size regardless of infection type (Mating status: $F_{1, 39}$= 2.75; $p = 0.110$; Mating status × Infection type: $F_{1, 39}$= 1.26; $p = 0.272$) (Figure 4.7b).

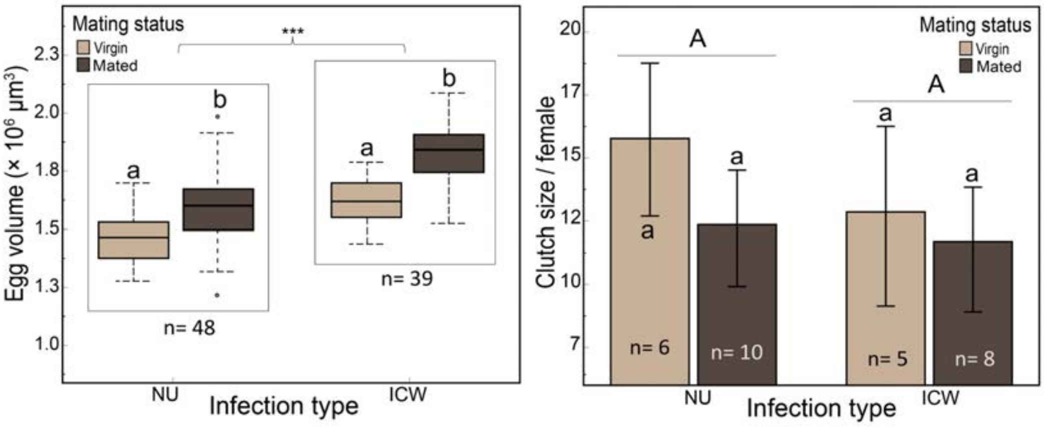


Figure 4.7 (a) Mean egg volume ± SE between naturally uninfected (NU) and co-infected (I_{CW}) females with different mating status (virgin and mated) (n = number of mothers examined). (b) Mean 1st clutch size (egg number) ± SE between virgin and mated NU and I_{CW} females (n = number of females examined). Different lowercase letters above the bars denote statistical differences between virgin and mated females ($p < 0.05$) within the corresponding infection types. Asterisks denote statistical difference levels between infection types under both mating status (***$p < 0.001$). Different uppercase letters above the lines denote statistical differences between offspring types and infection types ($p <$ 0.05 three-way ANOVA followed by Tukey test for multiple comparison).

4.5 Discussion

Our key finding is that endosymbionts can cause an increased host investment into egg size. This translates into higher chances of fertilization in infected femeales, resulting in more production of infected females which therefore facilitates endosymbiont spread. We showed that in *P. kellyanus*, females without *Cardinium* endosymbionts are more likely to produce male-biased broods due to the production of smaller eggs that, as a consequence, generally fail to be fertilised (Figure 4.2a). We also found that M(♀) harbouring *Cardinium*, irrespective of egg size, produced more daughters than uninfected ones. Given that we did not assess this across developmental stages, this could be associated with a higher survival of juvenile females, consequently affecting the offspring sex ratio produced by M(♀) (Figure 4.2b). Beyond this, we showed a complex host fitness cost-benefit interaction between *Wolbachia* and *Cardinium* under laboratory conditions. We found both indirect (via change in maternal resource allocation towards egg size) and direct endosymbiont effects on offspring fitness in terms of development time, survival rate, body size and longevity under dehydration stress (Figure 4.6).

4.5.1 Lower chance of female production in uninfected mated females: paternal disability in sperm transfer or lower maternal fitness?

In haplodiploids, highly male-biased offspring sex ratios despite mating can be due to constraints on sperm availability, such as failure in mating or sperm transfer (de la Filia et al. 2015). However, we confirmed the presence of motile sperm in all dissected mated females, including those that had produced only male offspring (Figure 4.3d). Our study also found no correlation of copulation duration with endosymbiont infection and with success in daughter production (Figure 4.3c). Therefore, lower daughter production by uninfected females is not due to mating failure and/or sperm transfer issues. Moreover, unlike in several parasitoid wasps where paternal size influences offspring sex allocation (e.g. Henter 2004; Boivin & Martel 2012), across all infection types, male size in *P. kellyanus* did not contribute to the offspring sex ratio (Figure 3c). In some insect species, male ejaculates contain substances that promote vitellogenesis and fertilization (Gillot 2003); but this (and a role for endosymbionts) has yet to be investigated in *P. kellyanus*.

Previous research on *P. kellyanus* has demonstrated maternal condition and egg size influence fertilization success (Katlav et al. 2021; Chapter 4). A positive relationship between egg size before mating and fertilization success after mating suggests a direct maternal effect on egg-size mediated sex allocation (Chapter 3). In this study, we showed that females lacking *Cardinium* produced smaller eggs than infected females. This size reduction was inflicted uniformly to all eggs (inferred from similar ERV among infection lines; see Figure 4S4c) and was even evident in virgin mothers. This indicates less resource availability and provisioning in uninfected females compared to those bearing *Cardinium*. Therefore, our study suggests that *Cardinium* may promote maternal resource allocation into egg size and facilitate egg size-mediated fertilization in mated females. This argument is consistent with several studies suggesting that maternally inherited symbionts can provision hosts with nutrients (e.g. Brownlie et al. 2009; Li et al. 2020). For example, in whiteflies, *Hamiltonella* and *Arsenophonus* are important in supplying B vitamins which probably facilitate oogenesis and fertilization (Wang et al. 2020).

4.5.2 *Cardinium* and *Wolbachia* fitness interactions in *P. kellyanus*

Disentangling endosymbiont interactions in the host is important for understanding their evolutionary and population dynamics, and history of host-endosymbiont associations (Weeks et al. 2007; Duron et al. 2008). Our study demonstrated interactive effects of *Wolbachia* and *Cardinium* on reproductive strategies and fitness traits of *P. kellyanus*. We showed that *Cardinium*-induced investment into egg size in both virgin and mated mothers was the highest when *Wolbachia* was absent (Figure 4.4a), indicating a cost of bearing *Wolbachia*. Furthermore, the fitness benefit of *Cardinium* may be partly utilized to counter the increased metabolic cost of bearing the two endosymbionts, resulting in fewer resources for egg provisioning. However, the cost of bearing *Wolbachia* on egg size did not influence offspring sex ratio. This may indicate that there is an egg size threshold for fertilization success (see Macke et al. 2011) that is still achieved by mated I_{CW} females. Sex-specific survivorship during development among infection lines is a potential source of sex ratio differences (Bondy & Hunter 2019). However, we showed a similar juvenile survival rate for I_{CW} and I_C lines in both sexes. Reproductive fitness benefits of bearing *Cardinium* have also been shown for a predatory mite, *M. occidentalis*, but only in terms of fecundity increase (Weeks & Stouthamer 2004). Conversely, in pharaoh ants, *Wolbachia* causes a higher reproductive investment and results in more female-biased sex ratios (Singh & Linksvayer 2020). However, to the best of our knowledge, there are few or no other reported examples of an endosymbiont causing larger egg sizes to facilitate fertilization and daughter production.

Higher investment into egg size by *Cardinium* came at the expense of smaller clutch size, suggesting a trade-off between egg size and number (Figure 4.5b; Katlav et al. 2021; Chapter 2; also see Fox et al. 1977). This can be related to maternal condition that determines how much a mother can invest into her offspring (Trivers & Willard 1973; Macke et al. 2011) and thus change the trade-off between egg size and number accordingly. Our finding shows that uninfected mothers, suffering lower fitness, reflected in their smaller size (Figure 4.3a), are selected to produce a larger number of smaller eggs (Chapter 3). Without measurement of egg size, we would have erroneously concluded that *Cardinium* imposed a cost in terms of lower reproductive output. Therefore, fecundity alone cannot be an accurate proxy of endosymbiont effects on reproductive outputs. The fecundity pattern in cured mothers was inconsistent across experimental cohorts, with clutch size being reduced in the last cohort, probably because of the two days delay in oviposition associated with our experimental design (Figure 4.1). Other studies in parasitoid wasps have attributed such lower fecundity to resorption of eggs as a female strategy of biological maintenance (Jervis & Kidd 1986; Kant et al. 2013). However, clutch size did not change with oviposition delay in either the I_{CW} or I_C, again suggesting a beneficial effect of *Cardinium*.

Consistent with our previous study, offspring fitness increased with egg size (Katlav et al. 2021; Chapter 2). Egg size increase due to *Cardinium* resulted in offspring with higher survival (Figure 4.6c), larger adult size (Figs. 4.6c) and higher longevity under dehydration stress (Figure 4.6d). However, we also found direct beneficial effects of *Cardinium* irrespective of egg size. In addition, the *Cardinium* fitness benefits were either reduced or unaffected by *Wolbachia*. Consistent with the effect on egg size, *Wolbachia* reduced the fitness advantage of bearing *Cardinium* in terms of adult forewing length, a reliable proxy of adult body size in thrips (Nakao 1994) that may influence other fitness parameters. Body size is an important indicator of reserved energy budget in animals (e.g. Qutton-Brock 1988; Navarro-Campos et al. 2011), with both larger females and males often enjoying higher reproductive success (Partridge 1988; Southwood 1966) and survival chance (Neems et al. 1990), and these were also demonstrated in our study. Furthermore, body size is an important indicator of mating success under sexual selection (Glaudas et al. 2020). Males may prefer to mate with larger females as they are more productive (Byrne & Rice 2006), and larger males are better competitors in acquiring mates (Calsbeek & Sinervo 2004). Several studies have shown positive impacts of *Wolbachia* on adult body size and survival (e.g. Joshi et al. 2014; Okayama et al. 2016). However, in our study we found this to be associated with *Cardinium* alone; and in co-infected thrips, *Wobachia* inflicted costs or was, at least, indifferent. Therefore, it is likely that *Wolbachia* in nature, other than CI, may rely on *Cardinium*'s beneficial fitness effect to expand its prevalence. To our knowledge, similar cases are quite scarce in the literature (Gotoh et al. 2007; White et al. 2009; 2011). One example is the parasitoid wasp *Encarsia inaron* in which *Cardinium* alone can increase adult survival, while co-infections with *Wolbachia* can mask this effect (White et al. 2011).

Additionally, we found that *Wolbachia* prolonged juvenile development time (i.e. time from egg to adult emergence) in all offspring types (Figure 4.6a). Such *Wolbachia*-induced developmental delay has also been shown in mosquitoes (e.g. McMeniman & O'Neill 2010; Ross et al. 2014). In our study, slower development might have been an adaptive strategy by *Wolbachia*-infected thrips to obtain more food during immature stages, compensating for a cost of reduced resource allocation at the embryonic stage, and maintain the fitness advantage conferred by *Cardinium* on survival and adult longevity. Slower development could, on the other hand, constitute a negative fitness aspect, with delaying the maturity and reducing population growth rate (Livdahl & Sugihara 1984; Suh et al. 2017). Prolonged development may also increase mortality rate before reproduction due to risks of predation and parasitism in nature (Prokopová et al. 2010). Moreover, it may increase the duration of exposure to any natural antibiotics before maturity that may consequently compromise *Wolbachia* titre, transmission efficiency and/or CI levels (Clancy & Hoffmann 1998). Therefore, we can conclude that I_{CW} thrips may be less competitive relative to I_C.

Finally, the more beneficial role of *Cardinium* may promote its transmission success in a haplodiploid population in which excessive male production (due to MD-CI) in incompatible mating (as demonstrated for *P. kellyanus*; Nguyen et al. 2017) may cast a serious hindrance for CI invasion efficiency (Egas et al. 2002), and may have evolved as an adaptation to overcome this hindrance. On the other hand, such a beneficial role may be less essential for *Wolbachia* to ensure an invasion success in *P. kellyanus* populations, given that compared to *Cardinium*, it induces a more efficient CI type (FM-CI) (Nguyen et al. 2017). Moreover, it has been also hypothesised that a less beneficial (or cost-inflicting) symbiont can 'hitchhike' with a more beneficial partner, and invade a host population more efficiently (Vautrin et al. 2007). Our study suggests that *Wolbachia* may fall in this category, justifying its considerable prevalence (although lower than *Cardinium*) in *P. kellyanus* populations (Nguyen et al. 2016), despite its seemingly parasitic effects revealed here. This finding may also explain as to why *Wolbachia*-only infected *P. kellyanus* are almost absent in nature (Nguyen et al. 2016; personal observation) despite *Wolbachia* induced complete CI and had a high transmission fidelity in laboratory crossing experiments (Nguyen et al. 2017) – two important parameters that are important for its invasion success (Zug & Hammerstein 2015). A similar scenario has been shown for the cost-inflicting X-type endosymbiont of the pea aphid which can only presist in populations in coinfection with *Hamiltonella* which provides a host benefit (Doremus & Oliver 2017).

4.5.3 Frequent absence of *Wolbachia* in field populations of *P. kellyanus*

The factors contributing to a lower prevalence of *Wolbachia* than *Cardinium* in Australian (native) populations of *P. kellyanus*, or its complete absence in invasive ranges (New Zealand and Mediterranean Region) are elusive (Nguyen et al. 2016). However, the findings of this study may attribute the lower prevalence of *Wolbachia* at least partly to its revealed fitness costs. Such a negative selection might have also been combined with a likely severe population bottleneck in introduced propagule (Ross et al. 1993), leading to a very low frequency of I_{CW} (below the equilibrium threshold required for establishment) and eventually a complete loss of I_{CW} (Turelli 1994). Alternatively, *Wolbachia* may have been acquired by native populations after introduction of I_C to the invasive ranges, and the lower prevalence of *Wolbachia* than *Cardinium* (Nguyen et al. 2016) and its higher fitness costs (Nguyen et al. 2017 and this study) might indicate a more recent association with the host. This requires further detailed study of the infection prevalences combined with host phylogeographic and population genomic analyses.

4.6 CONCLUSION

In summary, our study revealed that *Cardinium* and *Wolbachia*, already known to induce CI in *P. kellyanus* (Nguyen et al. 2017), also have other host fitness effects that may contribute to their invasion success. In particular, *Cardinium* biases sex allocation towards females by manipulating the egg size-mediated fertilization system. *Cardinium* achieves this by increasing host fitness and ensuing maternal resource allocation towards egg size. The fitness advantage of bearing *Cardinium* was also reflected in offspring size and survival. Lower fitness and resource allocation into egg size was seen in both cured and naturally uninfected thrips, suggesting that it is not a potential long term effect of microbiome disruption with antibiotic curing. More surprisingly, *Wolbachia* appeared to act antagonistically on the fitness advantage of bearing *Cardinium*, which may explain why it has lower prevalence in native populations and is absent in some invasive populations (Nguyen et al. 2016). However, we did not find direct evidence for fitness costs of *Wolbachia* in *P. kellyanus*. Further work could use population cage experiments with I_{CW} and I_C individuals to explore the effects of the two endosymbionts more directly and in competition. In addition, although we were unable to establish a I_W line, development of such a line would help decipher the net effect of *Wolbachia* on egg size-mediated fertilization success and other fitness traits. Finally, egg size-mediated fertilization might result from interactions between host-endosymbiont genetic conflicts (Werren 2011) and sexual conflicts (Shuker et al. 2009) over the sex ratio. Therefore, our findings direct future studies towards understanding the evolutionary forces driving egg size and sex allocation in haplodiploids.

4.7 APPENDIX III

4.7.1 Supplementary methods:

4.7.1.1 Establishment of thrips populations of different infection lines

For establishment of laboratory populations of *P. kellyanus*, sampling was conducted from lemon trees, in a citrus orchard in Kulnura, Mangrove Mountain, NSW, Australia on 11 January 2017. First a double-infected line (I_{CW}) (Figure 4S1A) was established by combining offspring of isofemale lines tested positive for co-infection with *Cardinium* and *Wolbachia* as explained in Katlav et al. (2021). This line was then expanded and maintained for seven generations in a controlled environmental chamber at 20±2 °C, 70±2% RH and 16:8 h (L:D) prior to antibiotic treatment. For each generation ten female offspring were randomly sampled to check for infection status using diagnostic PCR tests (see Katlav et al. 2021; Chapter 2) (see section 1.4 for more information). All samples of each generation were confirmed as double-infected (100%), indicating a very high transmission efficiency for *Cardinium and Wolbachia* as shown by Nguyen et al. (2017).

Antibiotic treatments were applied to the double-infected line (I_{CW}) with the aim to establish lines that are either *Cardinium*-only infected, *Wolbachia*-only infected, or completely uninfected. We followed the protocols established by Nguyen et al. (2017) with some modifications and additions:

Uninfected line (treated) (U): To establish the uninfected line (U) more than 1,000 newly emerged adult thrips (males + females) of I_{CW} line were treated with rifampicin (50 mg/ml) (Sigma-Aldrich) mixed with 50% w/v honey and distilled water, absorbed by cotton wool, for seven days, with provision of fresh antibiotic every 48 h (Figure 4S1B). This was repeated over three generations. The infection status of thrips was verified in each generation using nested PCR for *Wolbachia* and standard PCR for *Cardinium* (Table 1) as explained by Nguyen et al. (2020) to confirm that *Cardinium* and *Wolbachia* had been removed completely. The third generation was 100% uninfected. This line was maintained for more than seven generations for population expansion and recovery from antibiotic treatment before commencement of the experiment.

Naturally uninfected line (NU): In addition, we established a naturally uninfected (NU) line by combining offspring of two uninfected isofemale lines collected from the same source population as I_{CW} in the same date. These lines tested negative for *Cardinium* and *Wolbachia* using nested PCR for *Wolbachia* and standard PCR for *Cardinium* (Table 4.1). This NU colony has been kept for 12 generations before the experiment, but it was lost after the experiment (in generation 15) due contamination with predatory mesostigmatic mites.

Cardinium singly-infected line (I_C): More than 100 female pupae of the I_{CW} line were placed into a small Petri dish (diameter 30mm; height 15mm) with moistened filter paper and kept until adult emergence. Newly-emerged virgin females were treated with tetracycline hydrochloride (50 mg/ml) (Sigma-Aldrich) mixed with 50% w/v honey and distilled water and absorbed by cotton wool for 48 h (Figure 4S1C). Surviving females (N=38) were individually transferred to a small Petri dish harbouring ten uninfected males (from U line) for mating. After observation of mating the couples were individually transferred to a small container with lemon and pollen supply (38 isofemales). Nine isofemales died before reproduction. Once the adult offspring of the next generation emerged, isofemales (preserved in ethanol 100% after death) and 2-3 randomly sampled female offspring were tested for infection status with diagnostic PCR assays. Only three isofemales were *Cardinium*-only infected. These three isofemale lines were kept separately for one more generation. In the next generation, ten and five randomly sampled females and males respectively, from each line were subjected to diagnostic PCR assays to verify the infection status. All three lines were 100% *Cardinium*-only infected. Subsequently these three lines were combined and maintained in a larger container with lemon and pollen supply for more than seven generations for population expansion and recovery from antibiotic treatment before commencement of the experiment.

Unsuccessful efforts for establishment Wolbachia singly-infected line (I_W): Rifampicin and tetracycline treatments (using the same protocols as above) have not led to establishment of a *Wolbachia*-only infected line. Thus, more selective antibiotics for elimination of Cytophaga like bacteria (including *Cardinium*) was used (Morimoto et al. 2006). More than 200 female pupae of the I_{CW} line were transferred to a Petri dish (diameter 90 mm; height 15 mm) with moistened filter paper and kept until adult emergence. After emergence of adult females, 100 female individuals were treated with streptomycin sulfate (50 mg/ml) (Sigma-Aldrich) for 48 h and the remaining 100 females were treated with chloramphenicol >98% (50 mg/ml) (Sigma-Aldrich) for 48 h. After antibiotic treatments, the survivors of each treatment were transferred to a single Petri dish (supplied with pollen) harbouring ten uninfected males (from U line) for mating. After observation of mating the couples were individually transferred to a small container with lemon and pollen supply (64 isofemales). Subsequently, females of each treatment were individually transferred to a small container supplied with lemon and pollen. Once the next generation adult emerged isofemales (preserved in ethanol 100% after death) and 2-3 randomly sampled female offspring were tested for infection status with diagnostic PCR assays. All the tested individuals were still double infected (some with lower PCR signal for *Cardinium* or both endosymbionts in treatments with streptomycin sulfate), which suggested that these protocols were not suitable for establishment of a *Wolbachia* singly-infected line.

Different infection lines were maintained in separate boxes (in a separate incubator to avoid cross contamination) supplied with lemon on top of a thin vermiculite layer. Pollen of *Typha* sp. (cumbungi) were sprinkled on the lemons as an additional food source twice a week. Thrips population maintenance and experiments were done in controlled environmental chambers at 20±2 °C, 70±2% RH and 16:8 h (L:D) photoperiod.

4.7.1.2 Establishment of evenly-aged parental females and males

In each experimental cohort, we randomly sampled 200 females from each infection line and transferred them to eight separate small plastic containers (700 mL; diameter 10cm; height 11.5cm) each containing a single similar-sized fresh high-quality lemon with a small filter paper on the bottom (each container harboured 25 female individuals). Females were removed after they had laid eggs for 24 hours. After emergence of larvae we restricted the number of ~80 individuals per container to ensure equal larval density. The thrips larvae were kept on the individual lemons supplied with ad libitum *Typha sp.* pollen renewed every second day until the pupal stage at which different sexes are readily recognizable (Katlav et al. 2021). Female and male pupae of each container were separated and placed into small Petri dishes (diameter 30 mm; height 15 mm) with moistened filter paper and kept until adult emergence. This method established same-aged parental females and males reared under controlled population densities for the experiments.

4.7.1.3 Controlling for parental relatedness in crossing experiments

To control for parental relatedness in our crossing experiments we ensured that each mating event did not occur between siblings. As explained above (section 1.2), each infection line in each experimental cohort comprised eight small containers (with a single lemon) containing male and female offspring. Offspring of each container were produced by different set of female individuals. Each mating event in our crossing experiments took place between a randomly-sampled male of one container and randomly-sampled female of another container (both from the same experimental cohort and infection line) (Figure 4S2).

4.7.1.4 PCR diagnosis for verification of endosymbiont infection

The infection status of each line was verified before the experiment using 20 randomly selected individuals per line and, subsequently, for all individuals included in the fitness assays at the end of the experiment via diagnostic PCR. DNA extraction was conducted using a 5% Chelex solution (10 mM Tris-HCl; pH 8.0) following the method explained by Kobayashi et al. (2013). Standard PCR (and nested PCR if required) techniques (Nguyen et al. 2016) were used to test the infection status of each thrips with *Cardinium* and/or *Wolbachia* using *Cardinium*-specific and *Wolbachia*-specific primers (Table 4S1). The DNA quality of the individuals was evaluated by amplifying COI (Table 4S1).

4.7.1.5 Examining the unset of reproduction across different infection lines

To examine the effect of infection status on the onset of reproduction, we compared the pre-oviposition period of both virgin and mated females across different infection types. For this purpose, a set of 48 females (eight virgin and eight singly-mated females of each infection line) were individually transferred to oviposition chambers with ad libitum food supply (see Katlav et al. 2021; Chapter 2). Females were monitored every 24 hours until first oviposition occurred.

Two females died before first oviposition, and were thus excluded from the analysis. The analysis was performed using a general linear model (GLM) with ‘infection type’ and ‘mating status’ as fixed factors. The model did not detect an effect of infection type on pre-oviposition ($F_{2,46}$ = 0.89, p = 0.42); nor did it show an effect of mating status ($F_{1,46}$ = 0.18, p = 0.68) and its interaction with infection type ($F_{2,46}$ = 0.16, p = 0.94).

4.7.2 Supplementary tables

Table 4S.1 Primers used in this study for diagnostic PCR assays.

PCR	Primer name	Primer sequence (5’ -3’)	Target gene	Reference
Standard PCR	81F	TGGTCCAATAAGTGATGAAGAAAC	*Wsp (Wolbachia)*	Zhou et al. 1998
	691R	AAAAATTAAACGCTACTCCA		
Nested PCR	81F	AAAAATTAAACGCTACTCCA	*supergroup B specific (Wolbachia)*	Zhou et al. 1998
	522R	ACCAGCTTTTGCTTGATA		
Standard PCR	CLOf1	GGAACCTTACCTGGGCTAGAATGTATT	*16S rRNA Cardinium*	Duron et al. 2008
	CLOr1	GCCACTGTCTTCAAGCTCTACCAAC		
Standard PCR	LCO1490	GGTCAACAAATCATAAAGATATTGG	*COI*	Folmer et al. 1994
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA		

Table 4S.2 Supplementary results of statistical analyses of fitness traits (all factors and experimental cohorts analysed together) using Three-way ANOVA or ANCOVA with *F* and *p* values showing the effects of the fixed factors and covariate, if applicable.

Biological characteristic/factor	df, N	*F* (or χ^2)	*p*
Average egg volume			
Infection type	2, 181	21.34	**<0.001**
Mother type	2, 181	71.81	**<0.001**
Cohort	2, 181	0.61	0.543
Infection type × Mother type	4, 181	0.77	0.547
Infection type × Cohort	4, 181	0.34	0.847
Mother type × Cohort	4, 181	1.12	0.219
Infection type × Mother type × Cohort	8, 181	1.16	0.325
Covariate (mother forewing)	1, 181	12.22	**<0.01**
Maximum egg size			
Infection type	2, 181	23.01	**<0.001**
Mother type	2, 181	61.05	**<0.001**
Cohort	2, 181	0.102	0.903
Infection type × Mother type	4, 181	0.37	0.827
Infection type × Cohort	4, 181	0.63	0.641
Mother type × Cohort	4, 181	1.90	0.462
Infection type × Mother type × Cohort	8, 181	1.97	0.462
Covariate (mother forewing)	1, 181	3.94	**0.048**
Minimum egg size			
Infection type	2, 181	12.50	**<0.001**
Mother type	2, 181	50.17	**<0.001**
Cohort	2, 181	1.98	0.141
Infection type × Mother type	4, 181	1.29	0.270
Infection type × Cohort	4, 181	0.31	0.872
Mother type × Cohort	4, 181	0.68	0.606
Infection type × Mother type × Cohort	8, 181	0.48	0.868
Covariate (mother forewing)	1, 181	7.72	**<0.01**
Egg size range variation (ERV)			
Infection type	2, 181	0.91	0.405
Mother type	2, 181	1.19	0.308
Cohort	2, 181	1.85	0.161
Infection type × Mother type	4, 181	0.50	0.734

Infection type × Cohort	4, 181	1.21	0.309
Mother type × Cohort	4, 181	0.42	0.796
Infection type × Mother type × Cohort	8, 181	0.78	0.619
Covariate (mother forewing)	1, 181	1.04	0.309
Copulation time			
Infection type	2, 100	0.122	0.884
Mated mother type	1, 100	0.001	0.99
Cohort	2, 100	0.59	0.55
Infection type × Mother type	2, 100	0.25	0.779
Infection type × Cohort	4, 100	1.28	0.282
Mother type × Cohort	2, 100	0.39	0.676
Infection type × Mother type × Cohort	4, 100	0.68	0.602
Covariate (mother forewing)	1, 100	0.01	0.911
Covariate (father forewing)	1, 100	0.13	0.716
Onset of reproduction (day)			
Infection type	2, 53	0.34	0.714
Mother mating status (MMS)	1, 53	0.18	0.673
Infection type × MMS	2, 53	0.33	0.721
Mated mother size (forewing length)			
Infection type	2, 102	4.20	**0.018**
Mother type [M(♂) or M(♀)]	1, 102	6.61	**0.012**
Cohort	2, 102	0.50	0.609
Infection type × Mother type	2, 102	0.22	0.803
Infection type × Cohort	4, 102	0.82	0.519
Mother type × Cohort	2, 102	0.23	0.795
Infection type × Mother type × Cohort	4, 102	0.71	0.586
Father size (forewing length)			
Infection type	2, 102	0.17	0.837
Mother type [M(♂) or M(♀)]	1, 102	1.07	0.303
Cohort	2, 102	0.03	0.967
Infection type × Mother type	2, 102	0.30	0.739
Infection type × Cohort	4, 102	0.44	0.775
Mother type × Cohort	2, 102	0.07	0.974
Infection type × Mother type × Cohort	4, 102	0.46	0.762

4.7.3 Supplementary figures: *Figures 4S1-4S4*

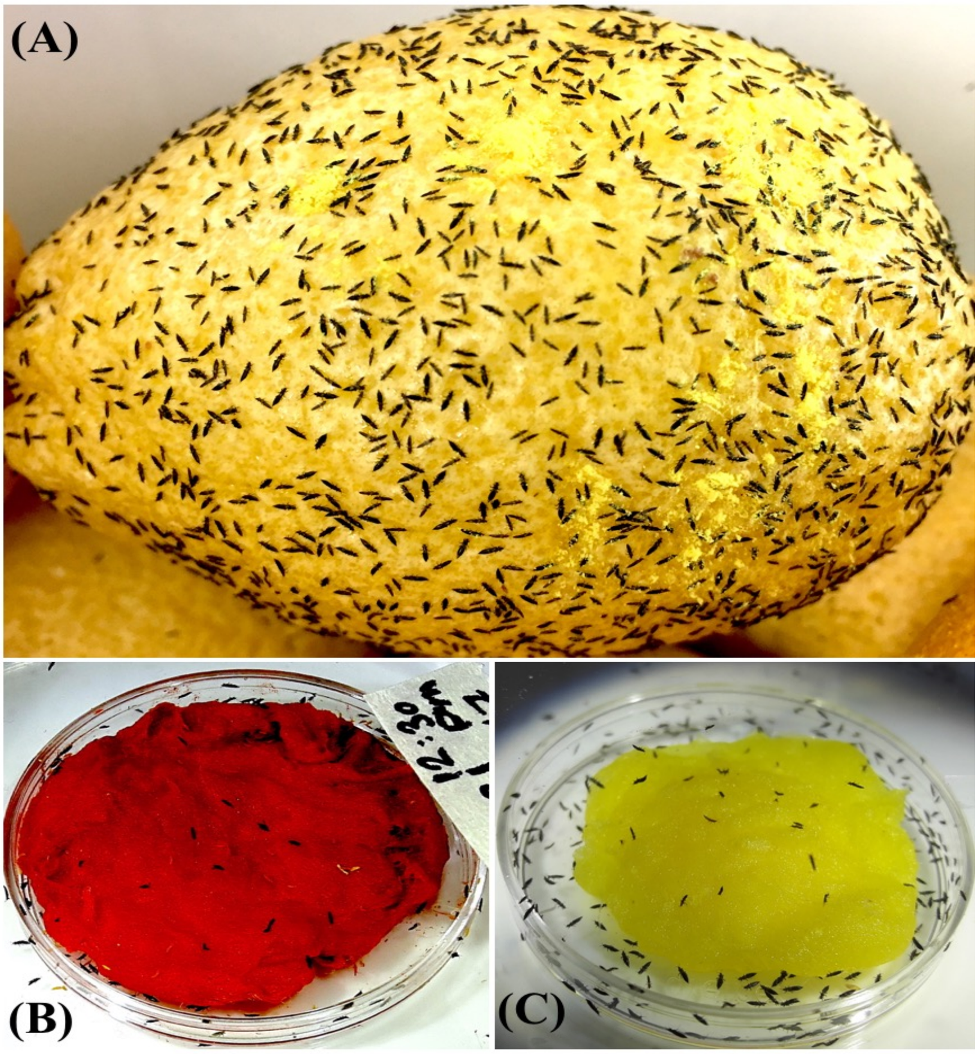


Figure 4S.1 (A) Laboratory population of *Pezothrips kellyanus* infected with *Cardinium* and *Wolbachia* (I_{CW} line); (B) Treatment with rifampicin (50 mg/ml) (Sigma-Aldrich) mixed with 50% w/v honey and distilled water absorbed by a cotton wool; (C) treated with tetracycline hydrochloride (50 mg/ml) (Sigma-Aldrich) mixed with 50% w/v honey and distilled water absorbed a by cotton wool.

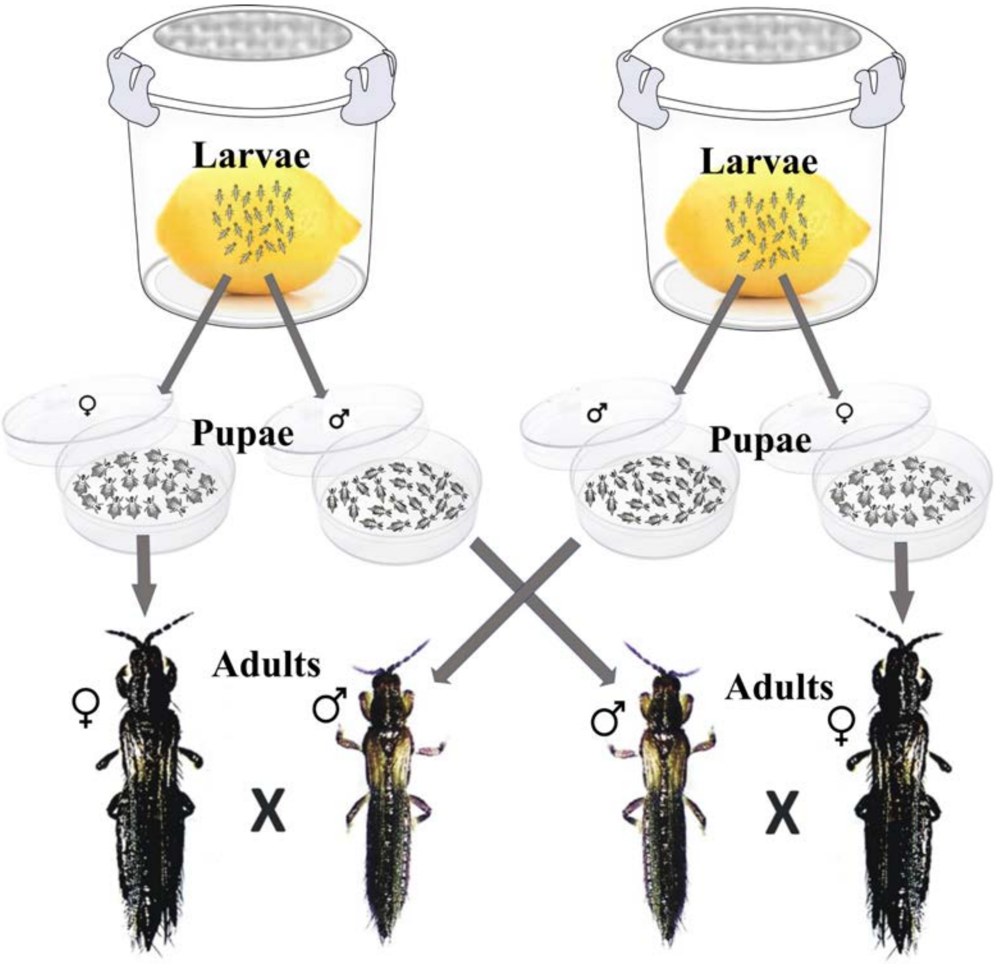


Figure 4S.2 Non-sib-mating protocol for controlling the parental relatedness in the crossing experiments. Each mating event took place between one randomly sampled male of one container and randomly sampled female of another container.

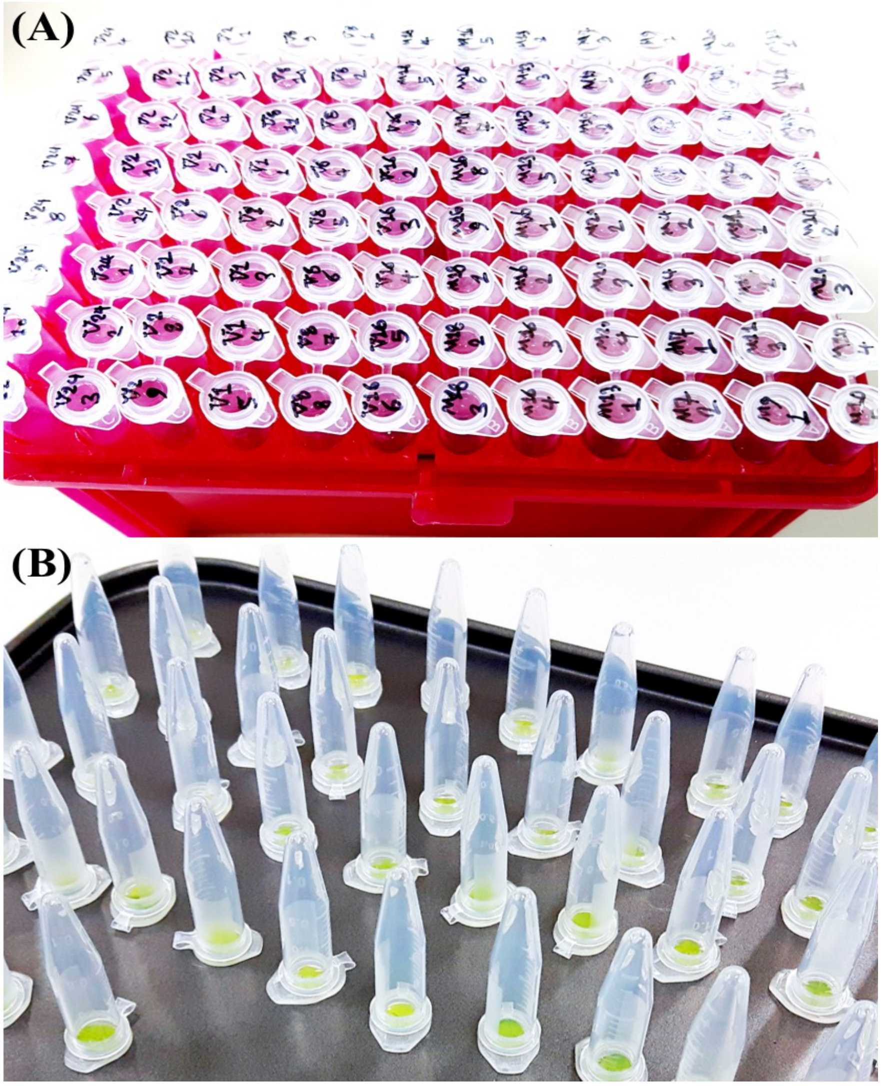


Figure 4S.3 (A) Embryonic chambers (PCR tubes with individual attached caps; 0.2ml) with each tube containing one egg of *Pezothrips kellyanus* placed on an agarose gel (0.6 % w/v; Agarose I, AMRESCO, USA) which covered the interior part of tube's cap. (B) Postembryonic chamber (1.5 ml microcentrifuge tubes) containing a fresh lemon leaf disc (0.8 cm diameter) placed in the interior part of lid on which an ad libitum amount of *Typha* sp. was sprinkled for the development of neonate larvae to adult stage.

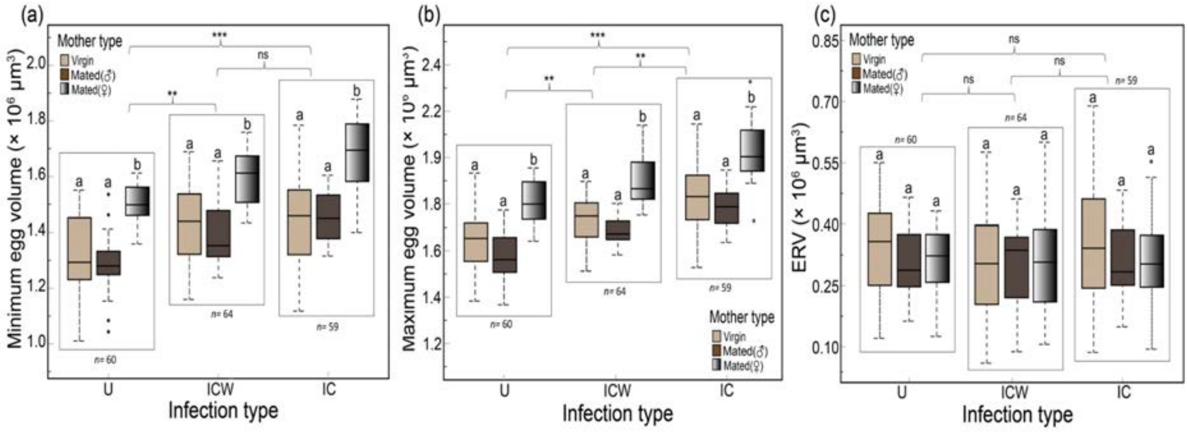
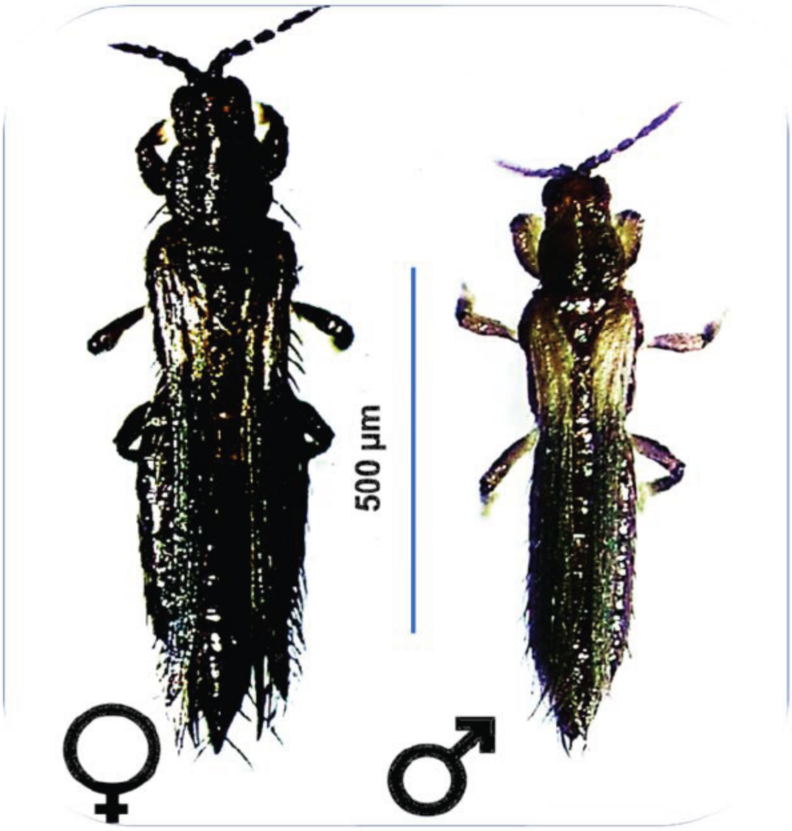


Figure 4S.4 (a) Minimum egg volume ± SE across different infection types and mother types. (b) Maximum egg volume ± SE across different infection and mother types. (c) Egg size range (egg size$_{max}$ - egg size$_{min}$) variation (ERV) ± SE across different infection and mother types. Different lowercase letters above the bars denote statistical differences between offspring types ($p < 0.05$) within the corresponding infection types. Asterisks denote statistical difference levels between infection types and mother types (**$p < 0.01$; ***$p < 0.001$; ns = not statistically significant; three-way ANOVA followed by Tukey test for multiple comparisons; n = number of mothers examined).

CHAPTER V

GENERAL DISCUSSION



Pezothrips kellyanus **female (left) and male (right)**

5.1 Overview

This PhD study investigated the mechanism of sex allocation in Kelly's citrus thrips, *Pezothrips kellyanus*, an economically important Australian native thrips species which is invasive in Mediterranean countries, New Zealand (Nguyen et al. 2016) New Caledonia (Bournier & Mound 2000) and Hawaii (Vassiliou 2011). It has also been reported from Chile (Vassiliou 2011) albeit this is questionable because extensive survey programs of the Chilean Ministry of Agriculture did not detect it there (personal communication Renato Ripa). For the first time for any haplodiploid insect, this study identified a finely tuned egg size-mediated fertilization mechanism which modulates sex ratio and is influenced by mating (see Chapter 2). The study further disentangled the underlying factor (maternal condition) that is responsible for the constrained sex allocation (extremely male-biased brood production) in a fraction of mated *P. kellyanus* females resulting in the expression of split sex ratios (see Chapter 3) as previously recorded in this species (Nguyen 2015). Furthermore, the study assessed the role of the two common bacterial endosymbionts *Wolbachia* and *Cardinium* in the reproductive biology of this species, and the mechanism by which they manipulate sex allocation. Both endosymbionts have previously been shown to independently induce cytoplasmic incompatibility (CI) in incompatible crosses which lead to male female mortality (caused by *Wolbachia*) or partial male development (caused by *Cardinium*) of fertilised embryos (Nguyen et al. 2017). This study demonstrated that *Wolbachia* and *Cardinium* in *P. kellyanus* also affect resource and sex allocation as well as trade-offs between reproductive and life history/fitness traits (see Chapter 4).

5.2 Key findings

5.2.1 Egg size-mediated sex allocation in *P. kellyanus* and the role of endosymbionts

Egg size matters for sex allocation

The present study showed that the mechanism whereby females in *P. kellyanus* regulate sperm access to her eggs to control sex allocation is dependent on egg size. Egg size-dependent fertilisation control is a strategy that allows sex-specific provisioning of resources towards individual eggs due to the sex-specific fitness returns from female and male offspring (Trivers & Willard 1973; Badyaev et al. 2006). This study showed that female thrips hatch from larger eggs, and male thrips from smaller ones. This sexual dimorphism in egg size has long been established for many bird species in which male eggs are generally larger than female eggs (Oddie 1998; Saino et al. 2020). We suggested that this asymmetric egg size provisioning in thrips occurs prior to fertilization. In many insects (including thrips), fertilization takes place just before oviposition (Boudreaux 1963; Chapman 1998; Helle 1967); therefore, unlike in birds (Cordero et al. 2000; Oddie 1998) a mother does not have an opportunity to invest more resources into female embryos after fertilization. Therefore, in *P. kellyanus*, investment into eggs before fertilization leads to oocytes with a broad range of sizes at the ovulation stage after which larger eggs are more likely fertilized than smaller eggs.

Mechanosensory mechanism of fertilization in insects

Many studies on haplodiploid and diplodiploid insects provide empirical evidence that sperm access to eggs is linked to direct mechanical stimulation of spermathecal muscles (see Chapman 1998). Dallai et al. (1996) detected several retractor muscles around the spermathecal duct of female thrips suggesting these muscles may control sperm supply to the fertilization chamber (vagina). Entrance of a matured oocyte into the fertilization chamber triggers a pressure on the terminal abdominal ganglia and the spermathecal opening that results in the contraction of the spermathecal duct muscles and compression of the spermatheca so that sperm is pushed into the fertilization chamber (Heming 1995; 1970). The present study found that there is a greater chance that larger eggs stimulate the spermathecal muscles and therefore receive sperm stored in the spermatheca. Egg size-dependent fertilisation has previously been demonstrated for the spider mite *T. urticae,* as eggs beyond a particular size threshold had a 93% chance of fertilization and developed into females (Macke et al. 2011). However the mechanism regulating this in mites remains unknown. In mites, sperm leaves the spermatheca and enters the ovarioles while the oocytes are still immature and vitellogenesis is still ongoing (Boudreaux 1963; Helle 1967). This is similar to what has been observed for a few hemipterans where sperm fertilises oocytes while they are still in the ovary or oviduct (Chapman 1998). Clearly, further studies are required to reveal mechanisms that regulate sex-specific provisioning of eggs in arthropod species with egg size sexual dimorphism where fertilization occurs during vitellogenesis and prior to ovulation and therefore not in the vagina.

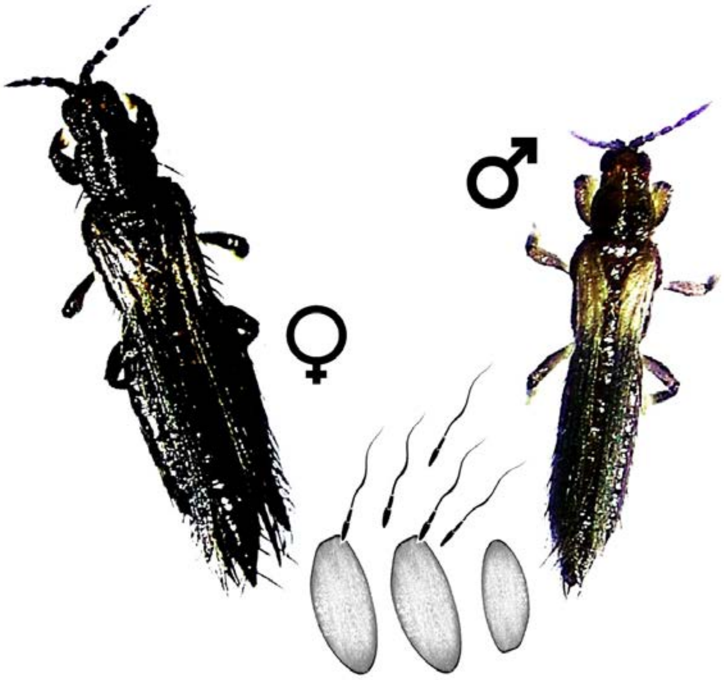


Figure 5.1 Egg size-mediated sex allocation in *Pezothrips kellyanus*. Females regulate access of sperm stored in their spermathecae to eggs depending on egg size after mating, and thereby control sex allocation. Larger eggs are more likely to be fertilized and develop to females, whereas smaller eggs remain unfertilized and become males.

In thrips, fertilization of eggs occurs in the vagina, by sperm entering through small V-shape pores, known as micropyles, situated posteriorly on the outer chorion (Heming 1995). It has been suggested that after ovulation an egg needs to be rotated by vaginal muscles in such a way that the micropyles face the opening of the spermathecal duct so that one or two sperm cells are pumped into the micropyles (Moritz 1982; 1988; Heming 1995; Jangra et al. 2020). The number of micropyles is variable among eggs and females although this is not extensively studied in thrips (see Heming 1995). A study on Lepidoptera has hypothesized that a reduced number of micropyles of an egg can increase the chance of infertility (Iossa et al. 2016). Therefore, it is possible that smaller eggs suffer from an absence or limited size and/or number of micropyles which may hinder the process of fertilization and syngamy (Lacerda et al. 2010).

On the other hand, a higher number of micropyles may also increase the risk of polyspermy and failure in embryonic development, as suggested for some insects (Iossa et al. 2016) and marine vertebrates (Zelazowska 2010), yet this remains to be explored for thrips.

Egg size adjustment as a strategy of sex allocation

The finding that female eggs are larger than male eggs is consistent with the sex allocation theory that predicts whenever one sex (here female) benefits more than the other from good conditions, offspring of this sex should be allocated more resources than those of the other sex (Trivers & Willard 1973). Therefore, egg size-mediated fertilization is not only a mechanism of sex ratio control but also a means to adjust resource allocation to individuals of one sex compared to the other (i.e. allocation to each female individual is higher than to male individuals). This is similar to the resource allocation strategy found in some parasitoid wasps, where female-destined eggs are oviposited in larger hosts and male-destined eggs in smaller hosts (Charnov et al. 1981). Likewise, in social bees, queens lay female-destined eggs in larger cells (highly provisioned cells) and male-destined ones in smaller cells (Radmacher & Strohm 2010). In both cases, females probably can decide to fertilize the eggs before oviposition based on the chemical and/or visual cues they receive from hosts' size and age, or the surface or size of the oviposition site (Mackauer et al. 1996; Ueno 1999; Stoepler et al. 2011).

Higher provisioning for female offspring is favourable as female offspring generally require more resources for development and survival than male offspring (Weatherhead & Teather 1994). However, the mechanism by which females permit or withhold the fertilization of eggs in response to the host size or amount of cell provisions is unknown. In some ichneumonid wasp species, for example, the pattern of abdominal tip movements during oviposition is a sign of a female's facultative control over the sex of oviposited eggs, with oviposition of female eggs taking longer than male ones. This is probably to increase abdominal pressure and the chance of sperm access to eggs (Cole 1981; Ueno 1995). On the other hand, host size-specific sex allocation in hymenopterans may be associated with a female's ability in selecting hosts based on sex allocation and the sequence of egg laying. A female might first lay clutches which contain more male offspring (Zhang et al. 2019) in smaller hosts and the subsequent clutches with more female offspring in larger hosts, and this may be an outcome especially when host resources are limited and variable in size. In this scenario, increase in fertilization success and female production in subsequent clutches could be due to an increase in maternal resource allocation with mother age as shown in spider mites (Macke et al. 2012a). Several studies have shown that females maximize the egg size in subsequent clutches during the reproductive life span according to the temporal sex ratio variation in subsequent clutches (Badyaev et al. 2002; Plaistow et al. 2007; Penteriani et al. 2010). Macke et al. (2012) showed that in the spider mite *T. urticae* females gradually increase the egg size in subsequent clutches whereby they can maximize fertilization probability until sperm depletion results in old (and singly-mated) females with reduced egg provisioning. Occurrence of egg size sexual dimorphism has also been found in some solitary wasps (Budrienė et al. 2013; O'Neill 1985) and, therefore, testing of egg size-dependent fertilization mechanisms may shed light on the sex allocation theories and associated evolutionary scenarios in hymenopterans.

Egg size sexual dimorphism is a source of sexual size dimorphism

A positive correlation between egg size and offspring fitness may entail a driving source for the evolution of sexual size dimorphism (SSD) (Tammaru et al. 2010; Teder 2014). The majority of insects, including many species of Thripidae, display considerable SSD with females being generally larger than males (Tyagi et al. 2008; Tammaru et al. 2010). Nevertheless, the developmental basis of SSD is not well understood. Theory suggests that apart from the effects of sexual selection, fecundity selection, food competition and sex-specific fitness effects of body size (Hedrick & Temeles 1989), SSD in adults might also result from selection on sex-specific costs during juvenile development (Stillwell et al. 2010; Teder 2014). For example, studies of SSD in butterflies suggest a role for sex-specific growth rates and development time (Teder 2014). However, another strategy to provision for sex-specific costs at the juvenile stage is sex-biased maternal investment into embryos. This can result in the evolution of SSD in eggs, as documented for birds (Weatherhead & Teather 1994). In *P. kellyanus*, sex-specific egg size differences revealed in laboratory and field-collected individuals of a thrips species with adult SSD may confirm effects of egg provisioning on SSD, as recently acknowledged in another study (Tammaru et al. 2010). Specifically, the present study on *P. kellyanus* suggests that SSD is not only caused by the slower development of females (also see Teder 2014) but also affected by egg size and egg-size mediated fertilization (Figure 5.2).

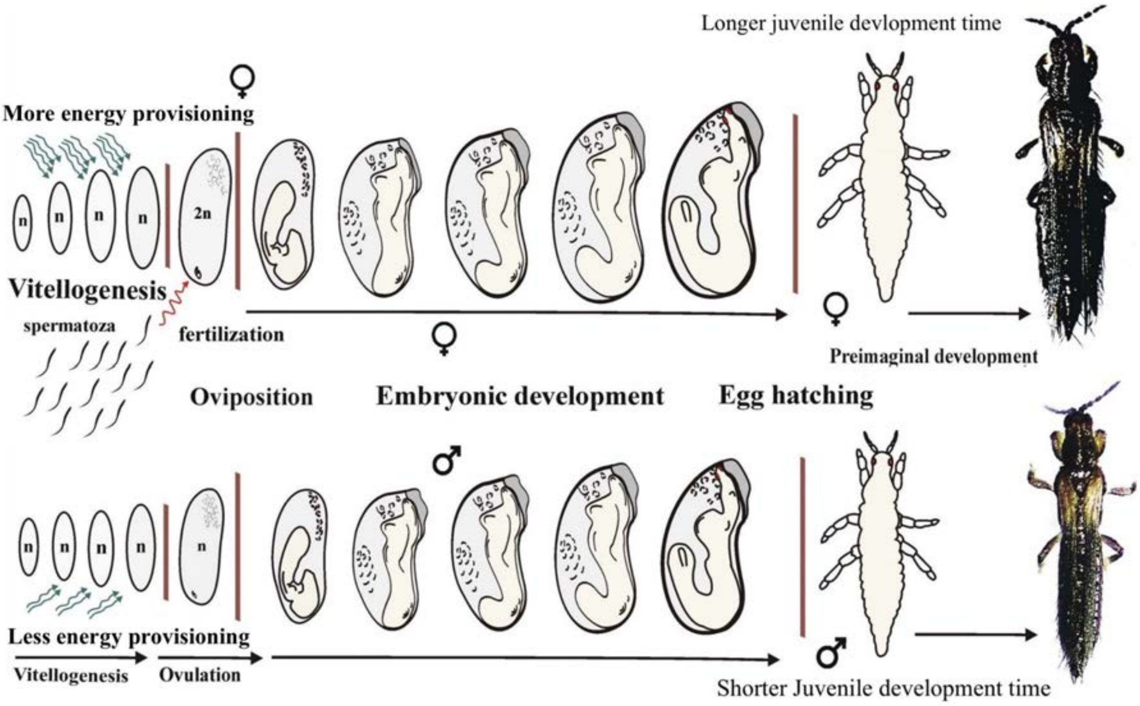


Figure 5.2 Conceptual diagram of the ontogenetic source of SSD in haplodiploid species such as *Pezothrips kellyanus* for which a size relationship was found between egg, larval and adult stages. Furthermore, the egg size difference between sexes occurred prior to fertilization and fertilization

occurred in a size-dependent fashion. Sex-specific differences in eggs size and juvenile development time can contribute to SSD.

Mating affects resource allocation towards eggs

Another key finding of this PhD study was that mating can change the patterns of resource allocation and reproductive strategies in terms of egg size and fecundity trade-off. In particular, mating caused mothers to produce larger eggs, and thereby more female offspring. In haplodiploid species males can only pass on their genes to daughters, since only females arise from fertilized eggs (Shuker et al. 2009; Gardner & Ross 2014). Therefore, incremental effect of mating on female resource allocation may be an expression of selection on males that favours production of daughters over sons. This selection is likely a consequence of sexual conflict between male and female preference over offspring sex ratio (Chapman et al. 2003; Helanterä & Ratnieks 2009; de la Filia et al. 2015). This potential conflict may be reflected in the fact that the effect of mating on egg size was not constant among all females, i.e. mating did not induce higher resource allocation in some females and they were thus constrained to produce male-biased offspring (see chapter 3 and general discussion – *section 5.2.2*).

Endosymbionts can affect fertilization success by manipulation of fitness and resource allocation

Maternally inherited endosymbionts favour higher female production in their host because these endosymbionts are not passed on by males (Hurst & Frost 2015). Therefore, they manipulate hosts reproduction to boost their maternal inheritance (Werren et al. 2008; Hurst & Frost 2015). Although, the analysis of some (but not all) endosymbiont host fitness effects might not always unveil beneficial impacts (sometimes detrimental, such as reduced fecundity and longevity loss), their overall impact on the hosts' reproduction should suffice their successful invasion of host populations (Zug & Hammerstein 2015). This is generally achieved by one of the four reproductive manipulations (as explained in Chapter 1) that promote the spread of endosymbiont in the host population by encouraging the production of infected female offspring over uninfected females (Stouthamer et al. 1993; Hurst & Jiggins 2000; Weeks & Breeuwer 2001). However, reproductive manipulation is not the only way whereby an endosymbiont can secure a successful invasion. Models developed for invasion dynamics of CI in haplodiploid species suggest that endosymbionts rely on several adaptive strategies other than CI to become established in host populations (Egas et al. 2002). One important example is an effect of *Wolbachia* on sex allocation such as increased daughter production in compatible matings (Werren & O'Neill 1997; Egas et al. 2002). The present PhD study established an effect of *Cardinium* in sex allocation for the first time. We showed that females infected with *Cardinium* only (or in co-infection with *Wolbachia*) had more resources for egg provision than uninfected females, as can be seen in their larger body size and egg volume. This higher fitness due to *Cardinium* reduced the probability of a female to have a constrained sex allocation and male-biased production, which is consistent with conditional sex allocation and egg size-mediated fertilization patterns revealed for *P. kellyanus*.

In *P. kellyanus*, *Cardinium* induced male development of fertilized eggs (MD-CI) in uninfected mothers mated with infected males (Nguyen et al. 2017). Theory suggests that MD-CI in haplodiploids minimises the invasion success of endosymbionts in host populations due to the higher production of uninfected males that increases the chance of compatible mating events and production of uninfected females (Egas et al. 2002). Therefore, manipulation of sex allocation towards more infected females by *Cardinium* may theoretically serve as a compensatory strategy to reduce the invasion threshold. Such a sex allocation effect has also been shown with other endosymbionts that do not induce CI or any other reproductive manipulation. For example, the bacterium *Hamiltonella* induces higher fertilization success and female-biased sex ratio that increase its prevalence among *B. tabaci* biotypes and other whiteflies species (Gueguen et al. 2010). Several *Hamiltonella* isolates in whiteflies have been found to be responsible for synthesis of five B vitamins (Rao et al. 2015). Wang et al. (2020) further found a similar function in another bacterium, *Arsenophonus*, and found that higher fertilization success induced by *Hamiltonella* and *Arsenophonus* is associated with their role in production of these B vitamins. Such a B vitamins biosynthetic contribution has likewise been shown for *Wolbachia* in some planthoppers that enhance reproductive effort in infected females (Ju et al. 2020). In another study *Wolbachia* and *Cardinium* co-infection in a spider species showed a pivotal effect on fat and amino acid synthesis in hosts. It is therefore important to see if the effect of *Cardinium* on *P. kellyanus* fitness and egg size mediated sex allocation is rendered through a change in host nutrition and metabolic functions.

5.2.2 Constrained sex allocation in *P. kellyanus*: causes, consequences and endosymbiont effects

Constrained sex allocation is driven by maternal condition

Bias in sex allocation can be driven by a variation in maternal fitness or condition (Trivers & Willard 1973; Charnov 1982). Theory suggests that female offspring, relative to male offspring, benefit more from increased maternal allocation (Trivers & Willard 1973). Therefore, mothers in good condition and with higher energy budgets are predicted to produce more offspring of the sex with higher fitness returns compared to those in improper condition (Trivers & Willard 1973). For the first time, this PhD study found a post-mating constrained sex allocation associated with maternal condition in a thrips species: smaller females produced M-broods, whereas larger females produced female-biased broods. This constraint in smaller females was further manifested in their smaller egg size prior to mating, suggesting a failure in fertilization (or withholding of fertilization) as a consequence of lower energy budget available for egg provisioning despite successful insemination. This is similar to the spider mite *T. urticae* where larger females can produce larger eggs to increase the chance of fertilization and female production (Macke et al. 2011; 2012a; b).

Constrained sex allocation is not associated with paternal factors

In arthropods, the ability of males to bias offspring sex ratios has recently become a focus in sex allocation studies (Henter 2004; Shuker et al. 2006; Boivin 2013; Chirault et al. 2019). As males in haplodiploidy can pass on genes to the next generation only via daughters, they may actively attempt to affect sex allocation in females (Hawkes 1992). A male's ability in changing a female's sex allocation can be associated with several factors (Shuker et al. 2006): 1) mating ability; 2) insemination success; 3) ejaculate size and quality; 4) chemical composition of accessory fluids accompanying the ejaculates that facilitate fertilization in female (Gillott 2003; Chapman et al. 2000); and 5) selfish genetic elements (SGEs) that can manipulate the sex ratio after fertilization (post-zygotic constraints) (Werren & Stouthamer 2003; Verspoor et al. 2020).

For the first time in thrips we tested the involvement of paternal factors constraining sex allocation. Particularly, we examined insemination success, paternal SGEs (PSR-like factors), and other factors representing paternal fitness such as mating duration and male body size. Irrespective of their offspring sex ratio, all tested *P. kellyanus* males successfully transferred motile sperm in large quantities during mating, which suggests that constrained sex allocation is not due to failure in insemination. Moreover, mating duration or males size did not affect sex allocation in their mates. Beyond this, *Wolbachia* and *Cardinium* did not affect the insemination success and mating duration in *P. kellyanus*. Although *Cardinium* increased fertilization success through enhancing female fitness (expressed in body and egg size), its potential role on male fertility was not directly addressed. A study on the beetle *Tribolium confusum* showed higher fertilization success in females mated with of *Wolbachia* infected males compared to uninfected ones and this was due to higher fertility of *Wolbachia* infected males (Wade & Chang 1995). Some other studies on Lepidoptera also showed that males provide females with a nuptial gift or accessory gland product upon mating that may affect reproductive effort and sex allocation (Thornhill & Alcock 1983). Therefore, future quantitative analyses of ejaculate size (sperm count) and quality (e.g. sperm viability, morphology and motility) and biochemical assessment of accessory compounds may help provide an insight into male factors (and their interactions with endosymbionts) that may influence fertilization success, yet are not manifested in the parameters we assessed.

Studies have shown that males can regulate sperm allocation upon mating in response to a female's promiscuity and body size (Pizzari et al. 2003). Males can achieve this by spending longer time in mating (Friberg 2006), as it is often suggested that copulation duration is under male control. Males may assess female mating status (Singh & Singh 2004; Firberg 2006) and size using chemical and visual cues from female's body to adjust sperm number transferring to his mate (Siva-Jothy & Stutt 2003). In *P. kellyanus* neither male nor female body size affected mating duration. Nevertheless, some studies in *Drosophila* fruit flies have demonstrated the role of female cuticular hydrocarbons in mating duration (Friberg 2006). This may prompt a similar test on *P. kellyanus* in the future.

Sometimes male-related constraints act on a postzygotic stage. Of particular importance in haplodiploids are SGEs (Stouthamer et al. 2002; Werren 2011). PSR elements are extreme examples of SGEs that have been documented from three families of parasitoid waps: *Nasonia vitripennis* (Pteromalidae) (Werren 1991); *Trichogramma kaykai* (Trichogrammatidae)

(Werren & Stouthamer 2003) and *Encarsia pergandiella* (Aphelinidae) (Hunter et al. 1993). For the first time beyond hymenopterans, the current study examined a thrips species for the presence of a PSR distorter; yet it did not provide any evidence that the M-brood production in *P. kellyanus* is heritable in a non-Mendelian manner from father to son, rejecting the the presence of a PSR-like factor.

Is constrained sex allocation an evolutionary constraint or a strategy?

Constrained mated *P. kellyanus* females had a similar fecundity when compared with unconstrained females (Chapters 2 and 3), which strongly suggests that the maternal effect on the reproductive effort is manifested via directional selection on egg size, but not number. Smaller females are selected to produce smaller size but same number of eggs as larger females. This is to avoid fertilization of eggs with non-optimal size for female development. In *T. urticae* the expression of maternal effects on sex ratio was proved to occur along a continuous spectrum, ranging gradually from less daughters in smaller mothers to more daughters in larger ones. Nevertheless, *P. kellyanus* had a binary mode, with sex ratio showing a prompt switch from M-brood to F-brood production. Therefore, the split sex ratio in *P. kellyanus* could not solely be a fitness constraint that limits the egg size for fertilization; rather the sex ratio could be an outcome of adaptive maternal strategy in which female facultatively adjusts sex ratio in response to her energy budget. It is likely that egg size change is a consequence of maternal adaptive decision to avoid unnecessary resource investment for the production of males (Carranza 2002). Indeed, these two scenarios (a passive effect of fitness constraint versus adaptive maternal decision) are not mutually exclusive, and together suggest that female’s constraints can drive adaptive decision at the individual level to cooperatively incline the sex ratio towards equal sex ratio at the population level (Carl Düsing 1884; Fisher 1930). A parallel pattern is known for birds where the change in hormonal levels in subsequent clutches could constrain resource allocation, yet females overcome this constraint by controlling the sequence in which sexes are produced according to hormonal levels (Badyaev et al. 2003). Sometimes in birds, post-conception mortality of one sex may constrain mothers in sex ratio adjustment, and selection may favour compensatory adaptive decision by mother to operate sex-biased abortion or sex-biased infanticide to compensate for sex ratio biases in the population (Frank 1990).

Endosymbionts as a potential driver of split sex ratio in P. kellyanus

Maternally inherited endosymbionts favour female hosts to produce as many females as possible, whereas it is beneficial to females to increase male production once the population sex ratio becomes female-biased (Egas et al. 2002). Such diverged inter-genomic conflicts between endosymbiont and host interests over sex allocation have received substantial attention in empirical studies and were described in several reviews (Hurst 1992; Werren et al. 1988; Ross et al. 2010). Interestingly, such conflicts may mean that the interests of the endosymbiont are often in line with the interests of genes in males of haplodiploid species where paternal genes are likewise only inherited to daughters. Therefore, endosymbionts may favour male adaptations that increase the fertilization success in females, such as increase in female resource allocation towards egg size after mating as shown in this study. However, such mating induced egg size increment was activated only in larger females, and smaller mated females still produced smaller eggs that remained unfertilized. This constrained sex allocation was more intense in uninfected females and may be a strategy to avoid CI by withholding fertilization of small eggs with compatible sperms. Therefore, constrained sex allocation and the resulting split sex ratio might have evolved as a response to CI and/or female-biased sex ratios due to endosymbionts. A similar strategy for minimizing risk of CI has been attributed to polyandry in which multiply mated females can potentially exploit post-copulatory sexual selection (Zeh & Zeh 1996; Champion de Crespigny et al. 2008; Price & Wedell 2008). For example, studies in *Drosophila* fruit flies have suggested that evolution of polyandry in females serve as an arms race to suppress the cost of CI by exploiting the higher sperm-competitive ability of uninfected males over infected ones (Champion de Crespigny & Wedell 2006).

Although constrained sex allocation may theoretically hinder the invasion dynamics of *Cardinium* and *Wolbachia*, these endosymbionts (particularly *Cardinium*) show very high prevalence in Australian *P. kellyanus* populations (Nguyen et al. 2016). Analysis of other fitness trade-offs of infection is important to better understand population dynamics of endosymbionts and examine this counterintuitive pattern (Egas et al. 2002). The present study revealed that *Cardinium* confers fitness benefits on *P. kellyanus* (larger body size, juvenile survivor rate and adult longevity). Although, *Wolbachia* effect on host fitness was, in comparison, absent (juvenile survivor rate and adult longevity) or antagonistic (resource allocation, body size and development time), it appears that the overall interaction of both endosymbionts is sufficient to maintain the coinfection in host. Finally, these overall fitness advantages as well as induction of CI and female-biased sex allocation might have altogether overweighed the impeding effect of constrained sex allocation on invasion and establishment of both endosymbiont in *P. kellyanus*.

5.3 Future research directions

The egg size-mediated sex allocation found in this study may shed light on how females can adjust sex allocation in response to population density and environmental cues. Moreover, the findings raise interesting questions regarding the evolutionary forces driving adjustments in sex and resource allocation in haplodiploids and their interactions with endosymbionts. Therefore, several research questions await empirical answers in future studies:

Are the sex ratio findings in the present research indicative of the overall sex allocation strategy in P. kellyanus?

Sex allocation studies in Thysanoptera have mostly looked at the secondary or tertiary sex ratios instead of the primary sex ratio (at the oviposition stage). Sex-specific developmental mortality is potentially an important confounding factor for accurately assigning the sex ratio results to sex allocation patterns (Bondy & Hunter 2019). Although present study did not directly look at the primary sex ratio (this study analysed the sex ratios at pupal stage where the sexes differences become readily recognizable), females and males in *P. kellyanus* had similar juvenile survival. Therefore, it is very likely that the sex ratio results in our study are a reliable proxy of sex allocation patterns. However, future studies on primary sex ratios of *P. kellyanus* should verify the accuracy of this argument. Direct observation of sperm pronucleus in eggs can provide a clearer picture of fertilization process. Moreover, cytological analyses of reproductive anatomy and egg morphological structure such as micropyle size and number and their correlation with fertilization success can increase our understanding of mechanism of egg-size mediated fertilization. Application of DAPI (4′,6-diamidino-2-phenylindole) staining protocol and confocal microscopy can help verify fertilization by observing sperm pronucleus within the egg to directly determine the primary sex ratio (Bondy & Hunter 2019). Moreover, the localization of *Cardinium* and *Wolbachia* in thrips germ line at different developmental stages using ‘fluorescence in situ hybridization’ (FISH) may help elucidate when the CI alteration of sperm chromatin occurs in males (see Doremus et al. 2020). While this is not directly informative on how endosymbionts can manipulate female resource allocation decisions, studies of female ovaries and oocytes with similar strategies may provide some insights as to why higher resource allocation towards eggs is induced by *Cardinium* but not *Wolbachia* in females.

Effect of multiple mating on egg size and sex allocation

Although mating is generally regarded as a costly process, polyandry is a widespread reproductive behaviour among many species (Taylor et al. 2014). Several studies have shown a range of negative to positive effects of polyandry on fecundity and fertility of females (Arnqvist & Nilsson 2000; Morrow et al. 2002; Moya-Laraño & Fox 2006; Bayoumy & Michaud 2014). However, the role of egg size also needs to be incorporated in analyses of polyandry effects on reproductive fitness. For example, Macke et al. (2012a) showed a significant reduction in the fecundity of *T. urticae* in multiply mated versus singly mated females, although without a change in egg size, leading to a reduction in the reproductive investment. Such a fecundity cost could arise form an energetic cost of copulation or sexual harassment, as it is known in several other insects (e.g. Sakurai and Kasuya 2008). Despite an increase in egg size, Macke et al. (2012a) showed that the sex ratio became more male-biased in multiply mated compared to singly mated females. This could be attributed to a male's ability to displace or kill the sperm in an already inseminated female to increase its own relative fitness (Harshman & Prout 1994; Macke et al. 2012a; Chapman et al. 1995). In a study of *Thrips tabaci* multiple matings proved to be costly in terms of fecundity and onset of reproduction (Li et al. 2015). However, in this species, multiple matings did not change fertilization rate and female production (Li et al. 2015). Nevertheless, egg and offspring size could have been taken into account to better assess the net reproductive investment.

In the present study about *P. kellyanus*, the effect of mating was assessed in singly mated females, despite laboratory observations show that females are receptive to subsequent mating events (A. Katlav, personal observation). Therefore, future studies should explore in more detail the mating behaviour (as explored by Webster et al. 2006) and the effects of polyandry on resource allocation into eggs, and consequently the sex ratio dynamics and other life-history patterns in *P. kellyanus*.

Furthermore, previous studies have shown that the effect of a first mating event is not always effective in securing successful fertilization. For example, in *Drosophila* fruit flies the first mating may not be effective, and females, therefore, require re-mating to overcome fertilization failure even though they have received suitable sperm from the first mating (Fisher et al. 2013). It will be important to test whether multiple matings of *P. kellyanus* can recuperate successful fertilization and female production in constrained females. Finally, re-mating willingness may also be another interesting pattern to be compared between constrained and unconstrained females.

Is population sex ratio of P. kellyanus *controlled by LMC?*

Theoretical models suggest that local mate competition (LMC) in haplodiploids promotes inbreeding and the evolution of female-biased sex ratios (Hamilton 1967; Charnov 1982). However, empirical and comparative studies supporting the idea that the sex ratio in *P. kellyanus* is adjusted in response to LMC is not available. So far, in thrips, the effect of LMC has been found in some gall-inducing thrips where males generally remain in or near their natal batches in the galls (Crespi et al. 1997; Willis et al. 2004; Tree & Walter 2009). However, the principle of equal population-level sex ratio (Düsing 1884; Fisher 1930) is more probable in a free-living thrips, like *P. kellyanus*, where random mating (panmixia) is more apparent (Higgins & Myers 1992; Crespi 1993). Nevertheless, even in free-living thrips species female-biased sex ratios are very common (Higgins & Myers 1992; Crespi 1993; Gerin et al. 1994; Kanara & Acharya 2014). Studies suggest that such bias might also arise under LMC as result of sib-mating before dispersal. This is because the chance of acquiring mate after leaving the natal patch is limited probably due the promiscuity of males and receptiveness of females upon emergence of adults (Birkhead & Møller 1998; Li et al. 2015) or their small body size which limit their dispersal ability specially in sever environmental conditions (Kranz et al. 2001; Bondy & Hunter 2019). In *P. kellyanus* individuals pupate in the soil underneath host plants (Mound & Jackman 1998), and adults disperse from the soil to find a mate. Webster et al. (2006) found male aggregations on young leaves in the evening and on the surface of ripe lemons where females were attracted to male pheromones for mating. Therefore, sib-mating is unlikely to be common in *P. kellyanus* populations.

The preliminary field observations of this study noticed highly female-biased sex ratio in some patches (closely spaced groups of flowers), while we also found some patches with males aggregations (A. Katlav, personal observations; Webster et al. 2006). Also, the field experiment part of Chapter 2 further showed a bimodal sex ratio: a small fraction of females produced male-biased offspring. These females were either virgin or constrained mated females (Chapter 3). Therefore, this bimodal pattern may further reduce the chance of sib-mating in the population, reducing the possibility of *P. kellyanus* sex ratio being LMC-driven. Split sex ratio was recorded for gall-inducing thrips species (Kranz et al. 2000) in which population sex ratio is bimodal, i.e. some galls were male biased and other galls female biased, but the overall population sex ratio was unbiased. Kranz et al. (2000) linked split sex ratios to virginity and density-dependent selection on mated (unconstrained) females to produce female-biased offspring, according to Godfray's model (Godfray 1990).

LMC and inbreeding are often interlinked, with high levels of LMC causing strong inbreeding. Under inbreeding, the optimal sex ratio for females is expected to incline towards female bias as predicted by the LMC theory (Herre 1985), because under high LMC level, mother-daughter relatedness increases; whereas, father-daughter relatedness remains unaffected. Crespi (1991) examined the heterozygosity level of a small sample of one Australian population of *P. kellyanus*, and showed a very low level of inbreeding which may further reject that role of LMC in shaping the sex allocation of this species. However, to confirm this argument more extensive genotyping from more populations is required to better estimate relatedness and inbreeding coefficients in the field.

Split sex ratio and its driving forces in P. kellyanus

We showed that females with a small body size and egg size produce extremely male-biased offspring while lager females produce highly female-biased offspring. However, the evolutionary forces driving this are yet to be uncovered. On one hand, split sex ratio could illustrate a kin-selection argument (Alonso et al. 2002), if egg size and offspring sex ratio reflects the quality of females and is correlated to their body size. This indicates that females benefit more than males from a larger size (Shine 1988; Macke et al. 2011). Alternatively, egg size and constrained sex allocation might be the result of an arms race between males (selected to produce more females) and females (selected to produce at least some males) (Chapman et al. 2003; de la Filia et al. 2015). Also, this could be a by-product of the genetic conflict between endosymbionts (selected to produce more females) and host female (selected to produce less females) over optimal sex ratio (Vala et al. 2003).

Constrained sex allocation and sexual selection

Constrained sex allocation after mating may affect selection on mate choice and mating frequency. Given that mating with smaller females may end up with constrained sex allocation, mating experiments that offer males a choice between small and large females would be very interesting. Moreover, the competitive ability of differently sized males in acquiring unconstrained females for female production is of high interest. Size-assortative mating is observed in a variety of insects (Hegde & Krishna 1997; Dorková et al. 2019). For example, in some beetles both large and small males prefer to mate with large females, although larger males successfully compete for large females and leave smaller males to mate with smaller females (Harari et al. 1999). Studies of size-related mating patterns in some thrips also showed that larger males are more successful in mate guarding and fighting with male rivals (Crespi 1989a). Crespi (1989) further showed population structure can also influence male and female mating patterns: in a small population, male size does not affect mating success. We hypothesise that constrained sex allocation may intensify sexual selection and males will selectively mate with larger females to avoid constrained sex allocation, yet this remains to be tested.

Does constrained sex allocation prevail in field populations of P. kellyanus*?*

The constrained sex allocation theory has so far been mostly tested on field-collected females by verification of insemination or ability of daughter production (Seidelmann et al. 2010; but see Henter 2004). In most cases the age and mating status of examined individuals are overlooked. For example, Kranz et al. (2000) attributed split sex ratios in populations of gall-inducing thrips with moderate to high portion of reproductive virgins. However, occurrence of constrained mated mothers is another likely source of split sex ratio that has not so far been addressed in populations of other thrips species. Therefore, integration of field population sex ratio analysis and laboratory crossing experiments can better unveil the mechanism and function of split sex ratios. The analysis of sex ratio of field collected individual females (Chapter 2) detected a clear bimodal sex ratio pattern as seen in the laboratory, yet the insemination status of the field collected individuals was not assessed. Therefore, future studies require spermathecal dissection of field collected individuals to distinguish between virgin and constrained mated females.

Effect of population density and food quality on constrained sex allocation in P. kellyanus*?*

Studies suggest that population density can significantly affect fitness components of individuals, behaviour and metabolism in insects (Applebaum & Heifetz 1999; Lazarevic 2004). For example, increased population density may shorten insect development time (Lord 1998; Mastrantonio et al. 2018), survival and reduce body weight and fecundity (Hirschberger 1999; Hooper et al. 2003). This is mainly due a reduction in food resource availability because of intraspecific competition for food (Mastrantonio 2018). In spider mites, developmental and sex ratio showed a marked reduction in higher population density regimes (Wrensch & Young 1978). This is consistent with the Trivers-Willard model (Trivers & Willard 1973) that has also been demonstrated for sexually dimorphic mammals and birds, where the offspring sex ratio is more inclined towards the less costly sex when population density increases (Clutton-Brock & Albon 1985; Koziel1 & Ulijaszek 2001). Therefore, given that under conditional sex allocation any changes in parental fitness may result in offspring sex ratio bias (Trivers & Willard 1973), we suggest that future studies should further investigate if variation in population density of *P. kellyanus* can lead to a change in offspring sex ratio. We hypothesise that increased population density may increase the frequency of females constrained to M-brood production due to lower resource availability and reduced fitness per individual.

Diet or host quality is another factor that can affect sex allocation in species with adaptive sex allocation adjustment. For example, in *Tetranychus cinnabarinus*, females reared on good quality leaves produced offspring with a slower developmental rate and more female-biased sex ratio than females reared on poor quality leaves (Wrensch & Young 1978). Several studies in thrips have shown the relative host quality on different life history parameters (Bondya & Hunter 2019). However, there are two different compelling arguments regarding the sex ratio adjustment under environmental condition. Under Fisher's principle in high-quality environment (Fisher 1930), there is no resource limitation, so fitness return of both sexes may become equal, inclining the offspring reproduction towards an even sex ratio. On the other hand, in accordance with the Trivers & Willard (1973) and Charnov (1982) hypotheses females may benefit relatively more than males with increased environmental condition. This may incline the reproductive decision towards more daughter production in an environment with high-quality food resources. The latter hypothesis has been shown for the mycophagous thrips *Elaphrothrips tuberculatus,* albeit with males benefiting more from maternal fitness. In this thrips species, females in good condition (with the ability to produce offspring with large size) produced male broods viviparously whereas those in lower condition produce female broods oviparously (Crespi 1988). However, in *P. kellyanus* as we found greater gains for female fitness with body size increase relative to males. Therefore, increase in host/food quality may result in higher number of unconstrained females with larger eggs size producing female broods, yet this hypothesis awaits testing in prospective studies.

Can Wolbachia and *Cardinium affect sex allocation affecting male fertility and sperm competition?*

The number of sperm produced is affected by various factors including male age (e.g. Beckenbach 1978; Awrahman et al. 2014), starvation (Gage & Cook 1994; Ianora et al. 1999) and parasites (Zuk 1987). One additional potential source of manipulation of sperm production sits with SGEs (Burt & Trivers 2006). This concept which has been largely understudied, suggests that males carrying SGEs frequently suffer from reduced sperm number and/or quality and lower competitive ability due to direct manipulation of spermatogenesis by SGE (Wedell

2013). For example, Champion de Crespigny & Wedell (2006) tested the impact of *Wolbachia* infection on sperm competitive ability in *D. simulans* and found reduced fertilisation of eggs of females mated with *Wolbachia* infected males' sperm compared to that of uninfected ones. This undermining effect of endosymbionts has so far not been tested in haplodiploid species and never been addressed for *Cardinium*. This can be tested by investigating the level of CI and the sex ratio patterns in uninfected females first mated with uninfected males and then with infected males (or vice versa) *in P. kellyanus,* in order to assess effect of male infection status and mating order on the expression of CI.

Sex allocation mechanism in other thrips species

Our findings may be of relevance to many species with similar sex determination systems (arrhenotoky) and thus a large diversity of thrips in Terebrantia. However, it might not apply to thrips species or other haplodiploids with a thelytokous sex-determination system in which female production is independent of mating and fertilization (Kumm & Moritz 2008). In endosymbiont-induced thelytoky sex allocation is 100% towards females anyway and, therefore, endosymbionts may not need to achieve female-biased sex ratios by egg size modulation. Yet, some species have both endosymbiont infected thelytokous lineages and uninfected arrhenotokous lineages and contrasting egg sizes between these will be interesting. Moreover, the female-biased conditional resource allocation might not apply to thrips with large males. For example, males of colonial/eusocial fungus-feeding species of Tubulifera are generally larger than females, and male body size is under selection for with male/male competition over mates and egg protection (Crespi 1986; Tyagi et al. 2008). Moreover, many thrips species are viviparous, with larvae developing fully before they reach the vagina and spermatheca (Crespi 1989b), and thus presumably preempting fertilization. Therefore, sex allocation in such groups may be controlled by different mechanisms.

5.4 Concluding remarks

Overall this study established the underlying mechanism of egg size-mediated sex and resource allocation for the first time in a haplodiploid insect. It further highlighted the role of maternal condition and egg size in the evolution of adaptive split sex ratios and the mechanism whereby *Cardinium* and *Wolbachia* can manipulate sex allocation – a complementary strategy that may boost their invasion success (along with CI induction) in host populations. Moreover, this study uncovered complicated interactive effects of *Cardinium* and *Wolbachia* on host fitness which further expand our current knowledge about ecological and epidemiological contexts of endosymbionts. Finally, the findings of this research contribute fundamentally to future studies of sex ratio dynamics of *P. kellyanus* and other haplodiploid arthropods, and are informative for the development of pest management programs. The findings are also important for studies of the population dynamics of *Cardinium* and *Wolbachia* in haplodiploid species and the development of biological control strategies such as the incompatible insect technique (IIT) to suppress populations of haplodiploid pests.

REFERENCES

Alonso, W. J., & Schuck-Paim, C. (2002). Sex-ratio conflicts, kin selection, and the evolution of altruism. *Proceedings of the National Academy of Sciences, 99*, 6843-6847.

Ameri, M., Kemp, D. J., Barry, K. L., & Herberstein, M. E. (2019). Age-specific reproductive investment and offspring performance in an orb-web spider, *Argiope radon*. *Evolutionary Biology*, *46*, 207-215.

Andersson, M. B. (1994). Sexual selection. Princeton University Press.

Andersson, M., & Simmons, L.W. (2006). Sexual selection and mate choice. *Trends in Ecology & Evolution*, *21*, 296-302.

Applebaum, S. W., & Heifetz, Y. (1999). Density-dependent physiological phase in insects. *Annual Review of Entomology*, *44*, 317-341.

Arakaki, N., Miyoshi, T., & Noda, H. (2001). *Wolbachia*–mediated parthenogenesis in the predatory thrips *Franklinothrips vespiformis* (Thysanoptera: Insecta). *Proceedings of the Royal Society of London B, 268*, 1011-1016.

Arnqvist, G., & Nilsson, T. (2000). The evolution of polyandry: multiple mating and female fitness in insects. *Animal Behaviour, 60*, 145-164.

Arnqvist, G., & Rowe, L. (2005). Sexual conflict. Princeton University Press.

Aron, S., Passera, L., & Keller, L. (1999). Evolution of social parasitism in ants: size of sexuals, sex ratio and mechanisms of caste determination. *Proceedings of the Royal Society of London B, 266*, 173-177.

Atkinson, D., & Begon, M. (1987). Reproductive variation and adult size in two co occurring grasshopper species. *Ecological Entomology*. *12,* 119-127.

Awrahman, Z. A., Champion de Crespigny, F., & Wedell, N. (2014). The impact of *Wolbachia,* male age and mating history on cytoplasmic incompatibility and sperm transfer in *Drosophila simulans*. *Journal of Evolutionary Biology*, *27*, 1-10.

Badyaev, A. V., Hill, G. E., Beck, M. L., Dervan, A. A., Duckworth, R. A., McGraw, K. J., Nolan, P. M., & Whittingham, L. A. (2002). Sex-biased hatching order and adaptive population divergence in a passerine bird. *Science, 295,* 316-318.

Badyaev, A.V., Acevedo Seaman, D., Navara, K. J., Hill, G. E., & Mendonca, M. T. (2006). Evolution of sex biased maternal effects in birds: III. Adjustment of ovulation order can enable sex specific allocation of hormones, carotenoids, and vitamins. *Journal of Evolutionary Biology, 19,* 1044-1057.

Baker, G. J., Jackman, D, Keller, M. A., Macgregor, A., & Purvis, S. (2002). Development of an integrated pest management system for thrips in citrus: Horticulture Australia, Sydney.

Baker, G., Keller, M., Crisp, P., Jackman, D., Barbour, D., & Purvis, S. (2011). The biological control of Kelly's citrus thrips in Australian citrus orchards. *IOBC/WPRS Bull. 62,* 267-274.

Bangham, J., Chapman, T., & Partridge, L. (2002). Effects of body size, accessory gland and testis size on pre-and postcopulatory success in *Drosophila melanogaster*. *Animal Behaviour, 64,* 915-921.

Bateman, A. J. (1948). Intra-sexual selection in Drosophila. *Heredity*. 2, 349-368.

Bayoumy, M. H., & Michaud, J. P. (2014). Female fertility in *Hippodamia convergens* (Coleoptera: Coccinellidae) is maximized by polyandry, but reduced by continued male presence. *European Journal of Entomology, 111,* 513-520.

Beckenbach, A. T. (1978). The" sex-ratio" trait in *Drosophila pseudoobscura*: fertility relations of males and meiotic drive. *The American Naturalist, 112,* 97-117.

Berrigan, D., & Locke, S. J. (1991). Body size and male reproductive performance in the flesh fly, *Neobellieria bullata*. *Journal of Insect Physiology, 37,* 575-581.

Berthouly, A., Helfenstein, F., Tanner, M., & Richner, H. (2008). Sex related effects of maternal egg investment on offspring in relation to carotenoid availability in the great tit. *Journal of Animal Ecology, 77,* 74-82.

Beukeboom, L. W. (1994). Phenotypic fitness effects of the selfish B chromosome, paternal sex ratio (PSR) in the parasitic wasp *Nasonia vitripennis*. *Evolutionary Ecology*. *8,* 1-24.

Beukeboom, L. W., & Perrin, N. (2014). The evolution of sex determination. Oxford University Press, USA.

Birkhead, T. R., & Møller, A. P. (eds) (1998). Sperm Competition and Sexual Selection. Academic Press, London.

Blank, R. H., & Gill, G. S. C. (1997). Thrips (Thysanoptera: Terebrantia) on flowers and fruit of citrus in New Zealand. *New Zealand Journal of Crop and Horticultural Science*. *25*, 319-332.

Boivin, G. (2013). Sperm as a limiting factor in mating success in Hymenoptera parasitoids. *Entomologia Experimentalis et Applicata*, *146*, 149-155.

Boivin, G., & Martel, V. (2012). Size-induced reproductive constraints in an egg parasitoid. *Journal of Insect Physiology*, *58*, 1694-1700.

Boller, E. F., Russ, K., Vallo, V., & Bush, G. L. (1976). Incompatible races of European cherry fruit fly, *Rhagoletis cerasi* (diptera: Tephritidae), their origin and potential use in biological control. *Entomologia Experimentalis et Applicata*, *20*, 237-247.

Boncoraglio, G., & Kilner, R. M. (2012). Female burying beetles benefit from male desertion: sexual conflict and counter-adaptation over parental investment. *PloS one*, *7*, 31713.

Bondy, E. C., & Hunter, M. S. (2019). Sex ratios in the haplodiploid herbivores, Aleyrodidae and Thysanoptera: A review and tools for study. *Advances in Insect Physiology*, *56*, 251-281.

Bordenstein, S. R., Uy, J. J., & Werren, J. H. (2003). Host genotype determines cytoplasmic incompatibility type in the haplodiploid genus *Nasonia*. *Genetics*, *164*, 223-233.

Bosch, J. (2008). Production of undersized offspring in a solitary bee. *Animal Behaviour*, *75*,

Bosch, J., & Vicens, N. (2006). Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. *Behavioral Ecology and Sociobiology*, *60*, 26-33.

Boudreaux, H. B. (1963). Biological aspects of some phytophagous mites. *Annual Review of Entomology*, *8*, 137-154.

Bourke, A. F. G., & Chan, G. L. (1994). Split sex ratios in ants with multiple mating. *Trends in Ecology & Evolution*, *9*, 120-122.

Bournier, J. P., & L. A. Mound (2000). Thysanoptera from New-Caledonia: an annoted check list. *Bulletin de la Société Entomologique de France*, *105*, 231-240.

Bourtzis, K., Nirgianaki, A., Markakis, G., & Savakis, C. (1996). *Wolbachia* infection and cytoplasmic incompatibility in *Drosophila* species. *Genetics*, *144*, 1063-1073.

Braendle, C., Miura, T., Bickel, R., Shingleton, A. W., Kambhampati, S., & Stern, D. L. (2003). Developmental origin and evolution of bacteriocytes in the aphid–*Buchnera* symbiosis. *PLoS Biology*. *1*, e21.

Breeuwer, J. A. (1997). *Wolbachia* and cytoplasmic incompatibility in the spider mites *Tetranychus urticae* and *T. turkestani*. *Heredity*, *79*, 41.

Brownlie, J. C., & Johnson, K. N. (2009). Symbiont-mediated protection in insect hosts. *Trends in Microbiology*, *17*, 348-354.

Buchner, P. (1953). Endosymbiose der Tiere Mitpflanzlichen Mikroorganismen. Birkhäuser Verlag Basel.

Budrienė, A., Budrys, E., & Nevronytė, Ž. (2013). Sexual size dimorphism in the ontogeny of the solitary predatory wasp *Symmorphus allobrogus* (Hymenoptera: Vespidae). *Comptes Rendus Biologies*, *336*, 57-64.

Bulmer, M. G. (1994). Theoretical Evolutionary Ecology (Sinauer, Sunderland, MA). *Evolutionary Ecology*, *10*, 457-462.

Burt A., & Trivers, R. (2006). Genes in Conflict: the Biology of Selfish Genetic Elements. Harvard University Press, Harvard.

Calsbeek, R., & Sinervo, B. (2004). Within clutch variation in offspring sex determined by differences in sire body size: cryptic mate choice in the wild. *Journal of Evolutionary Biology*, *17*, 464-470.

Cameron, E., Day, T., & Rowe, L. (2003). Sexual conflict and indirect benefits. *Journal of Evolutionary Biology*. *16*, 1055-1060.

Carranza, J. (2002). What did Trivers and Willard really predict?. *Animal Behaviour*, *63*, F1-F3.

Castañé, C., Riudavets, J., & Yano, E. (1999). Biological control of thrips. Albajes, R, Gullino, M. L., van Lenteren, J. C., Elad, Y. (Eds.), In: Integrated Pest and Disease Management in Greenhouse Crops, Kluwer Academic, Dordrecht, pp. 244-253

Cavalleri, A., & Mendonça Jr, M. D. S. (2020). Ectoparasitic thrips affect the behaviour of their aetalionid treehopper hosts. *Austral Entomology, 59*, 794-801.

Champion de Crespigny, F. E., & Wedell, N., 2006. *Wolbachia* infection reduces sperm competitive ability in an insect. *Proceedings of the Royal Society B*, *273*, 1455-1458.

Champion de Crespigny, F. E., Hurst, L. D., & Wedell, N., 2008. Do *Wolbachia* associated incompatibilities promote polyandry?. *Evolution: International Journal of Organic Evolution*, *62*, 107-122.

Chan, G. L., A. Hingle, and A. F. Bourke. 1999. Sex allocation in a facultatively polygynous ant: between-population and between-colony variation. *Behavioural Ecology*, *10*, 409-421.

Chapman, R. F. (1998). The insects: structure and function. Cambridge University Press.

Chapman, T. (2006). Evolutionary conflicts of interest between males and females. *Current Biology*. *16*, 744-754.

Chapman, T. (2018). Sexual conflict: mechanisms and emerging themes in resistance biology. *The American Naturalist*, *192*, 217-229.

Chapman, T., Arnqvist, G., Berrigan, J., & Rowe, L. (2003). Sexual conflict. *Trends in Ecology & Evolution*, *18*, 41-47.

Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F., & Partridge, L. (1995). Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature*, *373*, 241-244.

Charlat, S., Hurst, G. D., & Merçot, H. (2003). Evolutionary consequences of *Wolbachia* infections. *Trends in Genetics*, *19*, 217-223.

Charnov, E. L. (1978). Sex-ratio selection in eusocial Hymenoptera. *The American Naturalist, 112*, 317-326.

Charnov, E. L. (1982). The theory of sex allocation. Princeton University Press.

Charnov, E. L. (1993). Life history invariants: some explorations of symmetry in evolutionary ecology. Oxford University Press, USA.

Charnov, E. L., Los-den Hartogh, R. L., Jones, W. T., & Van den Assem, J. (1981). Sex ratio evolution in a variable environment. *Nature*, *289,* 27-33.

Chirault, M., Bressac, C., Goubault, M., & Lécureuil, C. (2019). Sperm limitation affects sex allocation in a parasitoid wasp *Nasonia vitripennis*. *Insect Science, 26*, 853-862.

Church, S. H., Donoughe, S., de Medeiros, B. A., & Extavour, C. G. (2019). Insect egg size and shape evolve with ecology but not developmental rate. *Nature*, *571*, 58-62.

Clancy, D. J., & Hoffmann, A. A. (1998). Environmental effects on cytoplasmic incompatibility and bacterial load in *Wolbachia* infected *Drosophila simulans*. *Entomologia experimentalis et Applicata*, *86*, 13-24.

Clutton-Brock, T. H. (1991). The evolution of parental care. Princeton University Press.

Clutton-Brock, T. H., & Parker, G. A. (1992). Potential reproductive rates and the operation of sexual selection. *The Quarterly Review of Biology*, *67*, 437-456.

Clutton-Brock, T. H., Albon, S. D., & Guinness, F. E. (1987). Interactions between population density and maternal characteristics affecting fecundity and juvenile survival in red deer. *The Journal of Animal Ecology*, *56*, 857-871.

Cole, L. R. (1981). A visible sign of a fertilization action during oviposition by an ichneumonid wasp, *Itoplectis maculator*. *Animal Behaviour*, *29*, 299-300.

Colonello, N. A., & Hartfelder, K. (2005). She’s my girl-male accessory gland products and their function in the reproductive biology of social bees. *Apidologie*, *36*, 231-244.

Conti, F., Tumminelli, R., Amico, C., Fisicaro, R., Frittitta, C., Perrotta, G., & Marullo, R., (2002). Monitoring *Pezothrips kellyanus* on citrus in eastern Sicily. Marullo R., & Mound

L. (Eds), In: Proceedings of the 7th International Symposium on Thysanoptera. Reggio Calabria, Italy pp. 207-210.

Cook, J. M. (2002). Sex determination in invertebrates. Hardy I. C. W. (Ed.), In: Sex Ratios: Concepts and Research Method, pp.178-194. Cambridge University Press, Cambridge, UK.

Cordaux, R., Bouchon, D., & Grève, P. (2011). The impact of endosymbionts on the evolution of host sex-determination mechanisms. *Trends in Genetics, 27,* 332-341.

Cordero, P. J., Griffith, S. C., Aparicio, J. M., & Parkin, D. T. (2000). Sexual dimorphism in house sparrow eggs. *Ecology and Sociobiology*, *48,* 353-357.

Crean, A. J., & Bonduriansky, R. (2014). What is a paternal effect?. *Trends in Ecology & Evolution*, *29,* 554-559.

Crespi, B. J. (1986). Territoriality and fighting in a colonial thrips, *Hoplothrips pedicularius*, and sexual dimorphism in Thysanoptera. *Ecological Entomology*, *11*, 119-130.

Crespi, B. J. (1988). Sex ratio selection in a bivoltine thrips. I. Conditional sex ratio manipulation and fitness variation. *Evolution*, *42,* 1199-1211.

Crespi, B. J. (1989a). Causes of assortative mating in arthropods. Animal Behaviour, *38*, 980-1000.

Crespi, B. J. (1989b). Facultative viviparity in a thrips. *Nature*, *337,* 357-358.

Crespi, B. J. (1991). Heterozygosity in the haploidiploid thysanoptera. *Evolution, 45,* 458-464.

Crespi, B. J. (1992). Eusociality in Australian gall thrips. *Nature*, 359, 724-726.

Crespi, B. J. (1993). Sex ratio selection in Thysanoptera. In: Wrensch, D. L., M. Ebbert, eds. Evolution and Diversity of Sex Ratio in Insects and Mites. Chapman and Hall, New York, pp. 214-234.

Crespi, B. J., Carmean, & D. A., & Chapman, T. W. (1997). Ecology and evolution of galling thrips and their allies. *Annual Review of Entomology, 42,* 51-71.

Crisp, P., & Baker, G. (2011). Soil mediated conservation biological control of Kelly's citrus thrips *Pezothrips kellyanus* pupae. *IOBC/WPRS Bulletin*, *62*, 239-246.

Cruickshank, R. H., & Thomas, R. H. (1999). Evolution of haplodiploidy in dermanyssine mites (Acari: Mesostigmata). *Evolution*, *53*, 1796-1803.

Czesak, M. E., & Fox, C. W. (2003). Evolutionary ecology of egg size and number in a seed beetle: genetic trade-off differs between environments. *Evolution*, *57*, 1121-1132.

Dallai, R., Del Bene, G., & Lupetti, P. (1996). Fine structure of spermatheca and accessory gland of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). *International Journal of Insect Morphology and Embryology*, *25*, 317-330.

Damiens, D., Bressac, C., & Chevrier, C. (2003). The effect of age on sperm stock and egg laying in the parasitoid wasp, *Dinarmus basalis*. *Journal of Insect Science*, *3,* p.22.

Danielsson, I. (2001). Antagonistic pre–and post–copulatory sexual selection on male body size in a water strider (*Gerris lacustris*). *Proceedings of the Royal Society of London B, 268*, 77-81.

Davies, N. B., Krebs, J. R., & West, S. A. (2012). An introduction to behavioural ecology. John Wiley & Sons.

De Barro, P. J., Liu, S. S., Boykin, L. M., & Dinsdale, A. B. (2011). *Bemisia tabaci*: a statement of species status. *Annual Review of Entomology, 56*, 1-19.

de la Filia, A. G., Bain, S. A., & Ross, L. (2015). Haplodiploidy and the reproductive ecology of Arthropods. *Current Opinion in Insect Science, 9*, 36-43.

Deaker, D. J., Foo, S. A., & Byrne, M. (2019). Variability in egg and jelly-coat size and their contribution to target size for spermatozoa: a review for the Echinodermata. *Marine and Freshwater Research*, *70*, 995-1006.

Dedeine, F., Bandi, C., Boulétreau, M., & Kramer, L. H. (2003). 16 Insights into *Wolbachia* obligatory symbiosis. *Insect Symbiosis*, *1*, 267–282.

Dedeine, F., Vavre, F., Fleury, F., Loppin, B., Hochberg, M. E., & Boulétreau, M. (2001). Removing symbiotic Wolbachia bacteria specifically inhibits oogenesis in a parasitic wasp. *Proceedings of the National Academy of Sciences, 98*, 6247-6252.

den Hollander, M., & Gwynne, D. T. (2009). Female fitness consequences of male harassment and copulation in seed beetles, *Callosobruchus maculatus*. *Animal Behaviour*, *78*, 1061-1070.

Dobson, S. L., Rattanadechakul, W., & Marsland, E. J. (2004). Fitness advantage and cytoplasmic incompatibility in *Wolbachia* single-and superinfected *Aedes albopictus*. *Heredity*. 93: 135.

Dobson, S., & Tanouye, M. (1996). The paternal sex ratio chromosome induces chromosome loss independently of *Wolbachia* in the wasp *Nasonia vitripennis*. *Development Genes and Evolution*, *206*, 207-217.

Doremus, M. R. & Oliver, K. M. (2017). Aphid heritable symbiont exploits defensive mutualism. *Applied and Environmental Microbiology*, *83*, 03276-16.

Doremus, M. R., Stouthamer, C. M., Kelly, S. E., Schmitz-Esser, S., & Hunter, M. S. (2020). *Cardinium* localization during its parasitoid wasp host's development provides insights into cytoplasmic incompatibility. *Frontiers in Microbiology*, *11*, 606399.

Dorková, M., Naďo, L., Jarčuška, B., & Kaňuch, P. (2019). Size dependent mating pattern in a nuptial gift giving insect. *Ecology and Evolution*, *9*, 454-462.

Douglas, A. E. (2009). The microbial dimension in insect nutritional ecology. *Functional Ecology*, *23*, 38-47.

Duron, O., Bouchon, D., Boutin, S., Bellamy, L., Zhou, L., Engelstädter, J., & Hurst, G. D. (2008). The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. *BMC Biology*, *6*, 27.

Dusenbery, D. B. (2009). Living at micro scale: the unexpected physics of being small. Harvard University Press.

Düsing, C. (1884). Die Regulierung des Geschlechtsverhältnisses bei der Vermehrung der Menschen, Tiere und Pflanzen. *Jenaische Zeitschrift für Naturwissenschaft, 17,* 593-940.

Eberhard, W. (1996). Female control: sexual selection by cryptic female choice. Princeton University Press.

Eberhard, W. G. (1985). Sexual selection and animal genitalia. Cambridge, MA: Harvard University Press.

Edwards, A. W. F. (1998). Natural selection and the sex ratio: Fisher's sources. *American Naturalist*, *151*, 564-569.

Egas, M., Vala, F., & Breeuwer, J. A. J. (2002). On the evolution of cytoplasmic incompatibility in haplodiploid species. *Evolution*, *56*: 1101-1109.

Engelstädter, J., & Hurst, G. D. (2009). The ecology and evolution of microbes that manipulate host reproduction. *Annual Review of Ecology, Evolution, and Systematics*, *40*, 127-149.

Engelstädter, J., & Hurst, G. D. D. (2006). Can maternally transmitted endosymbionts facilitate the evolution of haplodiploidy? *Journal of Evolutionary Biology*. *19*, 194-202.

Fedorka, K. M., & Mousseau, T. A. (2002). Nuptial gifts and the evolution of male body size. *Evolution*, *56*. 590-596.

Fine, P. E. (1975). Vectors and vertical transmission: an epidemiologic perspective. *Annals of the New York Academy of Sciences, 266*, 173-194.

Finkler, M. S., Van Orman, J. B., & Sotherland, P. R. (1998). Experimental manipulation of egg quality in chickens: influence of albumen and yolk on the size and body composition of near-term embryos in a precocial bird. *Journal of Comparative Physiology B, 168*, 17-24.

Fisher, D. N., Doff, R. J., & Price, T. A. (2013). True polyandry and pseudopolyandry: why does a monandrous fly remate?. *BMC Evolutionary Biology*, *13*, 1-11.

Fisher, R. A. (1930). The genetical theory of natural selection. Clarendon Press, Oxford p. 141-143.

Flatt, T. & Heyland, A. (2011). Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. OUP Oxford.

Floate, K. D., Kyei-Poku, G. K. and Coghlin, P. C. (2006). Overview and relevance of *Wolbachia* bacteria in biocontrol research. *Biocontrol Science and Technology, 16*, 767-788.

Folmer, O., Hoeh, W. R., Black, M. B., & Vrijenhoek, R. C. (1994). Conserved primers for PCR amplification of mitochondrial DNA from different invertebrate phyla. *Molecular Marine Biology and Biotechnology*, *3*, 294-299.

Ford, N. B., & Seigel, R. A. (2006). Intra individual variation in clutch and offspring size in an oviparous snake. *Journal of Zoology*, *268*, 171-176.

Fox, C. W. (1997). Egg-size manipulations in the seed beetle *Stator limbatus*: consequences for progeny growth. *Canadian Journal of Zoology*, *75*, 1465-1473. https://doi.org/10.1139/z97-769

Fox, C. W., & Czesak, M. E. (2000). Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology*, *45*, 341-369.

Fox, C. W., & T. A. Mousseau. (1998). Maternal effects as ad- aptations for trans generational phenotypic plasticity in insects. Pp. 159-177. in T. A. Mousseau and C. A. Fox, eds. Maternal effects as adaptations. Oxford Univ. Press, New York.

Fox, C. W., Czesak, M. E., Mousseau, T. A., & Roff, D. A. (1999). The evolutionary genetics of an adaptive maternal effect: egg size plasticity in a seed beetle. *Evolution*, *53*, 552-560.

Fox, C. W., Thakar, M. S., & Mousseau, T. A. (1997). Egg size plasticity in a seed beetle: an adaptive maternal effect. *The American Naturalist*, *149*, 149-163.

Frank, S. A. (1990). Sex allocation theory for birds and mammals. *Annual Review of Ecology and Systematics*, *21*, 13-55.

Freeland, S. J., & McCabe, B. K. (1997). Fitness compensation and the evolution of selfish cytoplasmic elements. *Heredity*, *78*, 391.

Friberg, U. (2006). Male perception of female mating status: its effect on copulation duration, sperm defence and female fitness. *Animal Behaviour*, *72*, 1259-1268.

Fry, A. J., & Rand, D. M. (2002). *Wolbachia* interactions that determine *Drosophila melanogaster* survival. *Evolution*, *56*,1976-1981.

Gage, M. J. G., & Cook, P. A. (1994). Sperm size or numbers? Effects of nutritional stress upon eupyrene and apyrene sperm production strategies in the moth *Plodia interpunctella* (Lepidoptera: Pyralidea). *Functional Ecology, 8*, 594-599.

Gardner, A., & Ross, L. (2014). Mating ecology explains patterns of genome elimination. *Ecology Letters*, 17, 1602-1612.

Gardner, A., Alpedrinha, J., & West, S. A. (2012). Haplodiploidy and the evolution of eusociality: split sex ratios. *The American Naturalist, 179*, 240-256.

Gebiola, M., Kelly, S. E., Hammerstein, P., Giorgini, M., & Hunter, M. S. (2016). “Darwin's corollary” and cytoplasmic incompatibility induced by *Cardinium* may contribute to speciation in *Encarsia* wasps (Hymenoptera: Aphelinidae). *Evolution*, *70*, 2447-2458.

Gebiola, M., Monti, M. M., Johnson, R. C., Woolley, J. B., Hunter, M. S., Giorgini, M., & Pedata, P. A. (2017). A revision of the *Encarsia pergandiella* species complex (Hymenoptera: Aphelinidae) shows cryptic diversity in parasitoids of whitefly pests. *Systematic Entomology*, *42*, 31-59.

Gerin, C., Hance, T. H., & Impe, G. V. (1994). Demographical parameters of *Frankliniella occidentalis* (Pergande) (Thysanoptera, Thripidae). *Journal of Applied Entomology, 118,* 370-377.

Ghislandi, P. G., Pekár, S., Matzke, M., Schulte Döinghaus, S., Bilde, T., & Tuni, C. (2018). Resource availability, mating opportunity and sexual selection intensity influence the expression of male alternative reproductive tactics. *Journal of Evolutionary Biology*, *31*, 1035-1046.

Gillott, C. (2003). Male accessory gland secretions: modulators of female reproductive physiology and behavior. *Annual Review of Entomology*, *48*, 163-184.

Giordano, R., O'Neill, S. L., & Robertson, H. M. (1995). *Wolbachia* infections and the expression of cytoplasmic incompatibility in *Drosophila sechellia* and *D. mauritiana*. *Genetics*. *140*, 1307-1317.

Giorgini, M., Bernardo, U., Monti, M. M., Nappo, A. G., & Gebiola, M. (2010). *Rickettsia* symbionts cause parthenogenetic reproduction in the parasitoid wasp *Pnigalio soemius* (Hymenoptera: Eulophidae). *Applied and Environmental Microbiology*. *76*, 2589-2599.

Giorgini, M., Monti, M. M., Caprio, E., Stouthamer, R., & Hunter, M. S. (2009). Feminization and the collapse of haplodiploidy in an asexual parasitoid wasp harboring the bacterial symbiont *Cardinium*. *Heredity*, *102*, 365.

Giron, D., & Casas, J., 2003. Mothers reduce egg provisioning with age. *Ecology Letters*, *6*, 273-277.

Glaudas, X., Rice, S. E., Clark, R. W., & Alexander, G. J. (2020). The intensity of sexual selection, body size and reproductive success in a mating system with male–male combat: is bigger better? *Oikos*, *129*, 998-1011.

Godfray, H. C. J. (1990). The causes and consequences of constrained sex allocation in haplodiploid animals. *Journal of Evolutionary Biology*, *3*, 3-17.

Godfray, H. C. J. (1994). Parasitoids: behavioral and evolutionary ecology. Princeton Univ. Press, Princeton, NJ.

Godfray, H. C. J., & Hardy, I. C. W. (1990). Estimating the frequency of constrained sex allocation in field populations of Hymenoptera. *Behaviour*, 114, 137-147.

Godfray, H. C. J., & Hardy, I. C. W. (1993). Sex ratio and virginity in haplodiploid insects. In: Evolution and diversity of sex ratio in insects and mites, Wrench, D. L., & Ebbert, M. A. (Eds.), pp. 402 – 417. Chapman & Hall, London, UK.

Godfray, H. C. J., & Waage, J. K. (1990). The evolution of highly skewed sex ratios in aphelinid wasps. *The American Naturalist*, *136*, 715-721.

Göth, A. & Booth, D. T. (2005). Temperature-dependent sex ratio in a bird. *Biology Letters 1*, 31-33.

Gotoh, T., Sugasawa, J., Noda, H., & Kitashima, Y. (2007). *Wolbachia*-induced cytoplasmic incompatibility in Japanese populations of *Tetranychus urticae* (Acari: Tetranychidae). *Experimental and Applied Acarology*, *42*, 1-16.

Gottlieb, D., Lubin, Y., & Harari, A. R. (2014). The effect of female mating status on male offspring traits. *Behavioral Ecology and Sociobiology, 68*, 701-710.

Grafen, A. (1986). Split sex ratios and the evolutionary origins of eusociality. *Journal of Theoretical Biology, 122*, 95-121.

Greenway, E. G., Dougherty, L. R., & Shuker, D. M. (2015). Mating failure. *Current Biology, 25*, R534-R536.

Gueguen, G., Vavre, F., Gnankine, O., Peterschmitt, M., Charif, D., Chiel, E., Gottlieb, Y., Ghanim, M., Zchori fein, E. I. N. A. T., & Fleury, F. (2010). Endosymbiont metacommunities, mtDNA diversity and the evolution of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex. *Molecular Ecology*, *19*, 4365-4376.

Guertin, D. S., Ode, P. J., Strand, M. R. & Antolin, M. F. (1996). Host-searching and mating in an outbreeding parasitoid wasp. *Ecolological Entomology, 21*, 27–33.

Hagimori, T., Abe, Y., Date, S., & Miura, K. (2006). The first finding of a *Rickettsia* bacterium associated with parthenogenesis induction among insects. *Current Microbiology, 52*, 97-101.

Hall, A. A., Morrow, J. L., Fromont, C., Steinbauer, M. J., Taylor, G. S., Johnson, S. N., & Riegler, M. (2016). Codivergence of the primary bacterial endosymbiont of psyllids versus host switches and replacement of their secondary bacterial endosymbionts. *Environmental Microbiology*, *18*, 2591-2603.

Hamilton, W. D. (1967). Extraordinary sex ratios. *Science*, 156, 477-488.

Hammerstein, P., & Parker, G. A. (1987). Sexual selection: games between the sexes. In: Sexual selection: testing the alternatives. Bradbury, J. W., & Andersson, M. B. (Eds.), pp. 119-142. Wiley, Chichester, UK.

Harcombe, W., & Hoffmann, A. A. (2004). *Wolbachia* effects in *Drosophila melanogaster*: in search of fitness benefits. *Journal of Invertebrate Pathology, 87*, 45-50.

Hardy, I. C. W. (2002). Sex ratios: concepts and research methods. Cambridge University Press, Cambridge, UK.

Hardy, I. C., Dijkstra, L. J., Gillis, J. E., & Luft, P. A. (1998). Patterns of sex ratio, virginity and developmental mortality in gregarious parasitoids. *Biological Journal of the Linnean Society, 64*, 239-270.

Harshman, L. G., & Prout, T. (1994). Sperm displacement without sperm transfer in *Drosophila melanogaster*. *Evolution, 48*, 758-766.

Harshman, L. G., & Zera, A. J. (2007). The cost of reproduction: the devil in the details. *Trends in Ecology and Evolution, 22*, 80-86.

Hawkes, P. G. (1992). Sex ratio stability and male-female conflict over sex ratio control in hymenopteran parasitoids. *South African Journal of Science, 88*, 423-430.

Head, M. L., Hunt, J., Jennions, M. D., & Brooks, R. (2005). The indirect benefits of mating with attractive males outweigh the direct costs. *PLoS Biology, 3*, 33.

Hedges, L. M., Brownlie, J. C., O'Neill, S. L., & Johnson, K. N. (2008). *Wolbachia* and virus protection in insects. *Science, 322*, 702-702.

Hedrick, A. V., & Temeles, E. J. (1989). The evolution of sexual dimorphism in animals: hypotheses and tests. *Trends in Ecology & Evolution, 4*, 136-138.

Hegde, S. N., & Krishna, M. S. (1997). Size-assortative mating in *Drosophila malerkotliana*. *Animal Behaviour, 54*, 419-426.

Heinz, K. M. (1996). Host size selection and sex allocation behaviour among parasitoid trophic levels. *Ecological Entomology, 21*, 218-226.

Helanterä, H., & Ratnieks, F. L. (2009). Sex allocation conflict in insect societies: who wins? *Biology Letters, 5*, 700-704.

Helle, W. (1967). Fertilization in the two spotted spider mite (*Tetranychus urticae*: Acari). *Entomologia Experimentalis et Applicata, 10*, 103-110.

Helms, K. R., Reuter, M., & Keller, L. (2005). Sex ratio conflict between queens and workers in eusocial Hymenoptera: mechanisms, costs, and the evolution of split colony sex ratios. *Evolution, 59*, 2626-2638.

Heming, B. (1970). Postembryonic development of the male reproductive system in *Frankliniella fusca* (Thripidae) and *Haplothrips verbasci* (Phlaeothripidae) (Thysanoptera). *Entomological Society of America*, *7*, 237-272.

Heming, B. S. (1995). History of the Germ Line in Male and Female Thrips. *In:* Parker BL, Skinner M, Lewis T. (eds) Thrips Biology and Management. NATO ASI Series (Series A: Life Sciences). Springer, Boston, MA.

Henter, H. J. (2004). Constrained sex allocation in a parasitoid due to variation in male quality. *Journal of Evolutionary Biology*, *17*, 886-896.

Herre E. A. (1985). Sex ratio adjustment in fig wasps. *Science*, *228*, 896-898.

Higgins, C. J., & Myers, J. H. (1992). Sex ratio patterns and population dynamics of western flower thrips (Thysanoptera: Thripidae). *Environmental Entomology*, *21*, 322-330.

Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A., & Werren, J. H. (2008). How many species are infected with *Wolbachia*? a statistical analysis of current data. *FEMS Microbiology Letters*, *281*, 215-220.

Himler, A. G., Adachi-Hagimori, T., Bergen, J. E., Kozuch, A., Kelly, S. E., Tabashnik, B. E., Chiel, E., Duckworth, V. E., Dennehy, T. J., Zchori-Fein, E., & Hunter, M. S. (2011). Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias. *Science*, *332*, 254-256.

Hirschberger, P. (1999). Larval population density affects female weight and fecundity in the dung beetle *Aphodius ater*. *Ecological Entomology*, *24*, 316-322.

Hoffmann, A. A., Clancy, D. J., & Merton, E. (1994). Cytoplasmic incompatibility in Australian populations of *Drosophila melanogaster*. *Genetics*, *136*, 993-999.

Hoffmann, A. A., Turelli, M., & Harshman, L. G. (1990). Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. *Genetics*, *126*, 933-948.

Hoffmann, A.A., Turelli, M., & Simmons, G.M., 1986. Unidirectional incompatibility between populations of *Drosophila simulans*. *Evolution*, *40*, 692-701.

Hooper, H. L., Sibly, R. M., Hutchinson, T. H., & Maund, S. J. (2003). The influence of larval density, food availability and habitat longevity on the life history and population growth rate of the midge *Chironomus riparius*. *Oikos, 102*, 515-524.

Hosken, David J., & Stockley, P. (2004). Sexual selection and genital evolution. *Trends in Ecology & Evolution*, *2*, 87-93.

Huffaker, C. B. (2012). Theory and practice of biological control. Elsevier.

Hunter, F. M., & Birkhead, T. R. (2002). Sperm viability and sperm competition in insects. *Current Biology*, *12*, 121-123.

Hunter, M. S., Perlman, S. J., & Kelly, S. E. (2003). A bacterial symbiont in the *Bacteroidetes* induces cytoplasmic incompatibility in the parasitoid wasp *Encarsia pergandiella*. *Proceedings of the Royal Society of London B*, *270*, 2185-2190.

Hunter, M.S., Nur, U., & Werren, J. H. (1993). Origin of males by genome loss in an autoparasitoid wasp. *Heredity*, *70*, 162-171.

Hurst, G. D., & Frost, C. L. (2015). Reproductive parasitism: maternally inherited symbionts in a biparental world. *Cold Spring Harbor Perspectives in Biology*, *7*, a017699.

Hurst, G. D., & Schilthuizen, M. (1998). Selfish genetic elements and speciation. *Heredity, 80*, 2-8.

Hurst, G. D., & Werren, J. H. (2001). The role of selfish genetic elements in eukaryotic evolution. *Nature Reviews Genetics*, *2*, 597.

Hurst, L. D. (1992). Intragenomic conflict as an evolutionary force. *Proceedings of the Royal Society of London B, 248*, 135-140.

Ianora, A., Miralto, A., Buttino, I., Romano, G., & Poulet, S. A. (1999). First evidence of some dinoflagellates reducing male copepod fertilization capacity. *Limnology and Oceanography*, *44*, 147-153.

Iossa, G., Gage, M. J., & Eady, P. E. (2016). Micropyle number is associated with elevated female promiscuity in Lepidoptera. *Biology Letters*, *12*, 20160782.

Jaenike, J. (2001). Sex chromosome meiotic drive. *Annual Review of Ecology and Systematics*. 32, 25-49.

Jaenike, J., Stahlhut, J. K., Boelio, L. M. and Unckless, R. L. (2010). Association between *Wolbachia* and *Spiroplasma* within *Drosophila neotestacea*: an emerging symbiotic mutualism?. *Molecular Ecology*, *19*, 414-425.

Janzen, F. J. (1994). Climate change and temperature-dependent sex determination in reptiles. *Proceedings of the National Academy of Sciences, 91*, 7487-7490.

Jervis, M. A., & Kidd, N. A. C. (1986). Host feeding strategies in hymenopteran parasitoids. *Biological Reviews*, *61*, 395-434.

Jeyaprakash, A., & Hoy, M. A. (2000). Long PCR improves *Wolbachia* DNA amplification: wsp sequences found in 76% of sixty three arthropod species. *Insect Molecular Biology*, *9*, 393-405.

Johnson, E. L., Cunningham, T. W., Marriner, S. M., Kovacs, J. L., Hunt, B. G., Bhakta, D. B., & Goodisman, M. A. (2009). Resource allocation in a social wasp: effects of breeding system and life cycle on reproductive decisions. *Molecular Ecology*, *18*, 2908-2920.

Joshi, D., McFadden, M. J., Bevins, D., Zhang, F., & Xi, Z. (2014). *Wolbachia* strain *w*AlbB confers both fitness costs and benefit on *Anopheles stephensi*. *Parasites & Vectors*, *7*, 336.

Joshi, D., Pan, X., McFadden, M. J., Bevins, D., Liang, X., Lu, P., Thiem, S., & Xi, Z. (2017). The maternally inheritable *Wolbachia w*AlbB induces refractoriness to *Plasmodium berghei* in *Anopheles stephensi*. *Frontiers in Microbiology*, *8*, 366.

Ju, J. F., Bing, X. L., Zhao, D. S., Guo, Y., Xi, Z., Hoffmann, A. A., Zhang, K. J., Huang, H. J., Gong, J. T., Zhang, X., & Hong, X. Y. (2020). *Wolbachia* supplement biotin and riboflavin to enhance reproduction in planthoppers. *The ISME Journal*, *14*, 676-687.

Kageyama, D., Narita, S., & Watanabe, M. (2012). Insect sex determination manipulated by their endosymbionts: incidences, mechanisms and implications. *Insect*, *3*, 161-199.

Kageyama, D., Ohno, M., Sasaki, T., Yoshido, A., Konagaya, T., Jouraku, A., Kuwazaki, S., Kanamori, H., Katayose, Y., Narita, S., & Miyata, M. (2017). Feminizing *Wolbachia* endosymbiont disrupts maternal sex chromosome inheritance in a butterfly species. *Evolution Letters*, 1, 232-244.

Kanara, H. G., & Acharya, M. F. (2014). Bionomics of rose thrips, *Frankiliniella occidentalis* Pergande. *Journal of Horticulture, 1*, 1-3.

Kant, R., Minor, M., Sandanayaka, M., & Trewick, S. (2013). Effects of mating and oviposition delay on parasitism rate and sex allocation behaviour of *Diaeretiella rapae* (Hymenoptera: Aphidiidae). *Biological Control*, 65, 265-270.

Katlav, A., Cook, J. M., & Riegler, M. (2021). Egg size-mediated sex allocation and mating-regulated reproductive investment in a haplodiploid thrips species. *Functional Ecology*, *35*, 485-498.

Katvala, M., Rönn, J. L., & Arnqvist, G. (2008). Correlated evolution between male ejaculate allocation and female remating behaviour in seed beetles (Bruchidae). *Journal of Evolutionary Biology*, *21*, 471-479.

Keller, L. (1999). Levels of selection in evolution. Princeton University Press.

Kemp, D. J., & Rutowski, R. L. (2004). A survival cost to mating in a polyandrous butterfly, *Colias eurytheme*. *Oikos 105*, 65-70.

Kempenaers, B., & Sheldon, B. C. (1997). Studying paternity and paternal care: pitfalls and problems. *Animal Behavior, 53,* 423–427.

Kern, P., Cook, J. M., Kageyama, D., & Riegler, M. (2015). Double trouble: combined action of meiotic drive and *Wolbachia* feminization in *Eurema butterflies*. *Biology Letters, 11*, 20150095.

Khanh, H. D. T., Bressac, C., & Chevrier C. (2005). Male sperm donation consequences in single and double matings in *Anisopteromalus calandrae*. *Physiological Entomology*, 30, 29-35.

Kindsvater, H. K., & Alonzo, S. H. (2014). Females allocate differentially to offspring size and number in response to male effects on female and offspring fitness. *Proceedings of the Royal Society of London B. 281*, 20131981.

King, B. H. (1987). Offspring sex ratios in parasitoid wasps. *The Quarterly Review of Biology*. *62*, 367-396.

King, B. H. (2000). Sperm depletion and mating behavior in the parasitoid wasp *Spalangia cameroni* (Hymenoptera: Pteromalidae). *Great Lakes Entomologist*, *33*, 4.

Kobayashi, K., Yoshimura, J., & Hasegawa, E. (2013). Coexistence of sexual individuals and genetically isolated asexual counterparts in a thrips. *Scientific Reports*. *3*, 3286.

Kolm, N., & Ahnesjö, I. (2005). Do egg size and parental care coevolve in fishes? *Journal of Fish Biology*, *66*, 1499-1515.

Komdeur, J., & Pen, I. (2002). Adaptive sex allocation in birds: the complexities of linking theory and practice. *Philosophical Transactions of the Royal Society B*, *357*, 373-380.

Koziel, S., & Ulijaszek, S. J. (2001). Waiting for Trivers and Willard: do the rich really favor sons? *American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists*, *115*, 71-79.

Kranz, B. D. (2005). Egg size and reproductive allocation in eusocial thrips. *Behavioral Ecology*, *16*, 779-787.

Kranz, B. D., Schwarz, M. P., Giles, L. C., & Crespi, B. J. (2000). Split sex ratios and virginity in a gall-inducing thrips. *Journal of Evolutionary Biology*, *13*, 700-706.

Kranz, B.D., Shibata, T., Tsuchida, K. and Okajima, S., 2002. Reproductive mode and split sex ratios in the facultatively ovoviviparous thrips, Bactrothrips brevitubus. *Evolutionary Ecology Research*, *4*, 1075-1092.

Krist, M. (2011). Egg size and offspring quality: a meta analysis in birds. *Biological Review*, *86*, 692-716.

Krueger, S., Mound, L. A. & Moritz, G. B. (2016). Offspring sex ratio and development are determined by copulation activity in *Echinothrips americanus* Morgan 1913 (Thysanoptera: Thripidae). *Journal of Applied Entomology*, *140*, 462-473.

Krueger, S., Mound, L. A., & Moritz, G. B. (2016). Offspring sex ratio and development are determined by copulation activity in *Echinothrips americanus* Morgan 1913 (Thysanoptera: Thripidae). *Journal of Applied Entomology, 140*, 462-473.

Kudo, S. I., Nakahira, T., & Saito, Y. (2006). Morphology of trophic eggs and ovarian dynamics in the subsocial bug *Adomerus triguttulus* (Heteroptera: Cydnidae). *Canadian Journal of Zoology, 84*, 723-728.

Kumm, S., & Moritz, G. (2008). First detection of *Wolbachia* in arrhenotokous populations of thrips species (Thysanoptera: Thripidae and Phlaeothripidae) and its role in reproduction. *Environmental Entomology, 37*, 1422-1428.

Kumm, S., & Moritz, G. (2010). Life cycle variation, including female production by virgin females in *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Journal of Applied Entomology*. *134*, 491-497.

Lacerda, L. D. M., Simões, Z. P., & Velthuis, H. H. W. (2010). The sharing of male production among workers and queens in *Scaptotrigona depilis* (Moure, 1942) (Apidae, Meliponini). *Insectes Sociaux, 57*, 185-192.

Lau, M. J., Ross, P. A., & Hoffmann, A. A. (2020). Infertility and fecundity loss of *Wolbachia*-infected *Aedes aegypti* hatched from quiescent eggs is expected to alter invasion dynamics. bioRxiv. https://doi.org/10.1101/2020.11.25.397240

Lazarevic, J., Peric-Mataruga, V., Vlahovic, M., Mrdakovic, M., & Cvetanovic, D. (2004). Effects of rearing density on larval growth and activity of digestive enzymes in *Lymantria dispar* L. (Lepidoptera: Lymantriidae). *Folia Biologica-Krakow, 52*, 105-112.

Lederhouse, R. C. (1981). effect of female mating frequency on egg fertility in the black swallowtail, *Papilio polyxenes* asterius (Papilionidae). *Journal of The Lepidopterists' Society, 35*, 226-227.

Lessells, C. K. M. (2006). The evolutionary outcome of sexual conflict. *Philosophical Transactions of the Royal Society B, 361*, 301-317.

Lewis, T. (1973). Thrips, their biology, ecology and economic importance. Academic Press Inc., London, 349 pp.

Li, C., He, M., Yun, Y., & Peng, Y. (2020). Co-infection with *Wolbachia* and *Cardinium* may promote the synthesis of fat and free amino acids in a small spider, *Hylyphantes graminicola*. *Journal of Invertebrate Pathology*, *169*, 107307.

Li, L. H., Zhang, Y., Zhu, D., & Zhou, X. N. (2018). Endosymbionts alter larva-to-nymph transstadial transmission of *Babesia microti* in *Rhipicephalus haemaphysaloides* ticks. *Frontiers in Microbiology, 9,* 1415.

Li, X. W., Fail, J., & Shelton, A. M. (2015). Female multiple matings and male harassment and their effects on fitness of arrhenotokous *Thrips tabaci* (Thysanoptera: Thripidae). *Behavioral Ecology and Sociobiology, 69,* 1585-1595.

Liu, P. C., Wei, H. X., Cao, D. D., & Wei, J. R. (2020). Relationships amongst sex ratio of progeny in *Anastatus disparis* (Hymenoptera: Eupelmidae), sperm depletion and decreased fecundity. *Applied Entomology and Zoology*, *55*, 25-30.

Liu, S. S., Colvin, J., & De Barro, P. J. (2012). Species concepts as applied to the whitefly *Bemisia tabaci* systematics: how many species are there? *Journal of Integrative Agriculture*, *11*, 176-186.

Livdahl, T. P., & Sugihara, G. (1984). Non-linear interactions of populations and the importance of estimating per capita rates of change. *The Journal of Animal Ecology*, *53*, 573-580.

Lord, C. C. (1998). Density dependence in larval *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, *35*, 825-829.

Mackauer, M. (1996). Sexual size dimorphism in solitary parasitoid wasps: influence of host quality. *Oikos*, *76*, 265-272.

Macke, E., Magalhaes, S., Bach, F., & Olivieri, I. (2012). Sex-ratio adjustment in response to local mate competition is achieved through an alteration of egg size in a haplodiploid spider mite. *Proceedings of the Royal Society B: Biological Sciences*, *279*, 4634-4642.

Macke, E., Magalhaes, S., Do-Thi Khanh, H., Frantz, A., Facon, B., & Olivieri, I. (2012a). Mating modifies female life history in a haplodiploid spider mite. *The American Naturalist*, *179*, 147-162.

Macke, E., Magalhaes, S., Khan, H. D. T, Luciano, A., Frantz, A., Facon, B., & Olivieri, I. (2011). Sex allocation in haplodiploids is mediated by egg size: evidence in the spider mite *Tetranychus urticae* Koch. *Proceedings of the Royal Society B, 278*, 1054-1063.

Macke, E., Olivieri, I., & Magalhães, S. (2014). Local mate competition mediates sexual conflict over sex ratio in a haplodiploid spider mite. *Current Biology*, *24*, 2850-2854.

Magrath, M. J., Van Lieshout, E., Visser, G. H., & Komdeur, J. (2004). Nutritional bias as a new mode of adjusting sex allocation. *Proceedings of the Royal Society B*, *271*, 347-349.

Majerus, M. E. (2003). Sex wars: genes, bacteria, and biased sex ratios. Princeton University Press.

Mastrantonio, V., Crasta, G., Puggioli, A., Bellini, R., Urbanelli, S., & Porretta, D. (2018). Cannibalism in temporary waters: Simulations and laboratory experiments revealed the role of spatial shape in the mosquito *Aedes albopictus*. *Plos one*, *13*, e0198194.

McGinley, M. A., Temme, D. H., & Geber, M. A. (1987). Parental investment in offspring in variable environments: theoretical and empirical considerations. *The American Naturalist, 130*, 370-398.

McLeish, M. J., Chapman, T. W., & Schwarz, M. P. (2007). Host-driven diversification of gall-inducing Acacia thrips and the aridification of Australia. *BMC Biology*, *5*, 3.

McMeniman, C. J., & O'Neill, S. L. (2010). A virulent *Wolbachia* infection decreases the viability of the dengue vector Aedes aegypti during periods of embryonic quiescence. *PLOS Neglected Tropical Diseases*, *4*, 748.

Medina, M., & Sachs, J. L. (2010). Symbiont genomics, our new tangled bank. *Genomics*, *95*, 129-137.

Merhej, V., Royer-Carenzi, M., Pontarotti, P., & Raoult, D. (2009). Massive comparative genomic analysis reveals convergent evolution of specialized bacteria. *Biology Direct*, *4*, 13.

Metzger, M., Bernstein, C., & Desouhant, E. (2008). Does constrained oviposition influence offspring sex ratio in the solitary parasitoid wasp *Venturia canescens*? *Ecological Entomology*, *33*, 167-174.

Moran, N. A., & Wernegreen, J. J. (2000). Lifestyle evolution in symbiotic bacteria: insights from genomics. *Trends in Ecology & Evolution*, *15*, 321-326.

Moran, N. A., McCutcheon, J. P., & Nakabachi, A. (2008). Genomics and evolution of heritable bacterial symbionts. *Annual Review of Genetics*, *42*, 165-190.

Moreira, L. A., Iturbe-Ormaetxe, I., Jeffery, J. A., Lu, G., Pyke, A. T., Hedges, L. M., Rocha, B. C., Hall-Mendelin, S., Day, A., Riegler, M., et al. (2009). A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell*, *139*, 1268-1278.

Morimoto, S., Kurtti, T. J., & Noda, H. (2006). In vitro cultivation and antibiotic susceptibility of a Cytophaga-like intracellular symbiote isolated from the tick *Ixodes scapularis*. *Current Microbiology*, *52*, 324-329.

Moritz, G. (1982). Zur Morphologie und Anatomie des Fransenfliiglers *Aeolothrips intermedius* Bagnall, 1934 (Aeolothripidae, Thysanoptera, Insecta). I Mitteilung. *Zoologische Jahrbücher Abteilung für Anatomie und Ontogenie der Tiere 107*, 557-608.

Moritz, G. (1988). The ontogenesis of Thysanoptera (Insecta) with special reference to the Panchaetothripine *Hercinothrips femoralis* (OM Reuter, 1891) (Thysanoptera, Thripidae, Panchaetothripinae) III. Praepupa and Pupa. *Zoologische Jahrbücher Abteilung für Anatomie und Ontogenie der Tiere*, *118*, 15-54.

Moritz, G. (1997). Structure, growth and development. Thrips as crop pests. In: Thrips as Crop Pests. Lewis, T. (Ed.), p.15-63. CAB International, Wallingford, CT.

Morrow, E. H., Arnqvist, G., & Pitcher, T. E. (2002). The evolution of infertility: does hatching rate in birds coevolve with female polyandry? *Journal of Evolutionary Biology*, *15*, 702-709.

Morrow, J. L., Hall, A. A., & Riegler, M. (2017). Symbionts in waiting: the dynamics of incipient endosymbiont complementation and replacement in minimal bacterial communities of psyllids. *Microbiome*, *5*, 58.

Mound, L. A., 2005. Thysanoptera: diversity and interactions. *Annual Review of Entomology*, *50*, 247-269.

Mound, L. A. (2011). Order Thysanoptera Haliday, 1836. In: Zhang, Z.-Q. (Ed.) Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. *Zootaxa, 3148*, 201-202.

Mound, L. A. (2014). Austral Thysanoptera: 100 years of progress. *Austral Entomology, 53*, 18-25.

Mound, L. A., & Jackman, D. J. (1998). Thrips in the economy and ecology of Australia. In *Proceedings of the sixth Australian Applied Entomological Research Conference*. pp. 472-478.

Moya-Laraño, J., & Fox, C. W. (2006). Ejaculate size, second male size, and moderate polyandry increase female fecundity in a seed beetle. *Behavioral Ecology, 17*, 940-946.

Nagelkerke, C. J., & Sabelis, M. W. (1998). Precise control of sex allocation in pseudo arrhenotokous phytoseiid mites. *Journal of Evolutionary Biology*, *11*, 649-684.

Nager, R. G., Monaghan, P., Griffiths, R., Houston, D. C., & Dawson, R. (1999). Experimental demonstration that offspring sex ratio varies with maternal condition. *Proceedings of the National Academy of Sciences*, *96*, 570-573.

Nakabachi, A., Yamashita, A., Toh, H., Ishikawa, H., Dunbar, H. E., Moran, N. A., & Hattori, M. (2006). The 160-kilobase genome of the bacterial endosymbiont *Carsonella*. *Science*, *314*, 267-267.

Nakamura, Y., Kawai, S., Yukuhiro, F., Ito, S., Gotoh, T., Kisimoto, R., & Noda, H. (2009). Prevalence of *Cardinium* bacteria in planthoppers and spider mites and taxonomic revision of "*Candidatus Cardinium* hertigii" based on detection of a new *Cardinium* group from biting midges. *Applied and Environmental Microbiology*, *75*, 6757-6763.

Nakamura, Y., Yukuhiro, F., Matsumura, M., & Noda, H. (2012). Cytoplasmic incompatibility involving *Cardinium* and *Wolbachia* in the white-backed planthopper *Sogatella furcifera* (Hemiptera: Delphacidae). *Applied Entomology and Zoology*, *47*, 273-283.

Nakao, S. (1993). Effects of temperature and photoperiod on wing form determination and reproduction of *Thrips nigropilosus* Uzel (Thysanoptera: Thripidae). *Applied Entomology and Zoology, 28*, 463-472.

Nakao, S. (1994). Effects of food type on wing form determination and development in female *Thrips nigropilosus* Uzel (Thysanoptera: Thripidae). *Applied Entomology and Zoology*, *29*, 273-278.

Navarro-Campos, C, Aguilar, A, & Garcia-Marí, F. (2011). Population trend and fruit damage of *Pezothrips kellyanus* in citrus orchards in Valencia (Spain). *IOBC/WPRS Bulletin*, *62*, 285-292.

Navarro-Campos, C., Pekas, A., Moraza, M. L., Aguilar, A., & Garcia-Marí, F. (2012). Soil-dwelling predatory mites in citrus: Their potential as natural enemies of thrips with special reference to *Pezothrips kellyanus* (Thysanoptera: Thripidae). *Biological Control*, *63,* 201-209.

Navarro Campos, C., Aguilar, A., & Garcia Marí, F. (2012). Aggregation pattern, sampling plan, and intervention threshold for *Pezothrips kellyanus* in citrus groves. *Entomologia Experimentalis et Applicata*, *142,* 130-139.

Neems, R. M., McLachlan, A. J., & Chambers, R. (1990). Body size and lifetime mating success of male midges (Diptera: Chironomidae). *Animal Behaviour*, *40*, 648-652.

Nguyen, D. T. (2015). Genetic diversity, reproductive systems and endosymbionts of two invasive thrips species (Doctoral dissertation, Hawkesbury Institute for the Environment, University of Western Sydney (Australia).

Nguyen, D. T., Morrow, J. L., Spooner-Hart, R. N., & Riegler, M. (2017). Independent cytoplasmic incompatibility induced by *Cardinium* and *Wolbachia* maintains endosymbiont coinfections in haplodiploid thrips populations. *Evolution, 71*, 995-1008.

Nguyen, D. T., Spooner-Hart, R. N., & Riegler, M. (2016) Loss of *Wolbachia* but not *Cardinium* in the invasive range of the Australian thrips species, *Pezothrips kellyanus*. *Biological Invasions*, *18,* 197-214.

Normark, B. B. (2003). The evolution of alternative genetic systems in insects. *Annual Review of Entomology*. *48*, 397-423.

Normark, B. B. (2004). Haplodiploidy as an outcome of coevolution between male-killing cytoplasmic elements and their hosts. *Evolution*, *58*, 790-798.

Nur, U., Werren, J. H., Eickbush, D. G., Burke, W. D., & Eickbush, T. H. (1988). A" selfish" B chromosome that enhances its transmission by eliminating the paternal genome. *Science, 240*, 512-514.

O'Neill, K. M. (1985). Egg size, prey size, and sexual size dimorphism in digger wasps (Hymenoptera: Sphecidae). *Canadian Journal of Zoology*, *63*, 2187-2193.

O'Neill, S. L., & Karr, T. L. (1990). Bidirectional incompatibility between conspecific populations of *Drosophila simulans*. *Nature*, *348*, 178.

Ochman, H., & Moran, N. A. (2001). Genes lost and genes found: evolution of bacterial pathogenesis and symbiosis. *Science*, *292*, 1096-1099.

Oddie, K. (1998). Sex discrimination before birth. *Trends in Ecology and Evolution*, *13*, 130-131.

Ode, P. J., Antolin, M. F., & Strand, M. R. (1997). Constrained oviposition and female-biased sex allocation in a parasitic wasp. *Oecologia*, *109*, 547-555.

Okayama, K., Katsuki, M., Sumida, Y., & Okada, K. (2016). Costs and benefits of symbiosis between a bean beetle and *Wolbachia*. *Animal Behaviour*, *119*, 19-26.

Olsson, M. & Shine, R. (1997). The limits to reproductive output: offspring size versus number in the sand lizard (*Lacerta agilis*). *The American Naturalist*, *149,* 179-188.

Osborne, S. E., San Leong, Y., O'Neill, S. L., & Johnson, K. N. (2009). Variation in antiviral protection mediated by different *Wolbachia* strains in *Drosophila simulans*. *PLoS Pathog*, *5,* e1000656.

Otto, S. P., & Jarne, P. (2001). Haploids- hapless or happening? *Science*, *292*, 2441-2443.

Paini, D. R., Sheppard, A. W., Cook, D. C., De Barro, P. J., Worner, S. P., & Thomas, M. B. (2016). Global threat to agriculture from invasive species. *Proceedings of the National Academy of Sciences. 113*, 7575-7579.

Pakyari, H., Fathipour, Y., & Enkegaard, A. (2011). Effect of temperature on life table parameters of predatory thrips *Scolothrips longicornis* (Thysanoptera: Thripidae) fed on two spotted spider mites (Acari: Tetranychidae). *Journal of Economic Entomology*. *104*, 799-805.

Parker, G. A. (1970). Sperm competition and its evolutionary consequences in the insects. *Biological Review*, *45*, 525-567.

Parker, G. A. (1979). Sexual selection and sexual conflict. Sexual selection and reproductive competition in insects, In: Sexual selection and reproductive competition in insects. pp. 123-166. Academic Press, New York.

Parker, G. A. (2006). Sexual conflict over mating and fertilization: an overview. *Philosophical Transactions of the Royal Society B*, 361, 235-259.

Parker, G. A., & Begon, M. 1986. Optimal egg size and clutch size: effects of environment and maternal phenotype. *American Naturalist. 128*, 573-592.

Parker, G. A., & Simmons, L. W. (1994). Evolution of phenotypic optima and copula duration in dungflies. *Nature*, *370*, 53-56.

Partridge, L., & Harvey, P. H., 1988. The ecological context of life history evolution. *Science*, *241*, 1449-1455.

Pen, I., & Weissing, F. J. (2002). Optimal sex allocation: steps towards a mechanistic theory. In: Sex ratios: concepts and research methods. Hardy, I. C. W. (Ed.). Cambridge University Press, Cambridge. pp. 26-45.

Penteriani, V., Delgado, M. M., Pérez-García, J. M., Botella, F., García, S., Zapata, J. A. S., Ortega, M. L., Martínez, J. E., Calvo, J. F., Ortego, J., & Penteriani, G. (2010). Sex allocation from an owl perspective: clutch order could determine brood sex to reduce sibling aggression in the Eagle Owl Bubo bubo. *Ornis Fennica*, *87*, 135.

Perlman, S. J., Kelly, S. E., & Hunter, M. S. (2008). Population biology of cytoplasmic incompatibility: maintenance and spread of *Cardinium* symbionts in a parasitic wasp. *Genetics*, *178*, 1003-1011.

Perry, J. C. (2011). Mating stimulates female feeding: testing the implications for the evolution of nuptial gifts. *Journal of Evolutionary Biology*, *24*, 1727-1736.

Petrie, M., Schwabl, H., Brande-Lavridsen, N., & Burke, T. (2001). Maternal investment: sex differences in avian yolk hormone levels. *Nature, 412*, 498.

Pike, T. W., & Petrie, M. (2003). Potential mechanisms of avian sex manipulation. *Biological Reviews, 78*, 553-574.

Pischedda, A., Stewart, A. D., Little, M. K., & Rice, W. R. (2010). Male genotype influences female reproductive investment in Drosophila melanogaster. *Proceedings of the Royal Society of London B, 278*, 2165-2172.

Pizzari, T., Cornwallis, C. K., Løvlie, H., Jakobsson, S., & Birkhead, T. R. (2003). Sophisticated sperm allocation in male fowl. *Nature, 426*, 70-74.

Plaistow, S. J., St. Clair, J. J., Grant, J., & Benton, T. G. (2007). How to put all your eggs in one basket: empirical patterns of offspring provisioning throughout a mother's lifetime. *The American Naturalist*, *170*, 520-529.

Poinsot, D., & Merçot, H. (1997). *Wolbachia* infection in Drosophila simulans: does the female host bear a physiological cost? *Evolution*, *51*, 180-186.

Price, T. A., & Wedell, N. (2008). Selfish genetic elements and sexual selection: their impact on male fertility. *Genetica*, *134,* 99.

Prokopová, M., Veselý, P., Fuchs, R., & Zrzavý, J. (2010). The role of size and colour pattern in protection of developmental stages of the red firebug (*Pyrrhocoris apterus*) against avian predators. *Biological Journal of the Linnean Society, 100,* 890-898.

Provencher, L. M., Morse, G. E., Weeks, A. R., & Normark, B. B. (2005). Parthenogenesis in the *Aspidiotus nerii* complex (Hemiptera: Diaspididae): a single origin of a worldwide, polyphagous lineage associated with *Cardinium* bacteria. *Annals of the Entomological Society of America*. *98*, 629-635.

Radder, R. S., Pike D. A., Quinn, A. E., Shine, R. (2009). Offspring sex in a lizard depends on egg size. *Current Biology, 19,* 1102-1105. https://doi.org/10.1016/j.cub.2009.05.027

Radmacher, S., & Strohm, E. (2010). Factors affecting offspring body size in the solitary bee *Osmia bicornis* (Hymenoptera, Megachilidae). *Apidologie*, *41*, 169-177.

Rao, Q., Rollat-Farnier, P. A., Zhu, D. T., Santos-Garcia, D., Silva, F. J., Moya, A., Latorre, A., Klein, C. C., Vavre, F., Sagot, M. F., & Liu, S. S. (2015). Genome reduction and potential metabolic complementation of the dual endosymbionts in the whitefly *Bemisia tabaci*. *BMC genomics*, *16*, 1-13.

Rautiala, P., Helanterä, H., & Puurtinen, M. (2018). The evolutionary dynamics of adaptive virginity, sex allocation, and altruistic helping in haplodiploid animals. *Evolution*, *72,* 30-38.

Riegler, M., O'Neill, S. L., Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K-H., & Stackebrandt, E. (2006). The genus Wolbachia. In: The Prokaryotes: Volume 5: *Proteobacteria: Alpha and Beta Subclasses*. Falkow, S. Rosenberg, E., Schleifer, K-H, Stackebrandt, E. (Eds.), pp. 547 – 561. New York, NY: Springer.

Rollinson, N., & Rowe, L. (2015). Persistent directional selection on body size and a resolution to the paradox of stasis. *Evolution*, *69*, 2441-2451.

Rönn, J.L., Katvala, M., & Arnqvist, G. (2011). Correlated evolution between male and female primary reproductive characters in seed beetles. *Functional Ecology*, *25*, 634-640.

Rose, M. R. (1991). Evolutionary biology of aging (1st ed.). New York: Oxford University Press.

Ross, K. G., Vargo, E. L., Keller, L., & Trager, J. C. (1993). Effect of a founder event on variation in the genetic sex-determining system of the fire ant *Solenopsis invicta*. *Genetics*, 135, 843-854.

Ross, L., Pen, I., & Shuker, D. M. (2010). Genomic conflict in scale insects: the causes and consequences of bizarre genetic systems. *Biological Reviews*, *85,* 807-828.

Ross, P. A., Endersby, N. M., Yeap, H. L., & Hoffmann, A. A. (2014). Larval competition extends developmental time and decreases adult size of wMelPop *Wolbachia*-infected *Aedes aegypti*. *The American Journal of Tropical Medicine and Hygiene*, *91*, 198-205.

Ross, P.A., Turelli, M., & Hoffmann, A.A. (2019). Evolutionary ecology of *Wolbachia* releases for disease control. *Annual Review of Genetics, 53*, 93-116.

Sachs, J. L., Skophammer, R. G., & Regus, J. U. (2011). Evolutionary transitions in bacterial symbiosis. *Proceedings of the National Academy of Sciences*, *108*, 10800-10807.

Saino, N., Romano, M., Ambrosini, R., Ferrari, R. P., & Møller, A. P. (2004). Timing of reproduction and egg quality covary with temperature in the insectivorous Barn Swallow, *Hirundo rustica*. *Functional Ecology*, *18*, 50-57.

Sakurai, G., & Kasuya, E. (2008). The costs of harassment in the adzuki bean beetle. *Animal Behaviour*, *75*, 1367-1373.

Santolamazza Carbone, S., Nieto, M. P. & Rivera, A. C. (2007). Maternal size and age affect offspring sex ratio in the solitary egg parasitoid *Anaphes nitens*. *Entomologia Experimentalis et Applicata*, *125*, 23-32.

Santolamazza Carbone, S., Nieto, M. P. & Rivera, A. C. (2007). Maternal size and age affect offspring sex ratio in the solitary egg parasitoid *Anaphes nitens*. *Entomologia Experimentalis et Applicata*, 125,23-32.

Sarakatsanou, A., Diamantidis, A. D., Papanastasiou, S.A., Bourtzis, K., & Papadopoulos, N. T. (2011). Effects of *Wolbachia* on fitness of the Mediterranean fruit fly (Diptera: Tephritidae). *Journal of Applied Entomology*, *135*, 554-563.

Schärer, L., & Pen, I. (2013). Sex allocation and investment into pre-and post-copulatory traits in simultaneous hermaphrodites: the role of polyandry and local sperm competition. *Philosophical Transactions of the Royal Society B*, *368*, 20120052.

Schmidt-Nielsen, K. & Knut, S. N. (1984). Scaling: why is animal size so important? Cambridge University press.

Schneider, D., Miller, W.J. & Riegler, M. (2011). Arthropods shopping for *Wolbachia*. In: Manipulative Tenants Bacteria Associated with Arthropods. Zchori-Fein E. & Bourtzis, K. (Eds.) pp.149-173. Boca Raton, Fl: CRC Press.

Schneider, M. I., & Vinuela, E. (2007). Improvements in rearing method for *Hyposoter didymator* (Hymenoptera: Ichneumonidae), considering sex allocation and sex determination theories used for Hymenoptera. *Biological Control*, *43*, 271-277.

Schneweis, D. J., Whitfield, A. E., and Rotenberg, D. (2017). Thrips developmental stage-specific transcriptome response to tomato spotted wilt virus during the virus infection cycle in *Frankliniella occidentalis*, the primary vector. *Virology, 500*, 226-237.

Schrader, F., & Hughes-Schrader, S. (1931). Haploidy in metazoa. *The Quarterly Review of Biology*. *6,* 411-438.

Schuler, H., Köppler, K., Daxböck Horvath, S., Rasool, B., Krumböck, S., Schwarz, D., Hoffmeister, T. S., Schlick Steiner, B. C., Steiner, F. M., Telschow, A., & Stauffer, C. (2016). The hitchhiker's guide to Europe: the infection dynamics of an ongoing *Wolbachia* invasion and mitochondrial selective sweep in *Rhagoletis cerasi*. *Molecular Ecology*, *25*, 1595-1609.

Seidelmann, K., Ulbrich, K., & Mielenz, N. (2010). Conditional sex allocation in the Red Mason bee, *Osmia rufa*. *Behavioral Ecology and Sociobiology*, *64*, 337-347.

Shan, H. W., Luan, J. B., Liu, Y. Q., Douglas, A. E., & Liu, S. S. (2019). The inherited bacterial symbiont *Hamiltonella* influences the sex ratio of an insect host. *Proceedings of the Royal Society B*, *286*, 20191677.

Shigenobu, S., & Wilson, A. C. (2011). Genomic revelations of a mutualism: the pea aphid and its obligate bacterial symbiont. *Cellular and Molecular Life Sciences*, *68*, 1297-1309.

Shine, R. (1988). The evolution of large body size in females: a critique of Darwin's" fecundity advantage" model. *The American Naturalist*, *131*, 124-131.

Shuker, D. M., & Day, T. H. (2001). The repeatability of a sexual conflict over mating. *Animal Behaviour, 61*, 755-762.

Shuker, D. M., & West, S. A. (2004). Information constraints and the precision of adaptation: sex ratio manipulation in wasps. *Proceedings of the National Academy of Sciences, 101*, 10363-10367.

Shuker, D. M., Moynihan, A. M., & Ross, L. (2009). Sexual conflict, sex allocation and the genetic system. *Biology Letters*, 5, 682-685.

Shuker, D. M., Sykes, E. M., Browning, L. E., Beukeboom, L. W., & West, S. A. (2006). Male influence on sex allocation in the parasitoid wasp *Nasonia vitripennis*. *Behavioral Ecology and Sociobiology, 59,* 829-835.

Shukla, S., Shilpa, M. C., & Gadagkar, R. (2013). Virgin wasps develop ovaries on par with mated females, but lay fewer eggs. *Insectes Sociaux, 60*, 345-350.

Simmons L. W. (2001). Sperm competition and its evolutionary consequences in the insects (Vol. 68). Princeton University Press.

Singh, R., & Linksvayer, T. A. (2020). *Wolbachia*-infected ant colonies have increased reproductive investment and an accelerated life cycle. *Journal of Experimental Biology*, *223*.

Singh, S. R., & Singh, B. N. (2004). Female remating in *Drosophila*: comparison of duration of copulation between first and second matings in six species. *Current Science*, pp. 465-470.

Siva-Jothy, M. T., & Stutt, A. D. (2003). A matter of taste: direct detection of female mating status in the bedbug. *Proceedings of the Royal Society of London B, 270,* 649-652.

Smith, A. R., Kapheim, K. M., Kingwell, C. J., & Wcislo, W. T. (2019). A split sex ratio in solitary and social nests of a facultatively social bee. *Biology Letters*, *15,* 20180740.

Smith, C. C., & Fretwell, S. D. (1974). The optimal balance between size and number of offspring. *The American Naturalist*, *108,* 499-506.

Snook, R. R., Gidaszewski, N. A., Chapman, T. & Simmons, L. W. (2013). Sexual selection and the evolution of secondary sexual traits: sex comb evolution in *Drosophila*. *Journal of Evolutionary Biology*, *26*, 912-918.

Sokal, R. R., & Rohlf, J. (1995). Biometry, 3rd edn. W. H. Freeman, New York.

Southwood, T. R. E., May, R. M., Hassell, M. P., & Conway, G. R. (1974). Ecological strategies and population parameters. *The American Naturalist*, *108*, 791-804.

Stearns, S. C. (1989). Trade-offs in life-history evolution. *Functional ecology*, *3,* 259-268.

Stearns, S. C. (1992). The evolution of life histories. Oxford: Oxford University Press.

Stevens, L., & Wade, M. J. (1990). Cytoplasmically inherited reproductive incompatibility in *Tribolium* flour beetles: the rate of spread and effect on population size. *Genetics*, *124*, 367-372.

Stillwell, R. C., & Davidowitz, G. (2010). Sex differences in phenotypic plasticity of a mechanism that controls body size: implications for sexual size dimorphism. *Proceedings of the Royal Society of London B, 277*, 3819-3826.

Stillwell, R. C., Blanckenhorn, W. U., Teder, T., Davidowitz, G., & Fox, C. W. (2010). Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. *Annual Review of Entomology*, *55*, 227-245.

Stoepler, T. M., Lill, J. T., & Murphy, S. M. (2011). Cascading effects of host size and host plant species on parasitoid resource allocation. *Ecological Entomology*, *36*, 724-735.

Stouthamer, C. M., Kelly, S. E., Mann, E., Schmitz-Esser, S., & Hunter, M. S. (2019). Development of a multi-locus sequence typing system helps reveal the evolution of *Cardinium hertigii*, a reproductive manipulator symbiont of insects. *BMC Microbiology*. *19*, 266.

Stouthamer, R., Breeuwer, J. A. J., Luck, R. F., & Werren, J. H. (1993). Molecular identification of microorganisms associated with parthenogenesis. *Nature*, *361*, 66-68.

Stouthamer, R., Breeuwer, J. A., & Hurst, G. D. (1999). *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annual Reviews in Microbiology*, 53, 71-102.

Stouthamer, R., Hurst, G., D. & Breeuwer, J. A. (2002). Sex ratio distorters and their detection. In: Sex ratios: Concepts and research methods. Hardy, I. C. W. (ed). Cambridge University Press, Cambridge, pp. 195-215.

Stouthamer, R., Luck, R. F. & Hamilton, W. D. (1990). Antibiotics cause parthenogenetic Trichogramma (Hymenoptera/Trichogrammatidae) to revert to sex. *Proceedings of the National Academy of Sciences, 87*, 2424-2427.

Stouthamer, R., Tilborg, M. V., De Jong, J. H., Nunney, L., & Luck, R. F. (2001). Selfish element maintains sex in natural populations of a parasitoid wasp. *Proceedings of the Royal Society of London. Series B, 268*, 617-622.

Suh, E., Mercer, D. R., & Dobson, S. L. (2017). Life-shortening *Wolbachia* infection reduces population growth of *Aedes aegypti. Acta Tropica*, *172*, 232-239.

Tagawa, J., Yoshida, C., Hashimoto, T., & Sudare, A. (1987). Effects of mating on the oviposition behaviour of the parasitic wasp, *Apanteles glomeratus* L. (Hymenoptera: Braconidae). *Journal of Ethology*, 5, 37-41.

Tamames, J., Gil, R., Latorre, A., Peretó, J., Silva, F. J., & Moya, A. (2007). The frontier between cell and organelle: genome analysis of *Candidatus Carsonella* ruddii. *BMC Evolutionary Biology*, 7, 181.

Tammaru, T., Esperk, T., Ivanov, V., & Teder, T. (2010). Proximate sources of sexual size dimorphism in insects: locating constraints on larval growth schedules. *Evolutionary Ecology, 24*, 161-175.

Taylor, M. L., Price, T. A., & Wedell, N. (2014). Polyandry in nature: a global analysis. *Trends in Ecology & Evolution*, *29*, 376-383.

Teder, T. (2014). Sexual size dimorphism requires a corresponding sex difference in development time: A meta analysis in insects. *Functional Ecology*, *28*, 479-486.

Terry, L. I., & Kelly, C. K. (1993). Patterns of change in secondary and tertiary sex ratios of the Terebrantian thrips, *Frankliniella occidentalis*. Entomologia Experimentalis et Applicata, *66*, 213-225.

Thornhill, R., & Alcock, J. (1983). *The evolution of insect mating systems*. Harvard University Press.

Toft, C., & Andersson, S. G. (2010). Evolutionary microbial genomics: insights into bacterial host adaptation. *Nature Reviews Genetics*, *11*, 465.

Tree, D. J., & Walter, G. H. (2009). Diversity of host plant relationships and leaf galling behaviours within a small genus of thrips–*Gynaikothrips* and Ficus in south east Queensland, Australia. *Australian Journal of Entomology*, *48,* 269-275.

Tregenza, T., & Wedell, N. (2000). Genetic compatibility, mate choice and patterns of parentage: invited review. *Molecular Ecology*, *9*, 1013-1027.

Trivers, R. L., & Hare, H. (1976). Haploidploidy and the evolution of the social insect. *Science*, *191*, 249-263.

Trivers, R. L., & Willard, D. E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science*, *179*, 90-92.

Turelli, M. (1994). Evolution of incompatibility inducing microbes and their hosts. *Evolution*, *48*, 1500-1513.

Tyagi, K., Mound, L., & Kumar, V. (2008). Sexual dimorphism among Thysanoptera Terebrantia, with a new species from Malaysia and remarkable species from India in Aeolothripidae and Thripidae. *Insect Systematics & Evolution*, *39*, 155-170.

Ueno, T. (1995). Abdominal tip movements during oviposition by two parasitoids (Hymenoptera: Ichneumonidae) as an index of predicting the sex of depositing eggs. *Applied Entomology and Zoology*, *30*, 588-590.

Ueno, T. (1999). Host size dependent sex ratio in a parasitoid wasp. *Population Ecology*, *41*, 47-57.

Vala, F, Breeuwer, J. A., & Sabelis, M. W. (2000). *Wolbachia*–induced "hybrid breakdown" in the two–spotted spider mite *Tetranychus urticae* Koch. *Proceedings of the Royal Society of London B*, *267*, 1931-1937.

Vala, F., Egas, M., Breeuwer, J. A. J., & Sabelis, M. W. (2004). *Wolbachia* affects oviposition and mating behaviour of its spider mite host. *Journal of Evolutionary Biology*, *17*, 692-700.

Van den Assem, J. (1970). Courtship and mating in *Luriophagus disringwndus* (Forst) Kurdj. (Hymenoptera, Pteromalidae). *Netherlands Journal of Zoology*, *20*, 329-352.

Van den Assem, J. (1977). A note on the ability to fertilize following insemination. *Netherlands Journal of Zoology*, *27*, 230-235.

Van den Beuken, T. P., & Smallegange, I. M. (2018). Life-history consequences of bidirectional selection for male morph in a male-dimorphic bulb mite. *Experimental and Applied Acarology*, *76*, 435-452.

Van Noordwijk, A. J. & de Jong, G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. *The American Naturalist*, *128*, 137-142.

Varikou, K., Birouraki, A., Tsitsipis, I., & Sergentani, C. H. R. (2012). Effect of temperature on the fecundity of *Pezothrips kellyanus* (Thysanoptera: Thripidae). *Annals of the Entomological Society of America*, *105,* 60-65.

Vassiliou, V, A. (2007). Chemical control of *Pezothrips kellyanus* (Thysanoptera: Thripidae) in citrus plantations in Cyprus. *Crop Protection*, *26,* 1579-1584.

Vassiliou, V. A. (2010). Ecology and behavior of *Pezothrips kellyanus* (Thysanoptera: Thripidae) on citrus. *Journal of Economic Entomology*, *103,* 47-53.

Vassiliou, V. A. (2011). Botanical insecticides in controlling Kelly's citrus thrips (Thysanoptera: Thripidae) on organic grapefruits. *Journal of Economic Entomology, 104*, 1979-1985.

Vautrin, E., Charles, S., Genieys, S., & Vavre, F. 2007. Evolution and invasion dynamics of multiple infections with *Wolbachia* investigated using matrix based models. *Journal of Theoretical Biology, 245*, 197-209.

Vavre, F., Fleury, F., Varaldi, J., Fouillet, P., & Boulétreau, M. (2000). Evidence for female mortality in *Wolbachia*-mediated cytoplasmic incompatibility in haplodiploid insects: epidemiologic and evolutionary consequences. *Evolution*, *54*, 191-200.

Vavre, F., Fouillet, P., & Leury, F. (2003). Between and within host species selection on cytoplasmic incompatibility inducing *Wolbachia* in haplodiploids. *Evolution*, 57, 421-427.

Vavre, F., Girin, C., & Bouletreau, M. (1999). Phylogenetic status of a fecundity enhancing *Wolbachia* that does not induce thelytoky in *Trichogramma*. *Insect Molecular Biology*, *8,* 67-72.

Verspoor, R. L., Price, T. A., & Wedell, N. (2020). Selfish genetic elements and male fertility. *Philosophical Transactions of the Royal Society B*, *375,* 20200067.

Visser, M. E. (1994). The importance of being large: the relationship between size and fitness in females of the parasitoid *Aphaereta minuta* (Hymenoptera: Braconidae). *Journal of Animal Ecology*, *63,* 963-978.

von Engelhardt, N., Carere, C., Dijkstra, C., & Groothuis, T. G. G. (2006). Sex-specific effects of yolk testosterone on survival, begging and growth of zebra finches. *Proceedings of the Royal Society B, 273*, 65-70.

Wade, M. J., & Chang, N. W. (1995). Increased male fertility in *Tribolium confusum* beetles after infection with the intracellular parasite *Wolbachia*. *Nature*, *373*, 72-74.

Wang, Y. B., Ren, F. R., Yao, Y. L., Sun, X., Walling, L. L., Li, N. N., Bai, B., Bao, X. Y., Xu, X. R., & Luan, J. B. (2020). Intracellular symbionts drive sex ratio in the whitefly by facilitating fertilization and provisioning of B vitamins. *The ISME Journal*, *14*, 2923-2935.

Weatherhead, P. J., & Teather, K. L. (1994). Sexual size dimorphism and egg size allometry in birds. *Evolution*, *48*, 671-678.

Webster, K. W., Cooper, P, Mound, L. A. (2006). Studies on Kelly's citrus thrips, *Pezothrips kellyanus* (Bagnall) (Thysanoptera: Thripidae): sex attractants, host associations and country of origin. *Australian Journal of Entomology, 45*, 67-74.

Wedell, N. (2013). The dynamic relationship between polyandry and selfish genetic elements. *Philosophical Transactions of the Royal Society of London B, 368*, 20120049.

Wedell, N. (2020). Selfish genes and sexual selection: the impact of genomic parasites on host reproduction. *Journal of Zoology*, *311*, 1-12.

Wedell, N., & Price, T. A. (2015). Selfish Genetic Elements and Sexual Selection. In: Current Perspectives on Sexual Selection – What’s left after Darwin?. Hoquet, T. (Ed.). Springer, Dordrecht. pp. 165-190.

Weeks, A. R., & Breeuwer, J. A. J. (2001). *Wolbachia*–induced parthenogenesis in a genus of phytophagous mites. *Proceedings of the Royal Society of London B*, *268*, 2245-2251.

Weeks, A. R., & Stouthamer, R., 2004. Increased fecundity associated with infection by a Cytophaga–like intracellular bacterium in the predatory mite, *Metaseiulus occidentalis*. *Proceedings of the Royal Society of London B*, *271*, S193-S195.

Weeks, A. R., Marec, F., & Breeuwer, J. A. J. (2001). A mite species that consists entirely of haploid females. *Science*. *292*, 2479-2482.

Weeks, A. R., Turelli, M., Harcombe, W. R., Reynolds, K. T., & Hoffmann, A. A. (2007). From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. *PLoS Biology*, *5*, 114.

Weeks, A. R., Velten, R., & Stouthamer, R. (2003). Incidence of a new sex–ratio–distorting endosymbiotic bacterium among arthropods. *Proceedings of the Royal Society of London B, 270*, 1857-1865.

Weinert, L. A., Araujo-Jnr, E. V., Ahmed, M. Z., & Welch, J. J. (2015). The incidence of bacterial endosymbionts in terrestrial arthropods. *Proceedings of the Royal Society of London B, 282*, 20150249.

Werren, J. H. (1991). The paternal-sex-ratio chromosome of *Nasonia*. *The American Naturalist 137*, 392-402.

Werren, J. H. (1997). Biology of *Wolbachia*. *Annual Review of Entomology*, *42*, 587-609.

Werren, J. H. (2011). Selfish genetic elements, genetic conflict, and evolutionary innovation. *Proceedings of the National Academy of Sciences*, *108*, 10863-10870.

Werren, J. H., & Beukeboom, L. W. (1998). Sex determination, sex ratios, and genetic conflict. *Annual Review of Ecology and Systematics*, *29*, 233-261.

Werren, J. H., & O'Neill, S. L. (1997). The evolution of heritable symbionts. In: Influential passengers: inherited microorganisms and arthropod reproduction. O'Neill, S. L., Hoffman, A. A., Werren, J. H. (Eds.) pp.1-41. Oxford: Oxford Univ. Press

Werren, J. H., & Stouthamer, R. (2003). PSR (paternal sex ratio) chromosomes: the ultimate selfish genetic elements. *Genetica*, *117*, 85-101.

Werren, J. H., Baldo, L., & Clark, M. E. (2008). *Wolbachia*: master manipulators of invertebrate biology. *Nature Reviews Microbiology*, *6*, 741-751.

Werren, J. H., Nur, U. and Wu, C. I. (1988). Selfish genetic elements. *Trends in Ecology & Evolution*, 3, 297-302.

Werren, J. H., O'Neill, S. L., & Hoffman, A. (1997). Influential passengers: inherited microorganisms and arthropod reproduction. The evolution of heritable symbionts. Oxford University Press, Oxford, pp.1-41.

West, S. A. (2009). Sex allocation. Princeton, NJ: Princeton University Press.

West, S. A., & Sheldon, B. C. (2002). Constraints in the evolution of sex ratio adjustment. *Science, 295*, 1685-1688.

West, S. A., Compton, S. G., Vincent, S. L., Herre, E. A., & Cook, J. M. (1998). Virginity in haplodiploid populations: a comparison of estimation methods. *Ecological Entomology*, *23*, 207-210.

White, J. A., Kelly, S. E., Perlman, S. J., & Hunter. M. S. (2009). Cytoplasmic incompatibility in the parasitic wasp *Encarsia inaron*: disentangling the roles of *Cardinium* and *Wolbachia* symbionts. *Heredity 102*, 483-489.

White, M. J., 1984. Chromosomal mechanisms in animal reproduction. *Italian Journal of Zoology, 51*, 1-23.

Wild, G., Gardner, A., & West, S.A. (2009). Adaptation and the evolution of parasite virulence in a connected world. *Nature*, 459, 983-986.

Williams, G. C. (1966). Adaptation and natural selection. Princeton University Press, Princeton, N.J.

Wills, T. E., Chapman, T. W., Mound, L. A., Kranz, B. D., & Schwarz, M. P. (2004). Natural history and description of *Oncothrips kinchega*, a new species of gall inducing thrips with soldiers (Thysanoptera: Phlaeothripidae). *Australian Journal of Entomology*, *43*, 169-176.

Winkler, D. W., & Wallin, K. (1987). Offspring size and number: a life history model linking effort per offspring and total effort. *The American Naturalist*, *129*, 708-720.

Wong Muñoz, J., Anderson, C. N., Munguía Steyer, R., & Córdoba Aguilar, A. (2013). Body size and morph as drivers of copulation duration in a male dimorphic damselfly. *Ethology 119*, 407-416.

Wrensch, D. L., & Ebbert, M. A. (1993). Evolution and diversity of sex ratio in insects and mites. New York, NY: Chapman & Hall.

Wrensch, D. L., & Young, S. S. Y. (1978). Effects of density and host quality on rate of development, survivorship, and sex ratio in the carmine spider mite. *Environmental Entomology*, *7*, 499-501.

Xiao, N., Pan, L. L., Zhang, C. R., Shan, H. W., & Liu, S. S. (2016). Differential tolerance capacity to unfavourable low and high temperatures between two invasive whiteflies. *Scientific Reports*, *6*, 1-10.

Xie, R. R., Chen, X. L., & Hong, X. Y. (2011). Variable fitness and reproductive effects of *Wolbachia* infection in populations of the two-spotted spider mite *Tetranychus urticae* Koch in China. *Applied Entomology and Zoology, 46*, 95-102.

Zabalou, S., Riegler, M., Theodorakopoulou, M., Stauffer, C., Savakis, C., & Bourtzis, K. (2004). *Wolbachia*-induced cytoplasmic incompatibility as a means for insect pest population control. *Proceedings of the National Academy of Sciences, 101*, 15042-15045.

Zchori-Fein, E., Gottlieb, Y., & Coll, M. (2000). *Wolbachia* density and host fitness components in *Muscidifurax uniraptor* (Hymenoptera: Pteromalidae). *Journal of Invertebrate Pathology*. *75*, 267-272.

Zchori-Fein, E., Gottlieb, Y., Kelly, S. E., Brown, J. K., Wilson, J. M., Karr, T. L. & Hunter, M. S. (2001). A newly discovered bacterium associated with parthenogenesis and a change in host selection behavior in parasitoid wasps. *Proceedings of the National Academy of Sciences*, *98*, 12555-12560.

Zchori-Fein, E., Perlman, S. J., Kelly, S. E., Katzir, N., & Hunter, M. S. (2004). Characterization of a '*Bacteroidetes*' symbiont in *Encarsia* wasps (Hymenoptera: Aphelinidae): proposal of '*Candidatus Cardinium* hertigii'. *International Journal of Systematic and Evolutionary Microbiology*, *54*, 961-968.

Zchori Fein, E., & Perlman, S. J. (2004). Distribution of the bacterial symbiont *Cardinium* in arthropods. *Molecular Ecology*, *13*, 2009-2016.

Zeh, J. A., & Zeh, D. W. (1996). The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proceedings of the Royal Society of London B*, 263, 1711-1717.

Żelazowska, M. (2010). Formation and structure of egg envelopes in Russian sturgeon *Acipenser gueldenstaedtii* (Acipenseriformes: Acipenseridae). *Journal of Fish Biology*, *76*, 694-706.

Zélé, F., Denoyelle, J., Duron, O. and Rivero, A., 2018. Can *Wolbachia* modulate the fecundity costs of Plasmodium in mosquitoes? *Parasitology, 145*, 775-782.

Zhang, D. Y. & Jiang, X. H. (2002). Size dependent resource allocation and sex allocation in herbaceous perennial plants. *Journal of Evolutionary Biology*, *15,* 74-83.

Zhang, X. F., Zhao, D. X., & Hong, X. Y. (2012). *Cardinium* – the leading factor of cytoplasmic incompatibility in the planthopper *Sogatella furcifera* doubly infected with *Wolbachia* and *Cardinium*. *Environmental Entomology*, *41*, 833-840.

Zhang, X. W., Dunn, D. W., & Wang, R. W. (2020). Egg load is a cue for offspring sex ratio adjustment in a fig pollinating wasp with male eggs first sex allocation. *Journal of Evolutionary Biology*, *33*, 366-376.

Zhou, W., Rousset, F., & O'Neill, S. (1998). Phylogeny and PCR-based classification of *Wolbachia* strains using wsp gene sequences. *Proceeding of Royal Society of London B, 265*, 509-515.

Zhu, L. Y., Zhang, K. J., Zhang, Y. K., Ge, C., Gotoh, T., & Hong, X. Y. (2012). *Wolbachia* strengthens *Cardinium*-induced cytoplasmic incompatibility in the spider mite *Tetranychus piercei* McGregor. *Current Microbiology, 65*, 516-523.

Zug, R., & Hammerstein, P. (2012). Still a host of hosts for *Wolbachia*: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PloS one*, *7,* e38544.

Zug, R., & Hammerstein, P. (2015). Bad guys turned nice? A critical assessment of *Wolbachia* mutualisms in arthropod hosts. *Biological Reviews 90,* 89-111.

Zuk, M. (1987). The effects of gregarine parasites, body size, and time of day on spermatophore production and sexual selection in field crickets. *Behavioral Ecology and Sociobiology*, *21*, 65-72.