Biology and behaviour of New Zealand ladybirds: Insights into the establishment success of introduced species

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Thesis abstract

What factors affect the successful establishment of introduced species? Our understanding of establishment processes is limited and concentrated on mean trait differences. However, recent research suggests that high levels of variation in functionally important traits facilitate establishment success. Ladybirds are generalist predators that serve as an excellent model system to study establishment success as they are widely introduced as biocontrol agents against agricultural pests. In my PhD, I examined the phenotypic (morphological, behavioural and physiological) variation and phylogenetic relationships between introduced and native ladybirds in New Zealand. Contrasting the differences between successfully established introduced species and native species in recipient ecosystems provides insights about the mechanisms that drive establishment success and a foundation for future research into introduction biology.

Firstly, I developed a framework to study establishment processes using ladybirds as a model system. I identified fundamental gaps in research into unsuccessful species introductions, dispersal abilities and the quantification of both inter- and intraspecific variation in ladybirds. This provided the conceptual foundation for my subsequent research comparing inter- and intraspecific variation in the morphology, behaviour and physiology of New Zealand ladybirds.

Morphologically, I found that introduced ladybirds had wider bodies than native ladybirds. Further, introduced species had greater intraspecific variation in size than native species. Introduced ladybirds with wide bodies and high intraspecific variation may have a larger prey range compared to native ladybirds, thereby increasing the probability of successful establishment. Behaviourally, I found differences between species in their general activity levels and responses to simulated predator attacks but there was no difference between species in their intraspecific variation. Physiologically, there was inter- and intraspecific variation in survival during desiccation resistance assays.

Overall, body size appears to influence traits associated with establishment success. There was a strong positive correlation between body size and general activity levels and survival time during desiccation resistance assays in each species. I found that the widely dispersed introduced ladybird *Halmus chalybeus* had significantly higher levels of intraspecific variation in size and colour compared to other species of ladybirds. This suggests that high intraspecific variation may indeed increase establishment success and dispersal.

Finally, the molecular phylogeny I constructed strongly supported the monophyly of Coccinellidae and subfamilies Microweiseinae and Coccinellinae and moderately supported the monophyly of tribe Epilachnini and paraphyletic tribes Coccinellini, Scymnini, Chilocorini and Coccidulini. However, there was no indication that the successful establishment of introduced species of ladybirds increases with relatedness to native species in New Zealand.

This is the first study to compare phenotypic variation between introduced and native ladybirds in New Zealand and the first molecular phylogeny to include native species of New Zealand ladybirds. My thesis presents indirect support for the hypothesis that high levels of intraspecific variation increase establishment success in introduced species. I propose that assessing phenotypic variation in introduced species is key to understanding establishment processes and thereby improving the effectiveness of biocontrol and conservation programmes.

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Chapter 1

Introduction

1.1 Background

Why do some introduced species become successfully established while some other species do not, despite numerous introduction efforts? What effects do introduced species have on native biodiversity? Because of the challenges associated with anthropogenic change, answering these questions is a key aim of current ecological research (Elliott *et al.* 1996; Gordon 1998; Kumschick *et al.* 2015). An introduced species is a species outside their natural geographical range, that may or may not become established in a new environment. Successful establishment of introduced species is important to enhance the effectiveness of biocontrol programmes and re-introduction of native and endemic species in conservation programmes (Gibbs *et al.* 2008; Griffith *et al.* 2019; Oro *et al.* 2011; Samways *et al.* 1999; Santos *et al.* 2009; Sarrazin and Barbault 1996; Seddon 2010). However, the success rate of species establishment is very low (Cock *et al.* 2016), and this has serious implications for pest management and conservation programmes. On the other hand, some introduced species spread very quickly and drive adverse ecological and evolutionary impacts such as biotic homogenisation (Dick *et al.* 2013; Kumschick *et al.* 2015; Mollot *et al.* 2017; Olden and Poff 2004). When non-native species become established in an environment outside their natural geographical range and cause, negative impacts on the environment, human activities, or human health it is called an invasive species (Lee, 2002). Invasive species can cause the loss of native diversity and the restructuring or destabilization of energy flow in food webs (David *et al.* 2017; Mollot *et al.* 2017). Therefore, understanding the factors affecting establishment success will provide insights that will not only enhance conservation and pest management programmes but also the development of strategies to control and mitigate the adverse effects of invasive species.

1.1.1 Establishment success

Species are introduced for conservation, sport and biological control of pest species in integrative pest management (Cock *et al.* 2016). Successful establishment means that the introduced species can maintain a self-sustaining population and disperse naturally from a location that is outside its natural geographical range (Hodek et al., 2012). However, not all introduced species become established. In fact, recent analysis of the establishment success of introduced species in biocontrol programmes suggests that the maximum success rate is 32.6% (Cock *et al.* 2016). Introduced populations must overcome several barriers to become established (i.e. geography, captivity/cultivation, survival, reproduction and environment) (Blackburn et al., 2011).

The factors that influence species establishment and spread may vary within and between taxa. To date, literature has mostly considered factors and traits that enable an introduced population to go from establishment to the spreading stage, with much less attention given to factors that drive earlier stages of the introduction process (Blackburn et al., 2011). Factors such as propagule pressure, habitat match, species-specific traits, co-introduction of other species and diseases, anthropogenic factors and climate change are all suggested to influence the survival and reproduction of an introduced species (Bomford *et al.* 2010; Dufour *et al.* 2018; Liang *et al.* 2020; Marchetti *et al.* 2004). Although traits such as body size, resource acquisition (e.g., dietary range) and female fecundity (e.g., egg mass) are well understood (Moser and Obrycki 2009), there is conflicting evidence and few field experiments to understand how factors such as genetic variation, competition, cannibalism and intraguild predation influence the establishment and impact of introduced species (Dick *et al.* 2017; Forsman 2014). For example, long-lived fish have shown increased establishment success in California compared to short-lived fish (Marchetti *et al.* 2004). However, another study on fish in the Great Lakes of North America showed that short-lived fish had higher establishment success (Kolar and Lodge 2002). Therefore, although many factors are hypothesised to affect the establishment success of introduced species, there are relatively few studies and the evidence is controversial (e.g. see Dick *et al.* (2017) and associated commentary).

1.1.2 Impacts of introduced species

Introduced species may be harmful (i.e., become invasive), beneficial or have mixed effects on recipient ecosystems (Levine *et al.* 2003; Snyder and Evans 2006). For example, non-native and invasive crayfish affect all trophic levels of freshwater food webs due to their generalist diet (i.e. feeding on macrophytes, invertebrates) and aggressive behaviour (i.e. against other native crayfish, fish and amphibians) (Twardochleb *et al.* 2013). Additionally, invasive species can introduce exotic parasites and diseases leading to further adverse effects on ecosystem functioning (Majerus 1997; Strayer *et al.* 1999; Young *et al.* 2017).

The ecological impacts of introduced species are highly diverse and they can affect both community structure and ecosystem processes. The main ecological impact of introduced species on community structure is the direct displacement or decline of native species via competition (e.g., the invasive round goby *Apollonia melanostomus* negatively affects native benthic fishes by increasing resource competition) (Bergstrom and Mensinger 2009) and intraguild predation (e.g., intraguild predation of a native amphipod (*Gammarus duebeni celticus*) by invasive amphipods (*Gammarus pulex*)) (Piscart *et al.* 2009). Competition between introduced and native species is driven by numerous factors including differences in resource acquisition, female fecundity, foraging ability, development time, size, dispersal ability, intraspecific variation, resource pre-emption, resource degradation, defence mechanisms and disruption to mating systems (Bergstrom and Mensinger 2009; Cini *et al.* 2018; Jaeger 1972; Michaud 2002). In addition, co-introduction of exotic parasites and diseases with the focal introduced species can affect species in the recipient ecosystem. For example, co-introduction of the parasites *Cichlidogyrus tilapiae*, *Cichlidogyrus sclerosus*, *Cichlidogyrus halli* and *Trichodina heterodentata* with the African cichlid *Oreochromis mossambicus* (Wilson *et al.* 2019) has contributed to the invasibility of this species (Wilson *et al.* 2019).

Further, the parapoxvirus that was introduced with grey squirrel (*Sciurus carolinensis*) in the United Kingdom (UK) has played a major role in the decline of native red squirrel (*Sciurus vulgaris*) populations (Tompkins *et al.* 2003).

Introduced species can affect ecosystem processes by changing the composition of the ecosystem. For example, beech bark disease is spread through a scale insect (*Cryptococcus fagisuga*) and a pathogenic fungus (*Neonectria coccinea* var. *faginata*). It also causes changes in the species composition of the forest due to the slow growth and death of infected beech trees (*Fagus grandifolia*) (Lovett *et al.* 2006). This affects the nutrient cycle in either of two ways: 1) when beech trees are replaced by sugar maple trees (*Acer saccharum*), the forest floor mass of the ecosystem is reduced and nitrification is increased, or 2) when the beech is replaced by hemlock (*Tsuga canadensis* and *Tsuga caroliniana*), the forest floor mass is increased and nitrification is decreased (Lovett *et al.* 2006).

The ecological impacts of introduced species are compounded by anthropogenic factors such as land use, disturbance and use of pesticides or insecticides. Introduced species are often adapted to anthropogenic disturbances giving them a competitive advantage over native species, therefore anthropogenic disturbances can affect native species more than the introduced species (Gao and Reitz 2017). For example, irrigation resulting from agriculture and developing urban landscapes has resulted in increased soil moisture that facilitates native ant displacement by the Argentine ant *Linepithema humile* in California (Gao and Reitz 2017).

Climate change could result in changes to ecosystems, making them habitable to introduced species (Townhill *et al.* 2017). This could lead to range expansions of introduced or invasive species such as the Asian club tunicate (*Styela clava*) in the UK and Ireland (Cook *et al.* 2013), causing adverse effects on native marine species (Townhill *et al.* 2017). Most native species appear to be less adapted than introduced species to cope with climatic changes, such as those induced by global warming (Majerus *et al.* 2006). This gives a competitive advantage to introduced species in terms of resource acquisition that can lead to further displacement or decline of native populations. For example, warm waters are associated with the sudden and rapid growth of Pacific oyster (*Crassostrea gigas*) compared to native species in the Wadden Sea (Nehls and Büttger 2007; Wrange *et al.* 2010). This has increased competition between Pacific oysters and its native competitors, blue mussels (*Mytilus edulis*) (Nehls and Büttger 2007). Therefore, global climatic changes may influence the ecological impacts of introduced species on recipient ecosystems. It is important to understand the factors influencing the successful establishment of introduced species to predict their invasive potential and their subsequent ecological effects on recipient ecosystems.

In addition to ecological impacts, the economic impacts from introduced species can be considerable. Some human-mediated species introductions may be economically favourable (e.g., species introduced for biocontrol purposes, or species introduced for entertainment purposes such as flowers and other ornamental plants), but the mitigation or control of introduced species that spread beyond their introduced areas can have negative economic impacts. For instance, in 2016 the European Union list of 'invasive alien species' was adopted by 28 member nations to manage 14 invasive flora and 23 invasive fauna that spanned across 4 million km^2 across the 28 member nations (Kopf *et al.* 2017).

Finally, some introductions can be harmful to human health and this in turn has negative economic impacts (Medlock *et al.* 2012). For instance, Asian tiger mosquitoes (*Aedes albopictus*) which were introduced to Europe are a carrier for many diseases such as yellow fever (Medlock *et al.* 2012). The negative impacts of introduced species to human health (including the economy) typically increase over time, affecting social wellbeing and the interactions between people within their communities (World Health Organisation, 2015).

Over the years, ecologists and evolutionary biologists have attempted to describe the biological invasion process (both natural and human mediated invasions) to understand the mechanisms underlying invasiveness and range expansion and thereby predict the impacts of introduced species (Allen and Lee 2006; Blackburn *et al.* 2011; Ehrenfeld 2010). Yet, establishment is the least well-understood stage of the introduction process (Blackburn *et al.* 2011). Understanding the underlying mechanisms of establishment in introduced species will enable us to develop effective biological control and conservation programmes (Levine *et al.* 2003).

1.1.3 Ladybirds (Coleoptera: Coccinellidae)

Ladybirds (Coleoptera: Coccinellidae) are used as biocontrol agents against agricultural pests and have been introduced to New Zealand for the biological control of scale insects, mealybugs, mites and aphids (Lo 2000; Pugh *et al.* 2015; Wearing *et al.* 2010) from the early 1890s (Galbreath and Cameron 2015; Kuschel 1990). Classical biological control of agricultural pests using natural enemies is a popular, economical and effective method to manage agricultural pests around the world (Greathead 1972; Grozea *et al.* 2015; Lo and Chapman 2001; Tillman 2011). Despite their success in pest management programmes, there are also failed introductions of ladybirds (Alaniz *et al.* 2020; Kajita *et al.* 2000; Soares *et al.* 2017). Further, there are a few invasive ladybird species (e.g., *Harmonia axyridis* and *Adalia bipunctata* (Sato and Dixon 2004)). Therefore, ladybirds are an excellent model system to study the processes of species introductions.

Understanding the biology and behaviour of ladybirds will help us in two respects. Firstly, it will help us understand the underlying mechanisms of establishment success of introduced ladybirds. Secondly, it will help us predict the impact an introduced ladybird species (whether biocontrol agent, accidentally introduced or invasive) may have on native biodiversity and ecosystem functioning. Research on ladybirds to date has concentrated on their use as biocontrol agents rather than their general biology and impact on native ecosystems (Appendix A: Supplementary table 2.1). Very few species have been studied beyond their effectiveness as a biocontrol agent. Work to date has mostly concentrated on six species: *Harmonia axyridis, Coccinella septempunctata, Adalia bipunctata*, *Halmus chalybeus, Cryptolaemus montrouzieri* and *Rodolia cardinalis,* all of which are species of economic importance for biocontrol programmes (Kundoo and Khan 2017; Rondoni *et al.* 2020).

There are 19 introduced, one invasive and 25 native ladybird species recorded in New Zealand (Szawaryn and Leschen 2019; Maddison 2010). Very few studies have investigated ladybirds in New Zealand (Ceryngier and Romanowski 2017; Galbreath and Cameron 2015; Lo and Chapman 2001; Lo 2000). Similarly, few studies investigate or report native ladybird species in New Zealand (Emberson 1998;

Martin 2015; Szawaryn and Leschen 2019; Watt 1982). Therefore, we urgently need information on the biology, behaviour and ecology of native ladybirds.

1.1.4 Phenotypic variation in ladybirds

Studies of female fecundity, dietary range, body size and the habitat of introduced species have concentrated on quantifying mean trait values (González-Suárez *et al.* 2015; Moser and Obrycki 2009). However, there are relatively few studies that examine the role of intraspecific variation (genotypic and phenotypic) and propagule pressure on the establishment of introduced species, despite strong evidence that it affects establishment success (Forsman 2014; Forsman *et al.* 2012; González-Suárez *et al.* 2015). For example, high intraspecific variation in body size in bumble bees (*Bombus* spp) is associated with a reduced risk of declining population size in habitats with rapidly changing environmental conditions (Austin and Dunlap 2019). Similarly high intraspecific variation in body size is associated with establishment success in introduced and invasive mammals (González-Suárez *et al.* 2015). Therefore, the aim of my PhD is to investigate the biology and behaviour of ladybirds in New Zealand, comparing levels of phenotypic variation within and between introduced and native ladybird populations. Further, I have developed the first molecular phylogeny of native and introduced ladybirds in New Zealand.

1.2 Project outline

I collected ladybirds from the greater Auckland region, New Zealand (figure 1.1) over three field seasons; 1) from January 2017 to June 2017, 2) from November 2017 to June 2018 and 3) from November 2018 to June 2019. Collection was focussed within the Auckland region due to the need for intensive sampling to capture representative phenotypic variation within each population. Details on sample sites and collections are available in chapters 3-6.

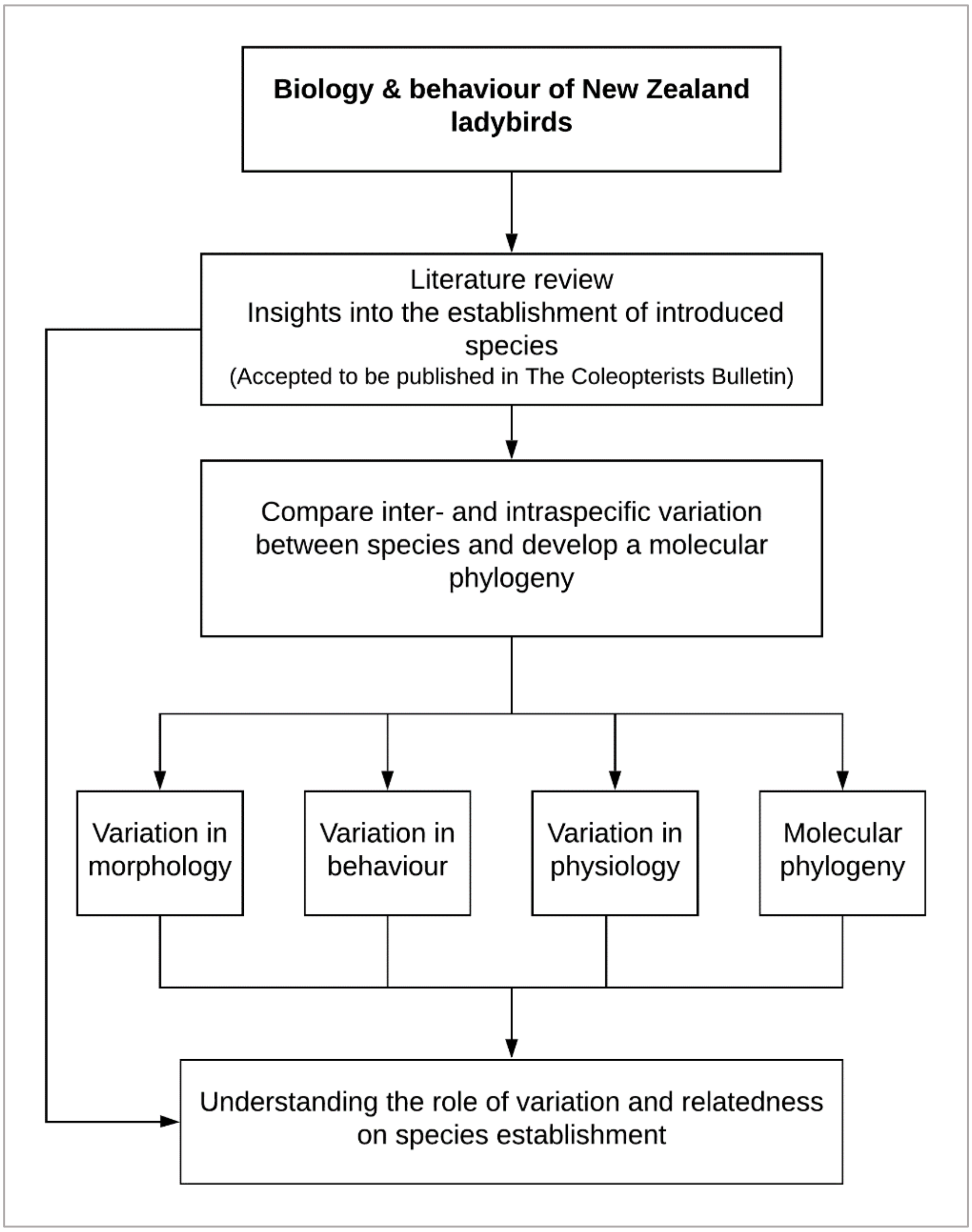

Figure 1.1 Research overview
Live specimens of each ladybird species were collected for studies of phenotypic variation (morphology, colour, general activity and antipredator behaviour, and desiccation resistance: Chapters 3-5 respectively) and phylogeny construction (Chapter 6). The phylogeny was supplemented with samples collected nationwide (sourced from the New Zealand Arthropod Collection).

1.3 Outline of my PhD thesis

My study is a thesis by publication; therefore, each chapter is a stand-alone chapter. It consists of one paper in press to be published (Chapter 2) and three papers in review or to be submitted to be published in peer reviewed, international journals.

Chapter 2 is a literature review investigating the factors affecting the successful establishment of introduced species using Coccinellini ladybirds (Coleoptera: Coccinellidae) as a model system. Here, building a framework influenced by invasive biology and biocontrol systems (*sensu* Abram and Moffat 2018), I categorised the factors affecting the successful establishment of introduced species: invasiveness, invasibility, and stochastic events. This framework provides a useful guide for researchers wanting to study establishment success and can be used as a checklist of assessment when introducing a new species for biocontrol or conservation. This chapter, titled 'Insights into the establishment of introduced species using coccinellines (Coleoptera: Coccinellidae) as a model system' is in press with The Coleopterists Bulletin (accepted 18/09/2020).

In Chapters 3 to 5, I compared inter- and intraspecific variation in traits hypothesised as functionally important for establishment success (Chapter 2) between introduced and native New Zealand ladybirds. Due to the logistical and ethical barriers to comparing establishment success directly, this approach identifies key targets for research in future introduction programmes in addition to providing insights into the biology and behaviour of New Zealand ladybirds.

In chapter 3, I discuss how body size, wing shape and colour variation differ between five introduced and five native species of ladybird in New Zealand. I found that there was greater intraspecific variation in body size in introduced than native ladybirds. Further, the widely distributed, introduced steel-blue ladybird *Halmus chalybeus* showed greater intraspecific variation in size and colour than all other introduced and native species. This chapter improves our understanding of the biology of introduced and native ladybirds, comprising the first detailed morphometric analyses of these species. It also provides a foundation for future research on the introduction, general biology and ecology of ladybirds.

In chapter 4, I compare inter- and intraspecific variation in activity levels and responses to a simulated predator attack in four introduced ladybird species. Having a high level of variation in functionally important traits such as dispersal ability may increase the establishment success of introduced species (Forsman 2014). Activity levels and the ability to withstand disturbances are major determinants of dispersal ability as they correlate with how fast and far a species can move (Hui and Pinter-Wollman 2014; Le Galliard *et al.* 2012). Therefore, variation in activity levels and the ability to withstand disturbances can provide useful insights into a species' establishment ability. I found that there was a strong positive effect of body size on activity levels. However, there was no significant difference in the level of intraspecific variation in general activity levels or responses to simulated predator attacks between species. I discuss how dispersal can help predict establishment success and the potential for range expansion in introduced species. The manuscript titled 'Inter- and intraspecific variation in ladybird (Coleoptera: Coccinellidae) activity' is in review at the Journal of Insect Behaviour (submitted 9/12/2020).

In chapter 5, I compare desiccation resistance between three introduced species of ladybird. Physiological hardiness (i.e. desiccation resistance and cold tolerance) and its variation may influence establishment success as physiological hardiness is associated with species richness, abundance, and dispersal (Block 1996; Bykova and Blatt 2019; Walther *et al.* 2009). I found ladybirds with larger body sizes had a lower risk of mortality than ladybirds with smaller body sizes. Species varied in their desiccation resistance, with *H. chalybeus* surviving the longest compared to *Serangium maculigerum* and *Illeis galbula*. Further, *S. maculigerum* had greater desiccation resistance and higher variation in desiccation resistance than *H. chalybeus* during starvation assays. I discuss how understanding desiccation resistance can help predict the invasive potential of ladybirds.

In Chapter 6, I present the first molecular phylogeny of New Zealand ladybird species. I discuss the evolutionary relationships between introduced and native ladybird species in New Zealand and how these relationships may have influenced the establishment success of introduced species in New Zealand. Further, I use the phylogeny to identify specimens previously undescribed in New Zealand.

In Chapter 7, I present a summary of the findings of this thesis, including recommendations for future work to understand the factors affecting the successful establishment of introduced species. Overall, my thesis improves our understanding of the biology, behaviour and ecology of introduced and native ladybirds in New Zealand and provides a foundation for future research predicting the establishment success of introduced and invasive ladybirds and mitigating their adverse effects on native ecosystems and biodiversity.

1.4 References

Alaniz, A. J., A. O. Soares, P. M. Vergara, E. B. de Azevedo, and A. A. Grez. 2020. The failed invasion of *Harmonia axyridis* in the Azores, Portugal: Climatic restriction or wrong population origin? Insect Science.1-13.

Allen, R. B., and W. G. Lee. 2006. *Biological invasions in New Zealand*, Springer - Verlag, Berlin.

Austin, M. W., and A. S. Dunlap. 2019. Intraspecific variation in worker body size makes North American bumble bees (*Bombus* spp.) less susceptible to decline. The American Naturalist 194: 381-394.

Bergstrom, M. A., and A. F. Mensinger. 2009. Interspecific resource competition between the invasive round goby and three native species: logperch, slimy sculpin, and spoonhead sculpin. Transactions of the American Fisheries Society 138: 1009-1017.

Blackburn, T. M., P. Pyšek, S. Bacher, J. T. Carlton, R. P. Duncan, V. Jarošík, J. R. Wilson, and D. M. Richardson. 2011. A proposed unified framework for biological invasions. Trends in Ecology & Evolution 26: 333-339.

Block, W. 1996. Cold or drought-The lesser of two evils for terrestrial arthropods? European Journal of Entomology 93: 325-340.

Bomford, M., S. C. Barry, and E. Lawrence. 2010. Predicting establishment success for introduced freshwater fishes: a role for climate matching. Biological Invasions 12: 2559-2571.

Bykova, O., and S. Blatt. 2019. Inclusion of physiological responses in insect pest risk analysis: evaluating species potential to achieve pest status. Journal of the Acadian Entomological Society 15: 1-13.

Ceryngier, P., and J. Romanowski. 2017. *Harmonia axyridis* (Pallas, 1773)(Coleoptera: Coccinellidae) and its parasite in south-western Bulgaria and northern Greece. BioInvasions Records 6: 307-310.

Cini, A., F. Cappa, I. Petrocelli, I. Pepiciello, L. Bortolotti, and R. Cervo. 2018. Competition between the native and the introduced hornets *Vespa crabro* and *Vespa velutina*: a comparison of potentially relevant life-history traits. Ecological Entomology 43: 351-362.

Cock, M. J. W., S. T. Murphy, M. T. K. Kairo, E. Thompson, R. J. Murphy, and A. W. Francis. 2016. Trends in the classical biological control of insect pests by insects: an update of the BIOCAT database. BioControl 61: 349-363.

Cook, E. J., S. Jenkins, C. Maggs, D. Minchin, F. Mineur, C. Nall, and J. Sewell. 2013. Impacts of climate change on non-native species. Marine Climate Change Impact Partnership: Science Review: 155-166.

David, P., E. Thébault, O. Anneville, P.-F. Duyck, E. Chapuis, and N. Loeuille. 2017. Chapter One-Impacts of invasive species on food webs: a review of empirical data. Advances in Ecological Research 56: 1-60.

Dick, J. T., K. Gallagher, S. Avlijas, H. C. Clarke, S. E. Lewis, S. Leung, D. Minchin, J. Caffrey, M. E. Alexander, and C. Maguire. 2013. Ecological impacts of an invasive predator explained and predicted by comparative functional responses. Biological Invasions 15: 837-846.

Dick, J. T. A., C. Laverty, J. J. Lennon, D. Barrios-O'Neill, P. J. Mensink, J. Robert Britton, V. Médoc, P. Boets, M. E. Alexander, N. G. Taylor, A. M. Dunn, M. J. Hatcher, P. J. Rosewarne, S. Crookes, H. J. MacIsaac, M. Xu, A. Ricciardi, R. J. Wasserman, B. R. Ellender, O. L. F. Weyl, F. E. Lucy, P. B. Banks, J. A. Dodd, C. MacNeil, M. R. Penk, D. C. Aldridge, and J. M. Caffrey. 2017. Invader Relative Impact Potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. Journal of Applied Ecology 54: 1259-1267.

Dufour, C. M., J. B. Losos, and A. Herrel. 2018. Do differences in bite force and head morphology between a native and an introduced species of anole influence the outcome of species interactions? Biological Journal of the Linnean Society 125: 576-585.

Ehrenfeld, J. G. 2010. Ecosystem consequences of biological invasions. Annual Review of Ecology, Evolution, and Systematics 41: 59-80.

Elliott, N., R. Kieckhefer, and W. Kauffman. 1996. Effects of an invading coccinellid on native coccinellids in an agricultural landscape. Oecologia 105: 537-544.

Emberson, R. M. 1998. The beetle (Coleoptera) fauna of the Chatham Islands. New Zealand Entomologist 21: 25-64.

Forsman, A. 2014. Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. Proceedings of the National Academy of Sciences 111: 302-307.

Forsman, A., L. Wennersten, M. Karlsson, and S. Caesar. 2012. Variation in founder groups promotes establishment success in the wild. Proceedings of the Royal Society B: Biological Sciences 279: 2800-2806.

Galbreath, R. A., and P. J. Cameron. 2015. The introduction of the eleven-spotted ladybird *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) to New Zealand in 1874: a spurious record created by cumulative misreporting. New Zealand Entomologist 38: 7-9.

Gao, Y., and S. R. Reitz. 2017. Emerging themes in our understanding of species displacements. Annual Review of Entomology 62: 165-183.

Gibbs, J. P., C. Marquez, and E. J. Sterling. 2008. The role of endangered species reintroduction in ecosystem restoration: tortoise–cactus interactions on Española Island, Galápagos. Restoration Ecology 16: 88-93.

González-Suárez, M., S. Bacher, and J. M. Jeschke. 2015. Intraspecific trait variation is correlated with establishment success of alien mammals. The American Naturalist 185: 737-746.

Gordon, D. R. 1998. Effects of invasive, non-indigenous plant species on ecosystem processes: Lessons from Florida. Ecological Applications 8: 975-989.

Greathead, D. J. 1972. The rediscovery in Kenya of 2 introduced coccinellids *Cryptolaemus montrouzieri* and *Rodolia cardinalis*. East African Agricultural and Forestry Journal 38: 214-214.

Griffith, T. C., I. D. Paterson, C. A. Owen, and J. A. Coetzee. 2019. Thermal plasticity and microevolution enhance establishment success and persistence of a water hyacinth biological control agent. Entomologia Experimentalis et Applicata 167: 616-625.

Grozea, I., M. Vlad, A. M. Virteiu, R. Stef, A. Carabet, L. Molnar, and V. Mazare. 2015. Biological control of invasive species *Metcalfa Pruinosa* Say (Insecta: Hemiptera: Flatidae) in ornamentals plants by using Coccinelids. Journal of Biotechnology 208: S112-S112.

Hodek, I., H. F. Van Emden, and A. Honěk. 2012. Ecology and behaviour of the ladybird beetles (Coccinellidae), Wiley-Blackwell, Chichester.

Hui, A., and N. Pinter-Wollman. 2014. Individual variation in exploratory behaviour improves speed and accuracy of collective nest selection by Argentine ants. Animal behaviour 93: 261-266.

Jaeger, R. G. 1972. Food as a limited resource in competition between two species of terrestrial salamanders. Ecology 53: 535-546.

Kajita, Y., F. Takano, H. Yasuda, and B. K. Agarwala. 2000. Effects of indigenous ladybird species (Coleoptera: Coccinellidae) on the survival of an exotic species in relation to prey abundance. Applied Entomology and Zoology 35: 473-479.

Kolar, C. S., and D. M. Lodge. 2002. Ecological predictions and risk assessment for alien fishes in North America. Science 298: 1233-1236.

Kopf, R. K., D. G. Nimmo, P. Humphries, L. J. Baumgartner, M. Bode, N. R. Bond, A. E. Byrom, J. Cucherousset, R. P. Keller, and A. J. King. 2017. Confronting the risks of large-scale invasive species control. Nature Ecology & Evolution 1: 1-4.

Kumschick, S., M. Gaertner, M. Vilà, F. Essl, J. M. Jeschke, P. Pyšek, A. Ricciardi, S. Bacher, T. M. Blackburn, and J. T. Dick. 2015. Ecological impacts of alien species: quantification, scope, caveats, and recommendations. BioScience 65: 55-63.

Kundoo, A. A., and A. A. Khan. 2017. Coccinellids as biological control agents of soft bodied insects: a review. Journal of Entomology and Zoology Studies 5: 1362-1373.

Kuschel, G. 1990. Beetles in a suburban environment: a New Zealand case study. The identity and status of Coleoptera in the natural and modified habitats of Lynfield, Auckland (1974-1989). DSIR Plant Protection Report 3: 119.

Lee, C. E. 2002. Evolutionary genetics of invasive species. Trends in Ecology & Evolution 17:386-391

Le Galliard, J. F., A. Remy, R. A. Ims, and X. Lambin. 2012. Patterns and processes of dispersal behaviour in arvicoline rodents. Molecular Ecology 21: 505-523.

Levine, J. M., M. Vila, C. M. Antonio, J. S. Dukes, K. Grigulis, and S. Lavorel. 2003. Mechanisms underlying the impacts of exotic plant invasions. Proceedings of the Royal Society of London B: Biological Sciences 270: 775-781.

Liang, S.-H., B. A. Walther, and B.-S. Shieh. 2020. Determinants of establishment success: Comparing alien and native freshwater fishes in Taiwan. PLOS ONE 15: e0236427.

Lo, P., and R. Chapman. 2001. Predation by *Halmus chalybeus* (Coleoptera: Coccinellidae) on *Ceroplastes destructor* and *C. sinensis* (Hemiptera: Coccidae: Ceroplastinae) infesting citrus in Northland, New Zealand. Biocontrol Science and Technology 11: 57-66.

Lo, P. L. 2000. Species and abundance of ladybirds (Coleoptera: Coccinellidae) on citrus orchards in Northland, New Zealand, and a comparison of visual and manual methods of assessment. New Zealand Entomologist 23: 61-65.

Lovett, G. M., C. D. Canham, M. A. Arthur, K. C. Weathers, and R. D. Fitzhugh. 2006. Forest ecosystem responses to exotic pests and pathogens in eastern North America. AIBS Bulletin 56: 395-405.

Maddison, P.M. 2010. Checklist of New Zealand Hexapoda. Order Coleoptera. [Chapter] NINE: Phylum Arthropoda: Subphylum Hexapoda: Protura, springtails, Diplura, and insects. *In*: Gordon, D. (Ed.), New Zealand Inventory of Biodiversity. Kingdom Animalia. Chaetognatha, Ecdysozoa, ich-nofossils Vol. 2. Canterbury University Press, Christchurch, New Zealand: 233–467.

Majerus, M., V. Strawson, and H. Roy. 2006. The potential impacts of the arrival of the harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera : Coccinellidae), in Britain. Ecological Entomology 31: 207-215.

Majerus, M. E. N. 1997. Interspecific hybridisation in ladybirds (Col.: Coccinellidae). Entomologist's Record and Journal of Variation 109: 11-23.

Marchetti, M. P., P. B. Moyle, and R. Levine. 2004. Invasive species profiling? Exploring the characteristics of non-native fishes across invasion stages in California. Freshwater Biology 49: 646-661.

Martin, N. 2015. Larvae and prey of three native ladybirds (Coleoptera: Coccinellidae). The Wētā 49: 16-22.

Medlock, J. M., K. M. Hansford, F. Schaffner, V. Versteirt, G. Hendrickx, H. Zeller, and W. V. Bortel. 2012. A review of the invasive mosquitoes in Europe: Ecology, public health risks, and control options. Vector-Borne and Zoonotic Diseases 12: 435-447.

Michaud, J. 2002. Invasion of the Florida citrus ecosystem by *Harmonia axyridis* (Coleoptera: Coccinellidae) and asymmetric competition with a native species, Cycloneda sanguinea. Environmental Entomology 31: 827-835.

Mollot, G., J. Pantel, and T. Romanuk. 2017. The effects of invasive species on the decline in species richness: a global meta-analysis. Advances in Ecological Research 56: 61-83.

Moser, S. E., and J. J. Obrycki. 2009. Competition and intraguild predation among three species of coccinellids (Coleoptera: Coccinellidae). Annals of the Entomological Society of America 102: 419-425.

Nehls, G., and H. Büttger. 2007. Spread of the pacific oyster *Crassostrea gigas* in the Wadden sea: causes and consequences of a successful invasion. Trilateral Workshop on Pacific Oyster Invasion in the Wadden Sea. Common Wadden Sea Secretariat, Wilhelmshaven, Germany 22: 54.

Olden, J. D., and N. L. Poff. 2004. Ecological processes driving biotic homogenization: testing a mechanistic model using fish faunas. Ecology 85: 1867-1875.

Oro, D., A. Martínez-Abraín, E. Villuendas, B. Sarzo, E. Mínguez, J. Carda, and M. Genovart. 2011. Lessons from a failed translocation program with a seabird species: Determinants of success and conservation value. Biological Conservation 144: 851-858.

Piscart, C., J. T. A. Dick, D. McCrisken, and C. MacNeil. 2009. Environmental mediation of intraguild predation between the freshwater invader *Gammarus pulex* and the native *G. duebeni celticus*. Biological Invasions 11: 2141.

Pugh, A. R., D. M. O'Connell, and S. D. Wratten. 2015. Further evaluation of the southern ladybird (*Cleobora mellyi*) as a biological control agent of the invasive tomato-potato psyllid (*Bactericera cockerelli*). Biological Control 90: 157-163.

Rondoni, G., I. Borges, J. Collatz, E. Conti, A. C. Costamagna, F. Dumont, E. W. Evans, A. A. Grez, A. G. Howe, E. Lucas, J.-É. Maisonhaute, A. Onofre Soares, T. Zaviezo, and M. J. W. Cock. 2020. Exotic ladybirds for biological control of herbivorous insects – a review. Entomologia Experimentalis et Applicata: 1-22

Samways, M. J., R. Osborn, H. Hastings, and V. Hattingh. 1999. Global climate change and accuracy of prediction of species' geographical ranges: establishment success of introduced ladybirds (Coccinellidae, Chilocorus spp.) worldwide. Journal of Biogeography 26: 795-812.

Santos, T., J. Pérez-Tris, R. Carbonell, J. L. Tellería, and J. A. Díaz. 2009. Monitoring the performance of wild-born and introduced lizards in a fragmented landscape: Implications for ex situ conservation programmes. Biological Conservation 142: 2923-2930.

Sarrazin, F., and R. Barbault. 1996. Reintroduction: challenges and lessons for basic ecology. Trends in Ecology & Evolution 11: 474-478.

Sato, S., and A. F. G. Dixon. 2004. Effect of intraguild predation on the survival and development of three species of aphidophagous ladybirds: consequences for invasive species. Agricultural and Forest Entomology 6: 21-24.

Seddon, P. J. 2010. From reintroduction to assisted colonization: moving along the conservation translocation spectrum. Restoration Ecology 18: 796-802.

Snyder, W. E., and E. W. Evans. 2006. Ecological effects of invasive arthropod generalist predators. Annual Review of Ecology, Evolution, and Systematics: 95-122.

Soares, A. O., A. Honěk, Z. Martinkova, J. Skuhrovec, P. Cardoso, and I. Borges. 2017. *Harmonia axyridis* failed to establish in the Azores: the role of species richness, intraguild interactions and resource availability. BioControl 62: 423-434.

Strayer, D. L., N. F. Caraco, J. J. Cole, S. Findlay, and M. L. Pace. 1999. Transformation of freshwater ecosystems by bivalves: a case study of zebra mussels in the Hudson River. BioScience 49: 19-27.

Szawaryn, K., and R. A. B. Leschen. 2019. Redescription and notes on the New Zealand ladybird species *Hoangus venustus* (Pascoe, 1875) (Coleoptera: Coccinellidae). Journal of Asia-Pacific Entomology 22: 226-232.

Tillman, P. G. 2011. Natural biological control of stink bug (Heteroptera: Pentatomidae) eggs in corn, peanut, and cotton farmscapes in Georgia. Environmental Entomology 40: 303-314.

Tompkins, D. M., A. R. White, and M. Boots. 2003. Ecological replacement of native red squirrels by invasive greys driven by disease. Ecology Letters 6: 189-196.

Townhill, B., J. Pinnegar, J. Tinker, M. Jones, S. Simpson, P. Stebbing, and S. Dye. 2017. Non-native marine species in north-west Europe: Developing an approach to assess future spread using regional downscaled climate projections. Aquatic Conservation: Marine and Freshwater Ecosystems 27: 1035-1050.

Twardochleb, L. A., J. D. Olden, and E. R. Larson. 2013. A global meta-analysis of the ecological impacts of nonnative crayfish. Freshwater Science 32: 1367-1382.

Walther, G.-R., A. Roques, P. E. Hulme, M. T. Sykes, P. Pyšek, I. Kühn, M. Zobel, S. Bacher, Z. Botta-Dukat, and H. Bugmann. 2009. Alien species in a warmer world: risks and opportunities. Trends in Ecology & Evolution 24: 686-693.

Watt, J. C. 1982. Terrestrial arthropods from the Poor Knights Islands, New Zealand. Journal of the Royal Society of New Zealand 12: 283-320.

Wearing, C. H., B. A. Attfield, and K. Colhoun. 2010. Biological control of woolly apple aphid, *Eriosoma lanigerum* (Hausmann), during transition to integrated fruit production for pipfruit in Central Otago, New Zealand. New Zealand Journal of Crop and Horticultural Science 38: 255-273.

WHO. 2015. Connecting Global Priorities: Biodiversity and Human Health, A State of Knowledge Review (Geneva, Switzerland). World Health Organization and Secretariat of the Convention on Biological Diversity.

Wilson, J. R., R. J. Saunders, and K. S. Hutson. 2019. Parasites of the invasive tilapia *Oreochromis mossambicus*: evidence for co-introduction. Aquatic Invasions 14: 332-349.

Wrange, A.-L., J. Valero, L. S. Harkestad, Ø. Strand, S. Lindegarth, H. T. Christensen, P. Dolmer, P. S. Kristensen, and S. Mortensen. 2010. Massive settlements of the Pacific oyster, *Crassostrea gigas*, in Scandinavia. Biological Invasions 12: 1145-1152.

Young, H. S., I. M. Parker, G. S. Gilbert, A. S. Guerra, and C. L. Nunn. 2017. Introduced Species, Disease Ecology, and Biodiversity–Disease Relationships. Trends in Ecology & Evolution 32: 41-54.

Chapter 2

Literature review

Insights into the establishment of introduced species using coccinellines (Coleoptera: Coccinellidae) as a model system

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Abstract

Species introductions occur around the world both inadvertently and deliberately (typically for conservation, agriculture or fisheries). However, not all introduced species become established. Understanding the factors that affect the establishment success of introduced species will help us improve species introductions for biocontrol and conservation purposes. Here we argue that important generalist arthropod predators, Coccinellini ladybirds (Coleoptera, Coccinellidae), are an ideal taxon for investigating the establishment process of introduced species. Coccinellini are introduced accidentally via plant exports and deliberately as biocontrol agents to reduce agricultural pests, and a few have become invasive species. Here, using work from invasive biology and biocontrol systems, we categorize the factors affecting the successful establishment of introduced species. These factors are 1) invasiveness of the species, 2) invasibility of the recipient ecosystem and 3) stochastic events that occur after the introduction. We review how factors such as diet and competition, dispersal ability, propagule population, disturbances and climate change can be studied within these three categories to better predict the establishment success of introduced ladybirds. Our review highlights that our current understanding of the differences between successful and unsuccessful species establishments is limited. To address this, we need direct comparisons of dispersal ability and both interspecific and intraspecific variation in ladybirds. We conclude that studies of ladybirds will help to develop theories that better characterize and predict establishment success and invasive potential.

Keywords: categorization, dispersal ability, intraspecific variation, habitat invasibility, intraguild predation

2.1 Introduction

Around the world, species are introduced into new locations both inadvertently and deliberately for biological control, conservation and sport. However, not all introduced species become established. A recent update on biological control agents suggests that only a maximum of 32.6% of introduced biocontrol agents become established after introduction (Cock *et al.* 2016). These low success rates have always been a problem in biological control programs. Therefore, it is important to understand the factors that affect the successful establishment of introduced species. Although introduced biological control agents are studied extensively in the screening process, we are still unable to predict their establishment success. To develop an overall model of establishment success of introduced species, Abram and Moffat (2018) suggested looking at the introduction of biological control agents as planned invasions to understand the factors enabling species to establish and disperse in a new environment. For instance, biological invasions are a four-part process comprising transport, introduction, establishment and spread (Blackburn *et al.* 2011). Current literature has mostly considered the factors that enable an introduced population to go from establishment to the spreading stage with much less attention to a) factors that drive earlier stages of the invasion process (Blackburn *et al.* 2011) and b) factors that affected failed introductions (Cock *et al.* 2016; Gariepy *et al.* 2015; Hariston *et al.* 1999; Meffe 1991; Zenni and Nunez 2013). The stage when most introductions fail is establishment—when the introduced population begins to reproduce and maintain (or grow) its population in its new habitat (Blackburn *et al.* 2011). Identifying the factors that drive the establishment of introduced species remains challenging and controversial (e.g. see Dick *et al.* [2017] and associated commentary). Therefore, we propose studying factors that promote and inhibit the successful establishment of introduced species.

To determine the establishment success of introduced species, we need to evaluate factors under three categories (*sensu* Abram and Moffat 2018): a) invasibility of the habitat, b) invasiveness of the introduced species and c) stochastic events (Fig. 2.1). We use the terms invasibility and invasiveness as defined in invasive biology, as we are considering species introductions as planned invasions to better understand the factors affecting successful establishment as an adaptation of the framework proposed by Abram and Moffat (2018) for biocontrol.

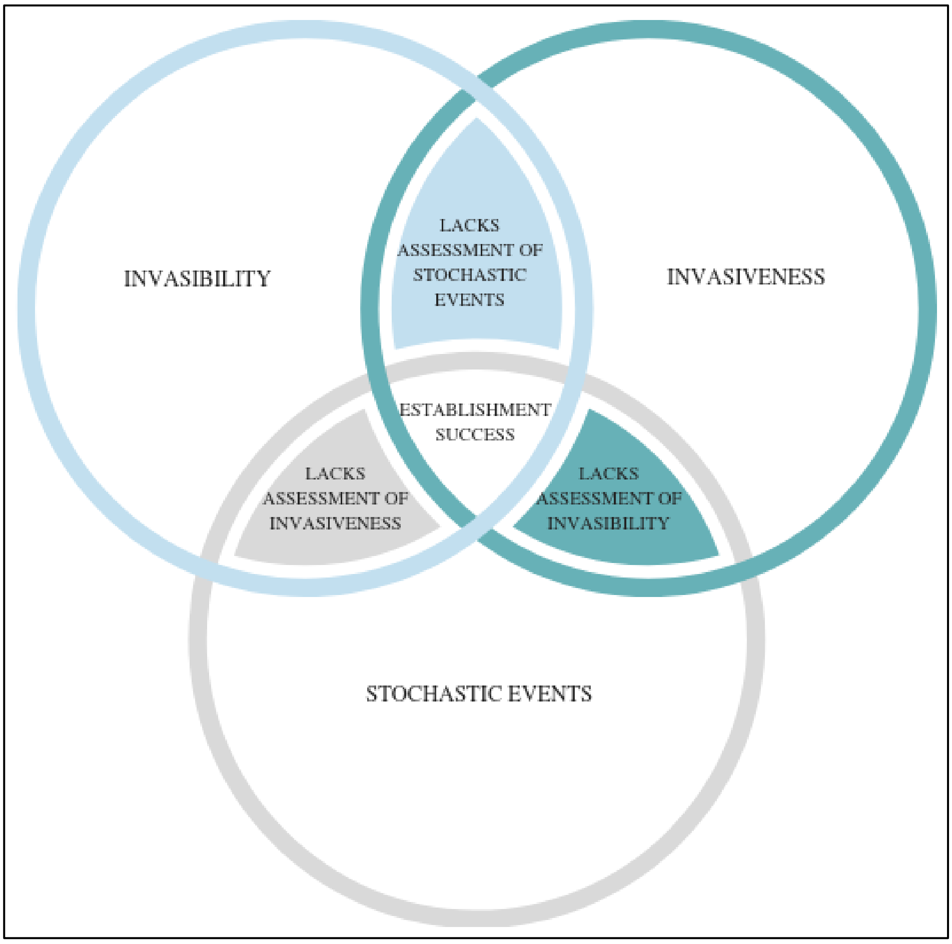

Figure 2.1 Categorization of factors to assess establishment success of introduced species.
Invasibility is defined as the characteristics of a habitat that make it vulnerable to invasion by the introduced species. Invasiveness is defined as traits of the introduced species that enable them to establish in a new area. Stochastic events are events that may change invasibility and invasiveness, thus affecting the establishment process. We predict that successful establishment will increase at the mid-point where all three categories overlap. However, in the absence of stochastic events that influence the establishment process, we may predict the success rate of establishment by assessing only the species' match in invasiveness with invasibility of the recipient ecosystem.

Our current approach assesses the characteristics of the introduced species to identify its effectiveness for the purpose for which it is going to be introduced (i.e., biocontrol) and assess whether the habitat parameters of the introduced area match the current known distribution or native habitats of the introduced species. This limits our understanding of how other factors in both species (i.e., variation and vulnerability to intraguild predation, see Table 2.1) and in the introduced area (i.e., other species present or absent, see Table 2.1) may affect establishment of the introduced species after its introduction. Potential interactions between the introduced species and recipient ecosystem are not studied enough. Using the proposed three categories to guide research effort would allow us to gain an overall understanding of species traits (invasiveness) and habitat parameters (invasibility) of the introduced area, how they interact with each other and any stochastic events occurring during the establishment process. For an introduced species to establish successfully in a new habitat, invasiveness of the species (species-specific characteristics) should match with habitat invasibility and finally should overcome any stochastic events (such as sudden weather changes) that may occur. For example, ladybirds generally acclimatize well in fluctuating temperatures, and this would not affect the establishment of introduced ladybirds in habitats with fluctuating temperatures (Singh *et al.* 2018). However, some species (i.e., *Harmonia axyridis* [Pallas, 1773]) require lower temperatures to induce and complete their overwintering diapause (Soares *et al.* 2008). Therefore, understanding variation in the temperature tolerance of the introduced species as well as the temperature profile of the introduced area will help us to predict how establishment of the introduced species would be affected by temperature fluctuations. Here we review studies of coccinelline ladybirds (Coleoptera: Coccinellidae) within the three-category framework to identify gaps in our understanding and suggest what we can do to improve the establishment success of ladybirds in biocontrol studies.

Table 2.1 Factors affecting the successful establishment of introduced ladybirds. Examples are illustrative rather than exhaustive.

Category	**Factor/ trait**	**Class**	**References**
Invasibility	Abiotic factors	Temperature (e.g., differences in performance during temperature extremes)	(Singh *et al.* 2018; Soares *et al.* 2008)
		Rainfall (e.g., high rainfall decreases probability of colonization)	(Comont *et al.* 2014a)
	Resource availability	Prey density	(Evans and Toler 2007; Moser and Obrycki 2009)
	Natural enemies	Parasites	(Dindo *et al.* 2016)
		Fungi	(Scorsetti *et al.* 2017)
		Pathogens	(Steele and Bjørnson 2019; Tayeh *et al.* 2012)

	Competitors		(Smith and Gardiner 2013; Soares *et al.* 2017)
	Intraguild predation risk	IG predator	(Kajita *et al.* 2000)
		IG prey	(Moser and Obrycki 2009)
		Colonization times	(Éric 2013)
		Spatial and temporal distribution	(Musser and Shelton 2003)
	Other flora and fauna	Innate responses to particular plant(s)	(Togni *et al.* 2016)
Invasiveness	Resource acquisition	Dietary range	(Berkvens *et al.* 2010; Emiljanowicz *et al.* 2017; Franzmann 2002; Majerus *et al.* 2006)
	Female fecundity		(Awad *et al.* 2013; Tayeh *et al.* 2012; Turgeon *et al.* 2011)
	Male reproductive success	Selection of traits that increase fitness in males in captive conditions	(Facon *et al.* 2011)

Vulnerability to natural enemies		(Riddick *et al.* 2009; Romanov 2019)
Defense mechanisms	Behavioral and physical adaptations	(Sato and Dixon 2004; Smith and Gardiner 2013; Snyder *et al.* 2004)
	Chemical defenses	(Kajita *et al.* 2014)
Mating systems	Adaptations to increase genetic diversity and avoid inbreeding in *Adalia bipunctata* Linnaeus.	(Saxena *et al.* 2019)
	Melanism	(Nedvěd *et al.* 2018)
	Voltinism	(Comont *et al.* 2014a)
Developmental times	Faster developmental times and higher investment in reproduction	(Raak-van den Berg *et al.* 2018; Tayeh *et al.* 2015)
Dispersal ability	Chemical cues	(Werf *et al.* 2000)

	Flight speed	(Lombaert *et al.* 2014)
Body size		(Dixon 2007; Éric 2013; Nóia *et al.* 2008)
Phenotypic variation	Phenotypic plasticity	(Boher *et al.* 2018; Lombaert *et al.* 2008)
	Seasonal fluctuations - morph frequencies	(Kawakami *et al.* 2019)
	Acclimatization	(Singh *et al.* 2018)
	Intraspecific variation	(Evans 2000)
Genetic variation	Genetic diversity	(Krafsur *et al.* 2005)
	Bridgehead effect	(Awad *et al.* 2015; Hodek and Ceryngier 2000; Lombaert *et al.* 2011)
	Multiple mating	
Cannibalism		(Tumminello *et al.* 2015)
Interspecific interaction	Aggressiveness of introduced species	(Kajita *et al.* 2014)

	Diapause		(Soares *et al.* 2008)
	Physiology	Eurythermicity	(Barahona-Segovia *et al.* 2015)
	Vulnerability to change/ disturbance	Rapid responses	(Kajita and Evans 2009)
Stochastic events	Propagule pressure		(Kajita *et al.* 2012)
	Climate change	Global warming	(Barahona-Segovia *et al.* 2015; Boher *et al.* 2018)
	Habitat degradation/ management/ land use/ disturbance	Increases in urban land cover	(Comont *et al.* 2014a)

Effects of traits on invasibility, invasiveness and stochastic events vary depending on the species and its interaction with the introduced habitat.

2.2 Coccinellines as a model system

Ladybirds, also called ladybird beetles, lady beetles, ladybugs and coccinellids (Hodek *et al.* 2012), are mostly predators that play a complex role in community dynamics (Rudolf 2007; Rudolf and Rasmussen 2013; Wallach *et al.* 2017). There are approximately 6000 described species of ladybird (Ślipiński *et al.* 2011). They are currently the most species-rich of 15 families in the superfamily Coccinelloidea (Robertson *et al.* 2015). The coccinellids have been the focus of several recent molecular phylogenetic studies (Escalona *et al.* 2017; Giorgi *et al.* 2009; Seago *et al.* 2011; Szawaryn *et al.* 2015), and based on these studies the classification has changed significantly, although it still needs further revision. The family is currently divided into two subfamilies, the less diverse Microweiseinae (Escalona and Ślipiński 2012) with only a handful of often cryptic genera and the larger Coccinellinae (Ślipiński 2007), containing up to 20 tribes whose relationships are poorly known and includes all of the taxa discussed in this review and familiar to most biologists. Ladybirds are generally thought of as specialized predators of stenorrhynchan Hemiptera, but their prey includes many other insects (including ladybirds) and arthropods (particularly mites) and they may also feed opportunistically on honeydew and pollen (Giorgi *et al.* 2009; Seago *et al.* 2011). Some, like the Epiplachnini, are plant feeding, while some Coccinellini are specialists on fungi (Escalona *et al.* 2017; Giorgi *et al.* 2009).

While ladybirds have a wide range of dietary specificities, coccinellines are important generalist arthropod predators (prey on soft-bodied arthropods) that have been and continue to be, introduced around the world for biological control of agricultural pests (Rudolf 2007; Rudolf and Rasmussen 2013; Wallach *et al.* 2017). Broad dietary range in generalist predators may increase their ability to establish in a wide range of habitats and increase their risk of becoming invasive (Emiljanowicz *et al.* 2017). Moreover, several Coccinellini species used in biocontrol programs are known to be invasive (Lombaert *et al.* 2014; Snyder *et al.* 2004). Ladybirds can have strong effects on the recipient ecosystem, and some introduced ladybird species have played a role in the displacement and decline of intraguild competitors, including other native ladybird species (Brown *et al.* 2011; Paul *et al.* 2015; Tumminello *et al.* 2015). Therefore, coccinellines serve as an ideal model system to evaluate the factors driving the establishment of introduced species. In this following section, we review the distribution of introduced Coccinellini and what is currently understood about the factors that influence their successful establishment. This review provides the first systematic compilation of our knowledge of factors affecting the successful establishment of introduced Coccinellini considering traits that influence both biological control and invasive potential.

2.2.1 Methods of introduction

Ladybirds are introduced into new habitats in two main ways: first, they are intentionally introduced as a biological control agent in agricultural crops (Gordon 1985; Snyder and Clevenger 2004), or second, they are accidentally introduced via exports of plants and other cargo (Schaefer *et al.* 1987). Current pest control and management programs include the introduction of ladybirds for a) classical biocontrol which involves the establishment of an introduced population for permanent suppression of pests and b) lab rearing of adults to augment wild populations for immediate reduction of pest populations such as the non-coccinellines *Cryptolaemus montrouzieri* Mulsant, 1853 and *Delphastus pusillus* (LeConte, 1852) (Michaud 2012; Obrycki and Kring 1998). Supplementary Table 2.1 provided in the Appendix summarizes studies on introduced ladybirds described under the proposed framework.

2.2.2 Establishment and distribution

Successful establishment occurs when a species that has settled into a location that is outside its natural geographical range can maintain a self-sustaining population with natural dispersal (Hokkanen and Sailer 1985; Howarth 1991). Introduced populations face several barriers to dispersal and the maintenance of a self-sustaining population such as: geography, captivity/cultivation, survival, reproduction and environment (Blackburn *et al.* 2011). Successful establishment occurs only once, and repeated introductions may not necessarily improve the probability of an introduced ladybird establishing (Day *et al.* 1994). Establishment success varies in ladybirds, with instances where only one introduction was sufficient for establishment, while in other instances several introductions did not result in population establishment (Kajita *et al.* 2000).

There are many factors hypothesized to influence the successful establishment and dispersal of introduced ladybird species in novel environments. Table 2.1 summarizes the factors that have been investigated to predict successful establishment. Note that this includes studies carried out on invasive species as well as considering species introductions for biological control and conservation.

2.3 Habitat invasibility

Many factors influence the invasibility of a habitat and hence the probability of establishment, including abiotic resistance, resource availability, the presence of natural enemies, mutualists, intraguild predators and other native flora and fauna (discussed in the following sections). Our rationale is to compile literature on ladybirds to provide hypotheses about habitat characteristics that may influence the invasibility of an introduced area. To date, less attention has been given to investigating invasibility in the recipient habitat than to the characteristics of the species to be introduced. Therefore, we gain insights into invasibility by contrasting traits between native and introduced species, which also in turn provides data on the invasiveness of the species. The exception to this is the effect of temperature on life history traits which has been studied extensively in order to understand the habitat match between native and introduced habitats (Facon *et al.* 2017; Singh *et al.* 2018). Temperature affects ladybird physiology. For example, winter diapause may affect reproductive success in *H. axyridis,* where one of the reasons for failed introductions in Azores is thought to be the unavailability of preferred winter temperatures (Soares *et al.* 2008). However, studies that compare photoperiodic regulation of diapause and its effect on reproduction in invasive and native populations of *H. axyridis* show that there is a weak effect of photoperiod on diapause induction (Reznik *et al.* 2015; Reznik *et al.* 2017). Therefore, the invasibility of the habitat should be investigated within the framework of the invasiveness of the species with respect to photoperiod, food quality and temperature (Reznik *et al.* 2015; Reznik *et al.* 2017). For instance, if the introduced species needs low rainfall, preferred prey with availability of alternative food resources and winter temperatures lower than 12°C to diapause then the habitat should be assessed within those parameters. Further, we need to understand how habitat invasibility would change following a stochastic event and how it would affect the invasiveness of the introduced species. For example, we need to identify the prey preferences of the introduced species (invasiveness) in addition to how prey densities and their fluctuations (invasibility) in the introduced habitat and changes in prey populations due to diseases or human mediated activities would interact during the establishment period. Therefore, below we discuss factors affecting the invasibility of the habitat, including interactions with species' invasiveness and potential stochastic changes, to better understand how these three categories affect successful establishment.

2.3.1 Abiotic factors

Rainfall and temperature factors directly affect the invasiveness and therefore establishment success of ladybirds. For example in ladybirds, increased rainfall decreases the probability of colonization locally (Comont *et al.* 2014a). As mentioned earlier, few studies compare the thermal capacities (see table of thermal constants of 25 ladybird species in Neved and Honek [2012]) of native and introduced species including ladybirds (Majerus *et al.* 2006; Singh *et al.* 2018; Zerebecki and Sorte 2011). In contrast to introduced ladybirds, most native ladybirds appear to be less able to cope with climatic changes, such as those induced by global warming (Majerus *et al.* 2006). Since most endemic ladybirds are habitat and niche specific (Majerus *et al.* 2006), they lack the ability to establish in novel habitats. On the other hand, introduced ladybirds often easily adapt/acclimatize to novel habitats as well as climatic changes (Singh *et al.* 2018). This gives them a competitive advantage over native ladybirds (Majerus *et al.* 2006). Comparative studies on the physiological performance of native and introduced species along a range of temperatures show that introduced species have broader temperature tolerance ranges than native species (Zerebecki and Sorte 2011). Higher temperature tolerances were related to broader geographic temperature ranges and higher maximum habitat temperatures but were unrelated to minimum habitat temperatures (Zerebecki and Sorte 2011). Therefore, it is more likely that introduced species that successfully establish in new environments have higher growth and survival rates at high temperatures and eurythermality (having a wider range of thermal comfort) than species that do not successfully establish (Hellmann *et al.* 2008; Zerebecki and Sorte 2011).

Some laboratory studies indicate that, contrary to field studies and predictions, both introduced and native ladybird species show eurythermality, although they show differences in performance (fecundity, locomotor performance, development time) in temperature extremes (Barahona-Segovia *et al.* 2015; Zerebecki and Sorte 2011). For example, neither native species (*Eriopis chilensis* Hofmann, 1970) nor introduced species (*H. axyridis*) performed well at high temperatures, but introduced species showed comparatively better performance at lower temperatures compared to *E. chilensis* (Barahona-Segovia *et al.* 2015). This suggests that there is variation in the degree that invasive/introduced ladybirds can respond to climate change (Barahona-Segovia *et al.* 2015). However, invasive species that perform better at lower temperatures, such as *H. axyridis*, may be challenged in terms of distribution following global warming due to a lack of thermal plasticity compared to native species (Boher *et al.* 2018). However, fluctuating temperatures can increase the reproductive capacity and development of ladybirds such as *Menochilus sexmaculatus* (Fabricius, 1781) (Singh *et al.* 2018). Investigating further the physiological performance of native and introduced species will help us better predict establishment success and reduce the number of failed introductions. For instance, assessing how different temperatures, temperature fluctuations (invasibility of the habitat) and changes in lowest and highest temperatures (stochastic events) due to climate change interact with preferred temperature ranges (invasiveness) of introduced ladybirds may provide insights into their establishment success.

In some species, variation in larval and adult coloration can be influenced by environmental factors such as temperature rather than through genetic inheritance (Majerus *et al.* 2006). Color patterns can therefore be an indication of climate during development. For example, *H. axyridis* has a wide range of color plasticity in pupae and adults which is linked to temperature (Michie *et al.* 2010). Final-instar larvae that experience low temperatures produce dark pupae which are adapted to absorb more heat than light-colored pupae, facilitating faster development and earlier eclosion in cool conditions (Majerus *et al.* 2006). Further, fluctuations in morph frequencies as a result of seasonal changes or temporality (temporal environmental heterogeneity) play an important role in controlling for the selection pressures faced by ladybirds due to changes in climatic conditions (Kawakami *et al.* 2019). We need comparisons of the level of color variation between native and introduced ladybird populations to identify their differences in invasiveness and tests of how color variation interacts with different climates or invasibility of different habitats.

2.3.2 Disturbance

Anthropogenic disturbance is a major factor influencing species establishment and distribution. A spatial distribution model of *H. axyridis* in France suggests that its establishment is predicted better by anthropogenic factors (such as urbanization, presence/absence of highways [disturbance], agriculture and vineyards) than environmental factors (temperature, wind speed and rainfall) (Veran *et al.* 2016). The establishment and rapid expansion of *H. axyridis* in urban areas of the UK is explained by its ability to use a wide range of habitats (such as having overwintering sites inside buildings) and prey species (Purse *et al.* 2015). Rapid responses to changes in the external environment provide a competitive advantage for introduced species (Rondoni *et al.* 2017), enabling successful establishment. Introduced species (*Coccinella septempunctata* Linnaeus, 1758) show more rapid responses to changes in prey density than the native species (*Coccinella tranversoguttata richardsoni* Brown, 1962), adopting oosorption as a strategy to conserve resources at low prey densities (Kajita and Evans 2009). Such rapid responses to sudden changes in prey densities may account for the greater reproductive success and abundance of introduced ladybirds over native ladybirds (Kajita and Evans 2009). Further, introduction of natural predators in the habitat can also affect the abundance of introduced species. For instance, populations of *Boreioglycaspis melaleucae* Moore, 1964 (an introduced weed biocontrol agent) were affected by the introduction of *H. axyridis* in Florida (Nimmo and Tipping 2009). Therefore, adaptability of reproductive behavior in response to anthropogenic disturbances provides a competitive advantage in the establishment of introduced species. Disturbances that are caused by habitat degradation and land use or urbanization reduces colonization success in ladybirds (Comont *et al.* 2014a). Therefore, assessing how the invasibility of the habitat in terms of the level of disturbance in the recipient ecosystem (sudden changes in prey and predator densities) and sudden changes to the environment such as deforestation (stochastic events) interact with the invasiveness of ladybirds by monitoring changes in their development, fecundity and dispersal will provide a better understanding of establishment processes.

2.3.3 Spatial and temporal distribution of intraguild competitors

The spatial and temporal distribution of ladybirds affects intraguild interactions between introduced species and native congeners (Musser and Shelton 2003). Changes in the spatial distribution of populations can allow individuals to reduce negative interactions with other species. The spatial and temporal distribution of native and introduced ladybirds and competitors (such as lacewings, braconids and other aphidophagous predators) are important regulators of the intensity of interactions between native and introduced populations. For example, the invasive species *H. axyridis* avoided interactions with native species, *Coleomegilla maculata* (De Geer, 1775) by changing its spatial distribution on host plants, where *H. axyridis* adults moved further away from *C. maculata* adults and larvae of *H. axyridis* move towards larvae of *C. maculata* (Musser and Shelton 2003). The different spatial distribution within the plant has been possible for *C. maculata* mainly because its distribution is not associated with that of aphid populations, therefore it is less vulnerable to predation by introduced species which have a spatial distribution associated with aphid density (Coderre *et al.* 1987; Harmon *et al.* 2000). However, a large-scale study showed that the abundance of native and other ladybirds was not influenced by the abundance of *H. axyridis* but was influenced by the abundance of aphids (Viglášová *et al.* 2017). Temporal changes in the distribution of ladybird species feeding on aphids can be caused by geometric and physiological constraints associated with size (Dixon 2007). Smaller aphidophagous species have an advantage over larger species, as they can exploit aphids when they are relatively uncommon. Hence, small aphidophagous species vary less in abundance and size than larger species each year (Dixon 2007). If we approach species introductions as planned invasions, understanding the presence of competitors and their spatial and temporal distribution (invasibility) may provide insights into the invasiveness of the introduced species, increasing the predictability of establishment success. This will further help us identify suitable times and places to introduce species and manage introduction programs to increase establishment success.

2.3.4 Other flora and fauna in recipient ecosystem

Despite ladybirds having been studied for years as biocontrol agents, few studies have examined interactions between introduced ladybird species and native flora and fauna other than native ladybirds. The dispersal and colonization of ladybirds are affected by innate responses to plants, e.g., the presence of coriander increased longevity and dispersal in *Cycloneda sanguinea* (Linnaeus, 1763) (Togni *et al.* 2016). However, there are few studies that have investigated these interactions to understand their effect on establishment success.

Ladybirds that feed on aphids can be negatively affected by ants protecting the honeydew-excreting hemipterans from their natural enemies (Powell and Silverman 2010). A laboratory study conducted to assess the interactions of native and introduced ladybirds with *Myrmica rubra* (Linnaeus, 1758) found no evidence that the introduced species have a competitive advantage over native ladybirds against aphid tending ants (Finlayson *et al.* 2009). Further, the intensity of attacks from ant populations are influenced by resource availability (Clark and Singer 2018; Takizawa and Yasuda 2006), therefore fluctuations in resources following introductions of ladybirds may change the intensity of attacks from mutualists such as ants and should be monitored closely to ensure successful establishment.

Further, ladybirds themselves have a variety of natural enemies including bacteria, nematodes and fungi to wasps and flies, and when these natural enemies are absent, ladybird establishment success may be increased (enemy release hypothesis, see review by Riddick *et al.* [2009]). Studies show that parasites and entomopathogenic fungi native to recipient ecosystems can reduce fitness in terms of delayed development, reduced female fecundity and higher mortality of introduced species than native species (Dindo *et al.* 2016; Scorsetti *et al.* 2017; Steele and Bjørnson 2019).

Introduced ladybird species can influence parasitism rates on native ladybirds. Parasitism rates of native ladybirds (*C. maculata*) decrease in the presence of introduced ladybirds (*H. axyridis*) as the introduced species are relatively non-suitable hosts and serve as a sink (ecological trap), decreasing parasitoid egg recruitment and native host death (Hoogendoorn and Heimpel 2002). However, the prevalence of natural enemies such as parasites is lower for introduced species than for native species during the early stages of establishment (Comont *et al.* 2014b). Saito and Bjormson (2008) studied the effects of a microsporidium transmitted horizontally from *Hippodamia convergens* Guérin-Méneville, 1842 to three other non-target ladybird species. Results suggest that native species (*Adalia bipunctata* [Linnaeus, 1758]) are a more suitable host for the microsporidium compared to the introduced species (*C. septempunctata* and *H. axyridis*) (Saito and Bjornson 2008). Similarly, *H. axyridis* is better able to cope with bacteria in the genus *Wolbachia*, which kill males early in development, compared to *A. bipunctata* (Goryacheva *et al.* 2015). However, *Dinocampus coccinellae* (Schrank, 1802) negatively affected the fitness of *H. axyridis* more than that of *A. bipunctata* (Dindo *et al.* 2016). Further, vulnerability to natural enemies such as parasites and pathogens may vary between species (Riddick *et al.* 2009; Romanov 2019), may change over time (i.e., increase sensitivity Knapp *et al.* [2019]) and may depend on invasibility factors such as resource availability and temperature (Steele and Bjørnson 2019). Comparative studies on the interactions and effects of these parasites on native and successfully established ladybirds with ladybirds that failed to establish will provide insights on how native natural enemies may influence establishment success.

2.4 Invasiveness of ladybirds

Factors affecting invasiveness are resource acquisition, female fecundity, mating system, developmental time, size, diapause, physiology, dispersal ability, inter- or intraspecific variation (phenotypic variation, genetic variation), social interactions, cannibalism, vulnerability to natural enemies, defense mechanisms and vulnerability to change / disturbance. We note that the invasiveness of species with regard to resource acquisition, cannibalism, competition and intraguild predation are closely associated with each other and with the invasibility of the habitat. For example, the effect of intraguild members present in the habitat (invasibility) depends on how vulnerable the introduced species is to these interactions or encounters (invasiveness). Therefore, below we discuss how the invasiveness of a species interacts with invasibility (and stochastic events) to better understand how invasiveness affects the establishment success of introduced ladybirds.

2.4.1 Diet and competition

Prey identity and density has a significant impact on the fitness of ladybirds, and therefore dietary range is a key factor influencing invasiveness in ladybirds and the establishment success of introduced populations. Predatory ladybirds have a wide dietary range, including Sternorrhyncha (e.g., aphids and whiteflies), other Hemiptera, Thysanoptera, young instars of holometabolan insects, phytophagous mites, fungi (mildew) and pollen (Berkvens *et al.* 2010; Hodek *et al.* 2012). Dietary range correlates with the range of habitats occupied by ladybirds. Ladybirds with wider dietary ranges are likely to occupy more habitats and disperse farther than ladybirds with narrow dietary ranges (Comont *et al.* 2012; Giorgi *et al.* 2009).

The voracity and reproductive capacity of ladybirds depends on prey density (Soares and Serpa 2007). However, the effect of prey density on larval survival is species-specific. For example, in successfully established introduced species such as *C. septempunctata* and *H. axyridis* survival increased with prey density, but native *C. maculata* population sizes remained relatively low in the presence of heterospecific larvae regardless of prey density (Moser and Obrycki 2009). Therefore, in the presence of heterospecific ladybird larvae (native and introduced species), high prey densities may increase the establishment success of the larvae of introduced species.

A wider dietary range increases invasiveness (Berkvens *et al.* 2010), which increases the ability to establish in a habitat with low densities of preferred prey by allowing ladybirds to feed on alternate prey (invasibility of the habitat). For example, *H. axyridis*, a biocontrol agent that has now become invasive, has a wide dietary range beyond target aphid and scale insects (Berkvens *et al.* 2010; Majerus *et al.* 2006). *Harmonia axyridis* consumes exotic pests in its native range, even in the presence of preferred native prey (Zhang *et al.* 2016). Berkvens *et al.* (2010) studied the development, reproduction and survival of *H. axyridis* on diets of fruits (apple, pear and raspberry), fungi (*Botrytis cinerea* Persoon, *Sclerotinia sclerotiorum* [Lib.] de Bary and *Rhizoctonia solani* Kühn) and pollen in the laboratory by offering one of the food sources to one individual. *Harmonia axyridis* larval and adult survival was prolonged when fruits were offered compared with water as a control, but *H. axyridis* maintained on a diet of fruits failed to complete development or reproduce (Berkvens *et al.* 2010). However, females that were fed solely on pollen in the laboratory successfully completed development and reproduced (Berkvens *et al.* 2010). Pollinivory probably provided complementary and sufficient nutrients for *H. axyridis* development and is an alternative food source in the absence of aphids and scale insects (Berkvens *et al.* 2010). Further, Tayeh *et al.* (2015) show that even though biocontrol populations have developed faster life histories than native and invasive populations during their laboratory rearing, invasive populations allocate more resources to reproduction than both biocontrol and native populations of *H. axyridis*. However, successful establishments are not simply associated with faster life histories and reproductive investments but with the ability to adapt and evolve life history strategies to match the introduced area (Tayeh *et al.* 2015).

As opposed to invasive *H. axyridis*, there are only a few studies that have investigated the effects of mixed diet on the growth and colonization of native coccinellid species (Evans *et al.* 1999). This limits our understanding of how having a mixed diet may increase establishment success by comparing differences between successfully established and native ladybirds. Egg production in both native and introduced aphidophagous ladybirds is higher when a mixed diet is provided as opposed to the essential food only (Evans *et al.* 1999). Most ladybirds that are introduced around the world are aphidophagous or polyphagous. However, the availability of aphid populations changes rapidly over the year, which relates to changes in the intensity of cannibalism and intraguild predation (IGP) in ladybirds (Yasuda *et al*. 2004). IGP is the killing and consumption of competitors or individuals of the same guild regardless of taxonomic relationship or mode of resource acquisition (see section 2.4.1.2 Intraguild predation) (Yasuda *et al.* 2004). Cannibalism and IGP are two major factors that determine population dynamics and the community structure of coccinellid assemblages.

2.4.1.1 Cannibalism

Reduced cannibalism in introduced species facilitates species establishment and provides a competitive advantage over native species. Cannibalism is observed in both native and introduced ladybirds, and rates of cannibalism differ between native and introduced species (Michaud 2003). Some studies show that the differences in the rate of cannibalism between native and introduced ladybirds did not change in absence or reduced prey densities (Michaud 2003), but contrastingly some native ladybird species showed higher levels of cannibalism compared to introduced ladybird species in the absence of prey (e.g., native species *Coccinella novemnotata* Herbst, 1793 and introduced species *C. septempunctata* [Cottrell 2005; Turnipseed *et al* 2015]). However, a recent study comparing cannibalism between *H. axyridis* larvae kept in isolation and in groups shows that the survival time of the last larva in a sample was increased for the larvae kept in groups in the presence of cannibalism (Reznik *et al.* 2018). This may suggest that in the absence of prey, having a higher cannibalism rate may influence survival of a population by increasing maximum survival time (Reznik *et al.* 2018). Cannibalism is a bigger threat for the eggs of introduced species like *H. axyridis* rather than IGP, because native ladybirds tend not to prey on *H. axyridis* (Cottrell 2005; Snyder *et al.* 2004). Rates of egg cannibalism in ladybirds change with sex, life stage, reproductive status and the presence of heterospecifics, regardless of prey availability (Bayoumy *et al.* 2016). A laboratory experiment that provided options to cannibalize conspecifics and intraguild predation revealed that the presence of heterospecifics reduced cannibalism in introduced ladybird species (such as *C. septempunctata*), promoting its dominance over native species (Tumminello *et al.* 2015). Therefore, the overall decline of native ladybird species like *C. novemnotata* could be a result of increased interactions between native and introduced ladybirds leading to asymmetric egg predation by introduced species (IGP) and cannibalism (Turnipseed *et al.* 2015).

2.4.1.2 Intraguild predation

Intraguild predation (IGP) can have a dramatic effect on the establishment success of introduced species (Éric 2013; Felix and Soares 2004; Nóia *et al.* 2008). An intraguild predation relationship occurs when one guild member (intraguild predator—IG predator) attacks another guild member (intraguild prey—IG prey) despite the presence of common prey (extraguild prey) (Janssen *et al.* 2006; Polis and Holt 1992). The direction, intensity and symmetry of IGP is affected by many factors including life stage, extraguild prey densities, intraguild prey densities and body size/mass (Éric 2013; Felix and Soares 2004; Nóia *et al.* 2008). The ability of an introduced species to become an IG predator (aggression, invasiveness) interacts with vulnerability of native species (invasibility of habitat) (Hindayana *et al.* 2001; Kajita *et al.* 2006). Similarly, the vulnerability of introduced species to become an IG prey (invasiveness) interacts with aggressiveness of native species (invasibility) in the introduced habitat (Hindayana *et al.* 2001; Kajita *et al.* 2000). This results in changes in community structure, niche shifts (mostly in intraguild prey), changes in foraging behavior and indirect effects on prey. For example, a laboratory study that investigated the role of native ladybirds (*H. axyridis* and *C. septempunctata*) in preventing the establishment of *A. bipunctata* in Japan revealed that despite high availability of prey, *H. axyridis* prolonged the development and changed the foraging behavior of *A. bipunctata* through IGP (Kajita *et al.* 2000).

The vulnerability of IG prey depends on the density, mobility, distastefulness, strength of the integument (processes on the body of ladybird larvae, such as non-coccinelline species, *Curinus coeruleus* [Mulsant, 1850]; Michaud and Grant [2003]) and size of the prey (Nóia *et al.* 2008). An increase in extraguild prey density results in significantly lower rates of IGP (Kajita *et al.* 2000; Tumminello *et al.* 2015). For example, *C. septempunctata* shows no signs of IGP against *A. bipunctata* when prey are abundant, yet in scarcity of aphids, adults and larvae of *C. septempunctata* prey on *A. bipunctata* (Kajita *et al.* 2006). Among many IGP types such as protective, competitive, nutritional (when the nutritive value of the IG prey exceeds the value of the alternative food sources available) and opportunistic (when prey selection is based on size selection rather than the guild), only nutritional and opportunistic IGP has been studied under laboratory conditions (Éric 2013).

The level of competition (invasibility) in the introduced habitat and vulnerability of the species to becoming intraguild prey (invasiveness) is a major factor influencing establishment success. The body size and weight of individuals in a population can indicate levels of competition in the environment (Moser and Obrycki 2009; Snyder *et al.* 2004) and hence the invasibility of the habitat. The body weight of native ladybirds (*C. maculata;* Moser and Obrycki [2009], *Eriopis connexa* [Germar, 1824]; Mirande *et al.* [2015b]) did not increase with prey density and presence of introduced ladybirds (*H. axyridis* and *C. septempunctata*) but the body weight of introduced ladybirds increased when they were reared in the presence of native ladybirds and native IG predators (Wells *et al.* 2017). The weight gains of introduced ladybirds are thought to be a competitive advantage because they could use native ladybirds as an additional food source (Moser and Obrycki 2009; Snyder *et al.* 2004). Similar results were found in a field cage study in which *H. axyridis* gained more weight when they were reared with native ladybirds (*C. maculata*) than with conspecifics (Hoogendoorn and Heimpel 2004). The body weight and mobility of individuals also influences the intensity of IGP (Felix and Soares 2004). Rates of IGP increase with larval instar when *H. axyridis* are the IG predator, but IGP does not change when *Coccinella undecimpunctata* Linnaeus, 1758 is the IG predator (Felix and Soares 2004). IGP almost always results when the IG predator and prey are very different in size (e.g., larvae vs. eggs, 4th-instar larvae vs 1st-instar larvae) and larvae in the same instar rarely prey on each other (Snyder *et al.* 2004). Further, ladybird species whose target prey are large or active have larger body sizes and lay larger eggs than ladybirds that target small or less active prey (Dixon and Hemptinne 2001). Although size influences IGP, mass does not seem to matter, as the rate of IGP does not change when the body weight is different between native and introduced ladybirds (Nóia *et al.* 2008). Therefore, when introducing ladybirds to a new habitat, understanding the differences in body sizes between native members of the guild in the introduced area and introduced species would help predict the level of competition and how that would influence the establishment success of the introduced species.

Developmental stage and the characteristics of other species in the ecosystem play a major role in deciding the intensity and direction of IGP. Small and slow larvae are the most vulnerable IG prey (Éric 2013; Nóia *et al.* 2008). However, foraging speed is influenced by body size and adults of larger ladybirds have been shown to have slower walking speeds compared to smaller ladybirds (Ghosh and Agarwala 2018). According to the slow growth high mortality hypothesis, faster growth reduces mortality (Clancy and Price 1987). Therefore, larvae of smaller predator species with faster developmental times could be larger than a co-occurring larvae of a larger predator species with slow developmental times, changing the direction of IGP (Evans 2004). Therefore, differences in colonization time (time when eggs start hatching during the season) could change the direction of IGP and affect the survival of offspring during IGP interactions (Éric 2013). Adverse effects of IGP on native species are strongest when interactions occur between older introduced and younger native larvae (Nóia *et al.* 2008; Yasuda *et al.* 2004). However, reproductive and development times of coccinellids may be affected by temperature and photoperiod (Singh *et al.* 2018; Wang *et al.* 2013). Therefore, it is important to investigate how changes in temperature and photoperiod may affect the intensity and direction of intraguild predation.

Relative body size influences the outcome of interspecific interactions by favoring larger individuals, which are more likely to become IG predators. For example, larvae of *H. convergens*, the smallest species, is preyed on by *H. axyridis*, *C. septempunctata* and *C. tranversoguttata* (Yasuda *et al.* 2004) when reared together. However, the intensity of the interactions were weaker when aphids were in abundance (Yasuda *et al.* 2004). Further, Snyder *et al*. (2004) argue that the likelihood of IGP is not related to size differences between predator and prey but is dependent on the ability to capture IG prey and the ability to avoid attack. Accordingly, small lacewing larvae are able to kill large ladybird larvae (Nedvěd *et al*. 2013). The survival of introduced aggressive ladybirds such as *C. septempunctata* and *H. axyridis* is higher when they share habitat with native species because natives become relatively easy IG prey, resulting in an increased probability of establishment (Moser and Obrycki 2009).

Cannibalism and egg predation by IG predators is common despite all ladybirds having alkaloid chemical defenses in all life stages (Braekman *et al.* 1999; Sloggett 2005). These alkaloids appear to have no harmful effects on the development or survival of first instars (Kajita *et al.* 2014) when digested by other ladybird species in IGP. Only *Calvia quatuordecimguttata* (Linnaeus, 1758) eggs were found to be well protected against *H. axyridis* (Katsanis *et al*. 2017). While introduced ladybirds may not have specific chemical defenses (nor do native species) to protect against IGP, introduced ladybirds are often more aggressive than native ladybirds, which results in asymmetric IGP (Kajita *et al.* 2014). However, invasive ladybirds have adaptations to protect eggs and larvae, which are advantageous for establishment in new habitats. For example, *H. axyridis* has a species-specific chemical defense system (alkaloids) to protect its eggs from predation (Sato and Dixon 2004; Smith and Gardiner 2013). In addition to chemical defenses, *H. axyridis* larvae also have strongly adhesive tarsi that facilitated more successful attacks and escapes against predation (Snyder *et al.* 2004).

Even though there is no evidence of egg predation by introduced ladybirds, being less vulnerable to predation by other generalist predators may increase the population growth of introduced species (Smith and Gardner 2013). Therefore, native and introduced species may have complex interactions, with introduced ladybirds having more effective chemical defenses and aggressive foraging behavior than native ladybirds (Hoogendoorn and Heimpel 2002) that indirectly result in exploitative and competitive relationships with native ladybirds (Smith and Gardiner 2013).

IGP directly favors IG predators by providing an additional nutritional source and indirectly favors IG predators by reducing exploitative competition (Yasuda *et al.* 2004). Therefore, when the IG predator is an introduced species, IGP directly and indirectly favors establishment of the introduced species (Yasuda *et al.* 2004). However, a recent study which investigated the unsuccessful introduction of *H. axyridis* suggests that both, a) the availability of resources sufficient to sustain large populations of large species, and b) the presence of less aggressive large coccinellid species which would not outcompete *H. axyridis*, are important to achieve successful establishment in a novel environment (Soares *et al.* 2017). Therefore, when introducing a species to a new habitat as a planned invasion, we should investigate the invasibility of the habitat based on body size and vulnerability to become IG prey of local members of the food guild, and potential interactions between these factors to better predict establishment success and plan introduction processes (Soares *et al.* 2017).

2.4.2 Dispersal ability

Dispersal ability is a critical factor that facilitates the establishment of introduced species (Harrison 1980; Tscharntke *et al.* 2002) and varies between individuals. Dispersal is movement creating gene flow and can be driven by many different behaviors such as foraging, finding mates, laying eggs, avoiding predators or even moving or drifting in wind (Benton and Bowler 2012). Understanding how invasibility and the dispersal ability of introduced species interact will help improve our ability to predict successful establishments. Ladybirds have specific machinery such as wings and specific developmental stages (adults) associated with dispersal. Therefore, wing polymorphism and variation in wing shape, size of the wings and flight muscles in monomorphic insects suggest that physiological mechanisms and ecological factors determine the dispersal ability of individuals (Zera and Denno 1997). However, flightless morphs of biocontrol agents such as *A. bipunctata* (Lommen *et al.* 2008) and *H. axyridis* (Seko *et al.* 2014; Seko *et al.* 2008) may be more effective in biocontrol due to an increased residence time in the target field compared to flying ladybirds. Migration behavior may also influence dispersal ability (Zhou *et al.* 1995). For example, adult *C. septempunctata* choose hibernation sites away from breeding and foraging sites and their dispersal ability and accumulation of nutrients and energy before hibernation plays a major role in their survival during and after hibernation (Zhou *et al.* 1995).

Dispersal ability can vary within a population. The ability of invasive populations of *H. axyridis* ladybirds to disperse is higher in individuals at the edge of the population than that at the core of the population, with an increase in flight speed at the edge of the population (Lombaert *et al.* 2014). Dispersal is also influenced by environmental factors. For example, a field experiment on *C. septempunctata* showed that dispersal and navigation of ladybirds towards foraging and mating habitats may be influenced by chemical cues (Werf *et al.* 2000). Further, species may disperse via human transportation and disperse into synanthropic areas such as walls of residential buildings (Borowski 2015). However, very little research has compared the dispersal abilities of native and introduced ladybird populations. This hinders our understanding of the importance of natural dispersal ability on establishment success.

2.5 Stochastic events affecting establishment success

Stochastic factors that may affect the establishment success of introduced species are propagule pressure, climate change and human mediated changes in the habitat (e.g., habitat degradation, management, land use and disturbance). For instance, a recent study suggests that atmospheric events such as changes in wind speed and direction may affect flying and migratory ladybirds such as *H. axyridis* and its invasion in the UK (Siljamo *et al.* 2020). We discuss how stochastic events such as climate change and disturbance interact with the invasiveness of species and invasibility of habitats in earlier sections. We review studies that assess propagule pressure to understand how it interacts with invasiveness, invasibility and the establishment success of introduced populations.

2.5.1 Propagule population and variation

Propagule pressure or characteristics of the propagule (i.e., founding) population, including the number of individuals in the propagule population (propagule size) and number of times the species is introduced to the recipient environment (propagule number), may strongly influence establishment success (Lockwood *et al.* 2005). While propagule pressure is hypothesized to influence establishment in diverse taxa, there are relatively few observational (Beirne 1975; Hopper and Roush 1993) or experimental studies that have investigated the effect of propagule pressure on the establishment success of introduced species (Moulton *et al.* 2012) including ladybirds. A population genetic study on *C. septempunctata* suggests higher propagule pressure has supported the establishment of this species in the USA (Kajita *et al.* 2012). Further, *H. axyridis* was first introduced in the USA in 1916 but was not established until 1988, which included several failed introductions including a single release of 32 specimens in 1979 and eight releases of 3781 specimens in 1980 (Chapin and Brou 1991). Further, investigating other stochastic events such as unusual weather patterns that occurred following introductions may help in understanding factors affecting the successful establishment of the propagule population (Moulton *et al.* 2012). Strong Allee effects may also influence the establishment success of introduced species. Allee effects may define establishment success only above a certain number of individuals (propagule size) independent of their variation (Drake and Lodge 2006) and may be influenced by repeated introductions or immigration events (Drake and Lodge 2006) and time between repeated introductions (McDermott and Finnoff 2016). Therefore, further investigation of strong and weak Allee effects in natural populations (Leung *et al.*

2004) may increase the predictability of establishment success of introduced ladybird species and the invasive potential of ladybirds such as *H. axyridis*. Further, a few studies suggest that failed establishment may have been influenced by other factors such as IGP (i.e., *A. bipunctata*; Kajita *et al.* [2000]). Species such as *Propylea quatuordecimpunctata* (Linnaeus, 1758) and *C. septempunctata* may have successfully established near port sites but lack of documentation may have implied failed introductions (Day *et al.* 1994). Additionally, the effect of propagule pressure is also influenced by genetic variability in the propagule, species-level traits and the location (such as level of disturbance, effects of other non-native species and the intensity of competition or predation) (Lockwood *et al.* 2005).

2.5.1.1 Genetic variation

Introduced populations should be less genetically diverse in introduced areas than in their native areas because they are founded by a small proportion of a larger metapopulation (Lombaert *et al.* 2011). Inbreeding depression can follow if the population remains small for several generations (Laugier *et al*. 2016). However, multiple introductions can increase the genetic variation of introduced populations to similar levels of source populations (Dlugosch and Parker 2008). Studies that quantitatively measure genetic loss due to introduction are limited, and there are few studies that compare the genetic variation of the source population to genetic variation of the introduced population in ladybirds (see study of *H. axyridis* by Krafsur *et al.* [2005] and Tayeh *et al.* [2012]).

A comparison of allele diversity and heterozygosities between native and introduced ladybird species in North America suggests that there is no relationship between genetic diversity and establishment success (Krafsur *et al.* 2005). However, the genetic variability of the population is related to its dietary range (Krafsur *et al.* 2005), with the most variation observed in populations of ladybirds that depend on ephemeral and discontinuously distributed prey (Krafsur *et al.* 2005).

Multiple mating, well documented in many ladybirds (Hodek and Ceryngier 2000), increases the genetic variability of the population. The mean number of males contributing to the fertilization of an *H. axyridis* egg cluster was 3.8 (Ueno 1996). The progeny of a single multiply-mated female can establish a new genetically variable population after an accidental dispersal to a new distant area (Awad *et al.* 2015; Awad *et al.* 2017). Studying genetic variation in invasive populations may also provide insights into how variability in gene flow may affect establishment success and how we can use genetic variability to increase establishment success in introductions carried out as planned invasions. For example, despite several unsuccessful attempts to actively introduce *H. axyridis* in North America for biocontrol purposes, successful establishment was not detected until after 1988 (Chapin and Brou 1991). However, *H. axyridis* has become invasive in later decades, suggesting that these invasive populations could have originated or established from successful invasive populations that were not previously detected rather than native ranges (Lombaert *et al.* 2010). This is an example of the invasive bridgehead effect in which an invasive population serves as the source for population expansions and successful subsequent invasions into new environments (Lombaert *et al.* 2010). Lombaert *et al.* (2011) examined the bridgehead population of *H. axyridis* to identify its source population and revealed an admixture of genetic markers from both eastern and western parts of its native area, increasing its natural genetic diversity, which facilitated it to further spread to other locations. Further, recent studies indicate that genetic bottlenecks are not as frequent in successfully established invasive populations as was once assumed (Lombaert *et al.* 2014) and that having an admixture may facilitate the establishment of introduced populations in novel environments or even help them become bridgehead populations (Lombaert *et al.* 2011).

2.5.1.2 Phenotypic variation

Phenotypic variation within and among populations can aid the establishment of a population in different environments because it generates variation in individual fitness (Kalaisekar *et al.* 2012). Similarly, individuals with the ability to adjust their phenotype in response to the environment are expected to have an increased probability of establishing compared to individuals with little phenotypic plasticity.

For a given genotype, environmental conditions may have a profound effect on the phenotype, including traits such as body size, color and/or size of the wings (Nijhout 2003). Adult body size represents the cumulative effect of genetic composition and diverse environmental conditions faced by the developing individual (Evans 2000). The body size of insects can correlate with fitness related traits such as fecundity, growth rate and survival (Honěk 1993). A comparative study between *C. septempunctata* (invasive in North America) and native North American ladybird species (*C. tranversoguttata, H. convergens, Hippodamia quinquesignata* [Kirby, 1837]*, Hippodamia sinuata* Mulsant, 1850*,* and *Hippodamia tredecimpunctata* [Linnaeus, 1758]), shows that *C. septempunctata* has greater variation in adult body size than native species (Evans 2000). A comparison of phenotypic variation between invasive and biocontrol populations of *H. axyridis* provided evidence of increased adaptive phenotypic plasticity of European invasive populations of *H. axyridis* compared to biocontrol populations (Lombaert *et al.* 2008). Nevertheless, we still have few comparisons of the level of phenotypic variation between sympatric native and introduced ladybird populations. Understanding how phenotypic variation in propagule populations increases the invasiveness of invasive populations and comparing phenotypic variation between successful and failed introductions may help us manage species introductions to increase the rate of successful establishment of introduced species.

2.6 Summary

Factors that affect the establishment of introduced populations can be categorized broadly into invasiveness of species, invasibility of the recipient ecosystem and stochastic events. Figure 2.2 summarizes how factors affecting successful establishment interact within the proposed framework of invasibility, invasiveness and stochastic events and how these factors may increase the predictability of establishment success. This provides a useful guide for researchers wanting to study establishment success and can be used as a checklist of assessments to be carried out when introducing a new species for biocontrol and conservation. For example, abiotic factors such as temperature may increase the invasibility of a habitat by interacting with the life history traits of introduced species such as female fecundity, body size, and developmental time. However, the outcomes of these interactions are affected by stochastic events such as climate change induced by global warming, fluctuations in prey populations, anthropogenic disturbances and biological homogenization. Therefore, to predict establishment success accurately we need to have insights into the interactions occurring between these factors.

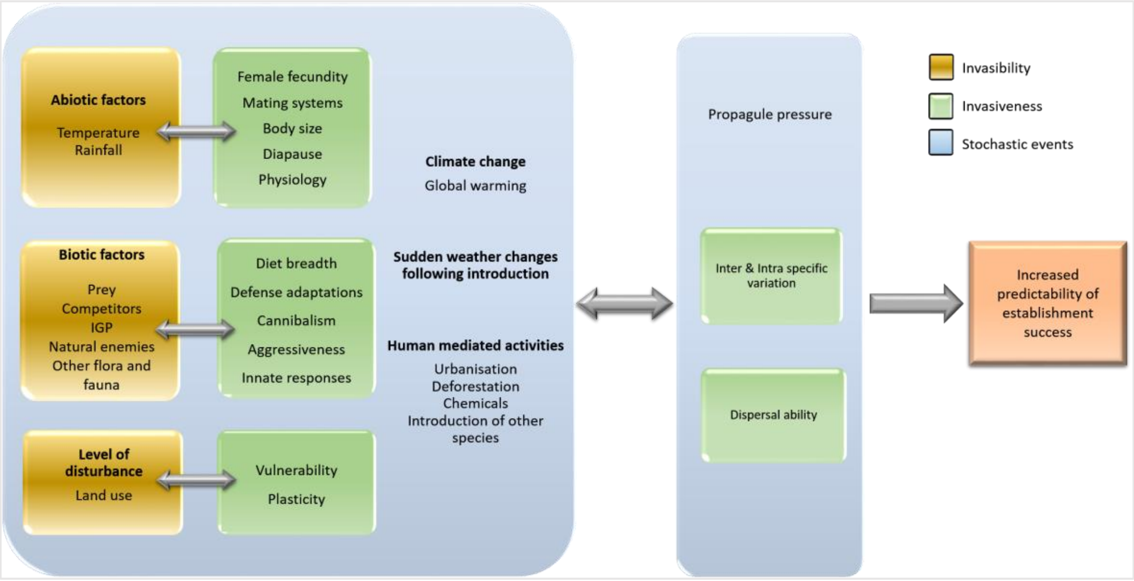

Figure 2.2 Summary of factors to assess within the proposed framework to increase predictability of establishment success

The presence and density of biotic factors such as preferred prey, alternate prey, natural enemies and native ladybirds affects the invasibility of a habitat and interacts with the invasiveness of the species by influencing its competitiveness and dietary range. The effect of both biotic and abiotic factors in the habitat interact with inter- and intraspecific variation and the dispersal ability of the introduced species, which may in turn depend on the characteristics of the propagule population.

Greater genotypic variation and phenotypic plasticity in functionally important traits have recently been hypothesized to increase establishment success, which implies that there are higher levels of variation in successfully established ladybirds than less successful populations (Forsman 2014). However, more studies comparing the genetic and phenotypic variation between introduced populations and native populations in the recipient ecosystems are needed (Kumschick *et al.* 2015). Investigating the level of correlation between trait variation and establishment success of invasive ladybirds will provide insights to improve the success of species introductions for biocontrol and conservation purposes. The characteristics of the propagule population and propagule pressure play a key role in the successful establishment of ladybirds (Kajita *et al.* 2012; Lockwood *et al.* 2005). The effect of propagule pressure depends on the biodiversity of the recipient population, climate and competition (Kajita *et al.* 2012; Lockwood *et al.* 2005). However, higher propagule pressure facilitates the establishment and range expansion of introduced populations by increasing the genetic variability of the introduced population (Lockwood *et al.* 2005).

Mating systems also influence the establishment success of introduced species by influencing the genetic variability and survival of the offspring. However, we need to assess how fecundity and egg viability is influenced by different mating systems to better understand how to improve the establishment of introduced species. Further, assessing rearing conditions in the laboratory before release, to ensure that we preserve genetic diversity in the population (Gariepy *et al.* 2015) will increase the establishment success of introduced species in biocontrol and conservation programs.

2.7 Conclusions and future directions

Ladybirds in the tribe Coccinellini are generalist predators introduced around the world for biological control purposes. The success of introduction is variable, and some introductions have inadvertently resulted in invasive populations. Research on invasive ladybirds, ladybird introductions and distributions can provide key insights into the factors that influence the successful establishment of introduced species. Currently, species introductions have very low establishment rates. We propose three categories based on invasion biology (*sensu* Abrams and Moffat 2018) that will aid research efforts to increase the establishment success of introduced ladybirds: investigating factors affecting invasibility, invasiveness and stochastic events (Table 2.1), and the interactions (Fig. 2.2) between these categories.

Most research on ladybirds has concentrated on a few widely distributed species and has focused on their effect on native ladybirds. Among the 16 coccinellines studied around the world for their use as biocontrol agents (Appendix A: Supplementary table 2.1), research assessing the success of these introductions is limited to only seven species: *H. axyridis, C. septempunctata, A. bipunctata, C. undecimpunctata, H. chalybeus*, *Rodolia cardinalis* (Mulsant, 1850) and the non-coccinelline *C. montrouzieri*. Future efforts may be helped via citizen science programs (Losey *et al.* 2007). Given the important role that ladybirds play as predators in ecosystems, there is an urgent need to diversify research (Sloggett 2005) into ladybird ecology to better understand ways to improve the establishment success of introduced ladybirds. Further, given the recent contradictory evidence on interactions between introduced and native ladybird populations (e.g., the relative influence of IGP on native ladybird population declines), we need more studies that assess these complex interactions in order to accurately project the impacts of introduced ladybird species establishments and assess their invasive potential.

Studies that have looked at unsuccessful establishments of introduced species—including ladybirds—are limited (Cock *et al.* 2016; Zenni and Nunez 2013). However, there are a few studies that have investigated failed invasions in *H. axyridis* (Evans *et al.* 2011; Soares *et al.* 2008; Soares *et al.* 2017; Zenni and Nunez 2013) which makes ladybirds an ideal model system to begin a comprehensive understanding of establishment success. Studying failed establishments will help us in two ways: 1) to understand the factors that limit establishment (Abram and Moffat 2018) and 2) predict the dispersal and distribution of introduced or invasive species accurately (Michael *et al.* 2012). Therefore, it is important to monitor and record species introductions, successful and unsuccessful establishments and use this information in distribution modelling to accurately predict the introduction success and dispersal of ladybirds.

Future work on ladybirds should concentrate on investigating differences in phenotypic and genetic variation between invasive/widely distributed ladybirds and less distributed/unsuccessful introductions of ladybirds. We also suggest investigation of the role of climatic parameters on species introductions and distributions, the role of climate change in range expansions and the role of IGP in native species declines. Further, understanding differences and effects of dispersal ability on species establishment will improve the predictability of establishment success of introduced species. Field experiments comparing life history traits between native and introduced or invasive ladybirds considering invasibility parameters of the introduced area (Raak-van den Berg *et al.* 2018) will provide insights into how abiotic and biotic factors interact with invasiveness of introduced species, improving our understanding of establishment success.

We believe the three categories (invasibility, invasiveness and stochastic events) will aid direct comparisons between successful and unsuccessful establishments for a cohesive theory of introduction biology. By targeting our research effort on the vulnerable establishment stage of introductions, we will be better placed to design and implement environmental management programs.

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2.9 References

Abram, P. K., and C. E. Moffat. 2018. Rethinking biological control programs as planned invasions. Current Opinion in Insect Science 27: 9–15.

Awad, M., P. Kalushkov, T. Nedvědová, and O. Nedvěd. 2013. Fecundity and fertility of ladybird beetle *Harmonia axyridis* after prolonged cold storage. BioControl 58: 657–666.

Barahona-Segovia, R. M., A. A. Grez, and F. Bozinovic. 2015. Testing the hypothesis of greater eurythermality in invasive than in native ladybird species: from physiological performance to life-history strategies. Ecological Entomology: 182–191.

Bayoumy, M. H., A. M. Abou-Elnaga, A. A. Ghanim, and G. A. Mashhoot. 2016. Egg cannibalism potential benefits for adult reproductive performance and offspring fitness of *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae). Egyptian Journal of Biological Pest Control 26: 35–42.

Beirne, B. 1975. Biological control attempts by introductions against pest insects in the field in Canada. The Canadian Entomologist 107: 225–236.

Benton, T. G., and D. E. Bowler. 2012. Dispersal in invertebrates: influences on individual decisions. *In* Dispersal Ecology and Evolution (eds J. Clobert, M. Baguette, T. G. Benton and J. M. Bullock). Oxford University Press, Oxford: 41–49.

Berkvens, N., C. Landuyt, K. Deforce, D. Berkvens, L. Tirry, and P. De Clercq. 2010. Alternative foods for the multicoloured Asian lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae). European Journal of Entomology 107: 189–195.

Blackburn, T. M., P. Pyšek, S. Bacher, J. T. Carlton, R. P. Duncan, V. Jarošík, J. R. Wilson, and D. M. Richardson. 2011. A proposed unified framework for biological invasions. Trends in Ecology and Evolution 26: 333–339.

Boher, F., F. M. Jaksic, S. I. Martel, M. J. Orellana, and F. Bozinovic. 2018. Does thermal physiology explain the ecological and evolutionary success of invasive species? Lessons from ladybird beetles. Evolutionary Ecology Research 19: 243–255.

Brown, P. M., R. Frost, J. Doberski, T. Sparks, R. Harrington, and H. E. Roy. 2011. Decline in native ladybirds in response to the arrival of *Harmonia axyridis*: early evidence from England. Ecological Entomology 36: 231–240.

Clark, R. E., and M. S. Singer. 2018. Differences in aggressive behaviors between two ant species determine the ecological consequences of a facultative food-for-protection mutualism. Journal of Insect Behavior 31: 510–522.

Cock, M. J. W., S. T. Murphy, M. T. K. Kairo, E. Thompson, R. J. Murphy, and A. W. Francis. 2016. Trends in the classical biological control of insect pests by insects: an update of the BIOCAT database. BioControl 61: 349–363.

Coderre, D., L. Provencher, and J. C. Tourneur. 1987. Oviposition and niche partitioning in aphidophagous insects on maize. The Canadian Entomologist 119: 195–203.

Comont, R. F., H. E. Roy, R. Harrington, C. R. Shortall, and B. V. Purse. 2014a. Ecological correlates of local extinction and colonisation in the British ladybird beetles (Coleoptera: Coccinellidae). Biological Invasions 16: 1805–1817.

Comont, R. F., H. E. Roy, O. T. Lewis, R. Harrington, C. R. Shortall, and B. V. Purse. 2012. Using biological traits to explain ladybird distribution patterns. Journal of Biogeography 39: 1772–1781.

Comont, R. F., B. V. Purse, W. Phillips, W. E. Kunin, M. Hanson, O. T. Lewis, R. Harrington, C. R. Shortall, G. Rondoni, and H. E. Roy. 2014b. Escape from parasitism by the invasive alien ladybird, *Harmonia axyridis*. Insect Conservation and Diversity 7: 334–342.

Cottrell, T. E. 2005. Predation and cannibalism of lady beetle eggs by adult lady beetles. Biological Control 34: 159–164.

Day, W., D. Prokrym, D. Ellis, and R. Chianese. 1994. The known distribution of the predator *Propylea quatuordecimpunctata* (Coleoptera: Coccinellidae) in the United States, and thoughts on the origin of this species and five other exotic lady beetles in eastern North America. Entomological News 105: 244–256.

Dick, J. T. A., C. Laverty, J. J. Lennon, D. Barrios-O'Neill, P. J. Mensink, J. Robert Britton, V. Médoc, P. Boets, M. E. Alexander, N. G. Taylor, A. M. Dunn, M. J. Hatcher, P. J. Rosewarne, S. Crookes, H. J. MacIsaac, M. Xu, A. Ricciardi, R. J. Wasserman, B. R. Ellender, O. L. F. Weyl, F. E. Lucy, P. B. Banks, J. A.

Dodd, C. MacNeil, M. R. Penk, D. C. Aldridge, and J. M. Caffrey. 2017. Invader relative impact potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. Journal of Applied Ecology 54: 1259–1267.

Dindo, M., S. Francati, A. Lanzoni, C. di Vitantonio, E. Marchetti, G. Burgio, and S. Maini. 2016. Interactions between the multicolored Asian lady beetle *Harmonia axyridis* and the parasitoid *Dinocampus coccinellae*. Insects 7: 67.

Dixon, A. 2007. Body size and resource partitioning in ladybirds. Population Ecology 49: 45–50.

Dixon, A., and J.-L. Hemptinne. 2001. Body size distribution in predatory ladybird beetles reflects that of their prey. Ecology 82: 1847–1856.

Dlugosch, K., and I. Parker. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology 17: 431–449.

Emiljanowicz, L. M., H. A. Hager, and J. A. Newman. 2017. Traits related to biological invasion: A note on the applicability of risk assessment tools across taxa. NeoBiota 32: 31.

Éric, L. 2013. Intraguild predation among aphidophagous predators. EJE 102: 351–364.

Escalona, H. E., and A. Ślipiński. 2012. Generic revision and phylogeny of Microweiseinae (Coleoptera: Coccinellidae). Systematic Entomology 37: 125–171.

Escalona, H. E., A. Zwick, H.-S. Li, J. Li, X. Wang, H. Pang, D. Hartley, L. S. Jermiin, O. Nedvěd, and B. Misof. 2017. Molecular phylogeny reveals food plasticity in the evolution of true ladybird beetles (Coleoptera: Coccinellidae: Coccinellini). BMC Evolutionary Biology 17: 151.

Evans, E. W. 2000. Morphology of invasion: Body size patterns associated with establishment of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in western North America. European Journal of Entomology 97: 469–474.

Evans, E. W., and T. R. Toler. 2007. Aggregation of polyphagous predators in response to multiple prey: Ladybirds (Coleoptera: Coccinellidae) foraging in alfalfa. Population Ecology 49: 29–36.

Evans, E. W., A. T. Stevenson, and D. R. Richards. 1999. Essential versus alternative foods of insect predators: Benefits of a mixed diet. Oecologia 121: 107–112.

Evans, E. W., A. O. Soares, and H. Yasuda. 2011. Invasions by ladybugs, ladybirds, and other predatory beetles. BioControl 56: 597–611.

Facon, B., A. Estoup, R. A. Hufbauer, J. Foucaud, and A. Tayeh. 2017. Mating status influences cold tolerance and subsequent reproduction in the invasive ladybird *Harmonia axyridis*. Frontiers in Ecology and Evolution 5: 108.

Felix, S., and A. O. Soares. 2004. Intraguild predation between the aphidophagous ladybird beetles *Harmonia axyridis* and *Coccinella undecimpunctata* (Coleoptera: Coccinellidae): The role of body weight. European Journal of Entomology 101: 237–242.

Finlayson, C., A. Alyokhin, and E. Porter. 2009. Interactions of native and non-native lady beetle species (Coleoptera: Coccinellidae) with aphid-tending ants in laboratory arenas. Environmental Entomology 38: 846–855.

Forsman, A. 2014. Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. Proceedings of the National Academy of Sciences 111: 302–307.

Franzmann, B. A. 2002. *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), a predacious ladybird new in Australia. Australian Journal of Entomology 41: 375–377.

Gariepy, V., G. Boivin, and J. Brodeur. 2015. Why two species of parasitoids showed promise in the laboratory but failed to control the soybean aphid under field conditions. Biological Control 80: 1–7.

Giorgi, J. A., N. J. Vandenberg, J. V. McHugh, J. A. Forrester, S. A. Ślipiński, K. B. Miller, L. R. Shapiro, and M. F. Whiting. 2009. The evolution of food preferences in Coccinellidae. Biological Control 51: 215–231.

Gordon, R. D. 1985. The Coccinellidae (Coleoptera) of America north of Mexico. Journal of the New York Entomological Society 93: 912 pp.

Goryacheva, I. I., A. V. Blekhman, B. V. Andrianov, T. V. Gorelova, and I. A. Zakharov. 2015. Genotypic diversity of *Wolbachia pipientis* in native and invasive *Harmonia axyridis* Pall., 1773 (Coleoptera, Coccinellidae) populations. Russian Journal of Genetics 51: 731–736.

Hariston, N. G., L. J. Perry, A. J. Bohonak, M. Q. Fellows, C. M. Kearns, and D. R. Engstrom. 1999. Population biology of a failed invasion: Paleolimnology of *Daphnia exilis* in upstate New York. Limnology and Oceanography 44: 477–486.

Harmon, J., A. Ives, J. Losey, A. Olson, and K. Rauwald. 2000. *Coleomegilla maculata* (Coleoptera: Coccinellidae) predation on pea aphids promoted by proximity to dandelions. Oecologia 125: 543–548.

Harrison, R. G. 1980. Dispersal polymorphisms in insects. Annual Review of Ecology and Systematics 11: 95–118.

Hellmann, J. J., J. E. Byers, B. G. Bierwagen, and J. S. Dukes. 2008. Five potential consequences of climate change for invasive species. Conservation Biology 22: 534–543.

Hodek, I., H. F. Van Emden, and A. Honěk. 2012. *Ecology and behaviour of the ladybird beetles (Coccinellidae)*, Wiley-Blackwell, Chichester.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos 66: 483–492.

Hoogendoorn, M., and G. E. Heimpel. 2002. Indirect interactions between an introduced and a native ladybird beetle species mediated by a shared parasitoid. Biological Control 25: 224–230.

Hoogendoorn, M., and G. E. Heimpel. 2004. Competitive interactions between an exotic and a native ladybeetle: a field cage study. Entomologia Experimentalis et Applicata 111: 19–28.

Hopper, K. R., and R. T. Roush. 1993. Mate finding, dispersal, number released, and the success of biological control introductions. Ecological Entomology 18: 321–331.

Janssen, A., M. Montserrat, R. HilleRisLambers, A. M. d. Roos, A. Pallini, and M. W. Sabelis. 2006. Intraguild predation usually does not disrupt biological control. Trophic and Guild in Biological Interactions Control 3: 21–44.

Kajita, Y., and E. W. Evans. 2009. Ovarian dynamics and oosorption in two species of predatory lady beetles (Coleoptera: Coccinellidae). Physiological Entomology 34: 185–194.

Kajita, Y., and E. W. Evans. 2010. Relationships of body size, fecundity, and invasion success among predatory lady beetles (Coleoptera: Coccinellidae) inhabiting alfalfa fields. Annals of the Entomological Society of America 103: 750–756.

Kajita, Y., H. Yasuda, and E. W. Evans. 2006. Effects of native ladybirds on oviposition of the exotic species, *Adalia bipunctata* (Coleoptera : Coccinellidae), in Japan. Applied Entomology and Zoology 41: 57–61.

Kajita, Y., F. Takano, H. Yasuda, and B. K. Agarwala. 2000. Effects of indigenous ladybird species (Coleoptera: Coccinellidae) on the survival of an exotic species in relation to prey abundance. Applied Entomology and Zoology 35: 473–479.

Kajita, Y., E. M. O'Neill, Y. Zheng, J. J. Obrycki, and D. W. Weisrock. 2012. A population genetic signature of human releases in an invasive ladybeetle. Molecular Ecology 21: 5473–5483.

Kajita, Y., J. J. Obrycki, J. J. Sloggett, E. W. Evans, and K. F. Haynes. 2014. Do defensive chemicals facilitate intraguild predation and influence invasion success in ladybird beetles? Journal of Chemical Ecology 40: 1212–1219.

Kalaisekar, A., V. Ramamurthy, J. Patil, A. Dhandapani, and N. Azad Thakur. 2012. Multivariate morphometrics of elytral colour polymorphism in seven-spotted ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). Current Science 102: 1418–1425.

Kawakami, Y., K. Yamazaki, and K. Ohashi. 2019. Intergenerational fluctuations in colour morph frequencies may maintain elytral polymorphisms in the ladybird beetle *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae). Biological Journal of the Linnean Society 128: 725–734.

Krafsur, E. S., J. J. Obrycki, and J. D. Harwood. 2005. Comparative genetic studies of native and introduced Coccinellidae in North America. European Journal of Entomology 102: 469–474.

Kumschick, S., M. Gaertner, M. Vilà, F. Essl, J. M. Jeschke, P. Pyšek, A. Ricciardi, S. Bacher, T. M. Blackburn, and J. T. Dick. 2015. Ecological impacts of alien species: Quantification, scope, caveats, and recommendations. BioScience 65: 55–63.

Lockwood, J. L., P. Cassey, and T. Blackburn. 2005. The role of propagule pressure in explaining species invasions. Trends in Ecology and Evolution 20: 223–228.

Lombaert, E., T. Malausa, R. Devred, and A. Estoup. 2008. Phenotypic variation in invasive and biocontrol populations of the harlequin ladybird, *Harmonia axyridis*. Biocontrol 53: 89–102.

Lombaert, E., T. Guillemaud, J.-M. Cornuet, T. Malausa, B. Facon, and A. Estoup. 2010. Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. PLOS ONE 5(3): e9743.

Lombaert, E., A. Estoup, B. Facon, B. Joubard, J. C. Grégoire, A. Jannin, A. Blin, and T. Guillemaud. 2014. Rapid increase in dispersal during range expansion in the invasive ladybird *Harmonia axyridis*. Journal of Evolutionary Biology 27: 508–517.

Lombaert, E., T. Guillemaud, C. Thomas, L. Lawson Handley, J. Li, S. Wang, H. Pang, I. Goryacheva, I. Zakharov, and E. Jousselin. 2011. Inferring the origin of populations introduced from a genetically structured native range by approximate Bayesian computation: Case study of the invasive ladybird *Harmonia axyridis*. Molecular Ecology 20: 4654–4670.

Lommen, S. T. E., C. W. Middendorp, C. A. Luijten, J. van Schelt, P. M. Brakefield, and P. W. de Jong. 2008. Natural flightless morphs of the ladybird beetle *Adalia bipunctata* improve biological control of aphids on single plants. Biological Control 47: 340–346.

Losey, J. E., J. E. Perlman, and E. R. Hoebeke. 2007. Citizen scientist rediscovers rare nine-spotted lady beetle, *Coccinella novemnotata*, in eastern North America. Journal of Insect Conservation 11: 415–417.

Majerus, M., V. Strawson, and H. Roy. 2006. The potential impacts of the arrival of the harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera : Coccinellidae), in Britain. Ecological Entomology 31: 207–215.

Meffe, G. K. 1991. Failed invasions of a southeastern blackwater stream by bluegills-implications for conservation of native communities. Transactions of the American Fisheries Society 120: 333–338.

Michael, P. J., P. B. Yeoh, and J. K. Scott. 2012. Potential distribution of the Australian native *Chloris truncata* Based on modelling both the successful and failed global introductions. PLOS ONE 7: e42140.

Michaud, J. 2012. Coccinellids in biological control. Ecology and behaviour of the ladybird beetles (Coccinellidae). *In* Ecology and behaviour of the ladybird beetles (eds Hodek I, van Emden HF, Honek A). Wiley-Blackwell, Oxford: 488–519.

Michie, L. J., F. Mallard, M. E. N. Majerus, and F. M. Jiggins. 2010. Melanic through nature or nurture: genetic polymorphism and phenotypic plasticity in *Harmonia axyridis*. Journal of Evolutionary Biology 23: 1699–1707.

Moser, S. E., and J. J. Obrycki. 2009. Competition and intraguild predation among three species of coccinellids (Coleoptera: Coccinellidae). Annals of the Entomological Society of America 102: 419–425.

Moulton, M. P., and W. P. Cropper. 2016. Propagule size and patterns of success in early introductions of Chukar Partridges (*Alectoris chukar*) to Nevada. Evolutionary Ecology Research 17: 713–720.

Moulton, M. P., W. P. Cropper, and M. L. Avery. 2012. Historical records of passerine introductions to New Zealand fail to support the propagule pressure hypothesis. Biodiversity and Conservation 21: 297–307.

Musser, F. R., and A. M. Shelton. 2003. Factors altering the temporal and within-plant distribution of coccinellids in corn and their impact on potential intra-guild predation. Environmental Entomology 32: 575–583.

Nedvěd, O., M. Awad, G. Laugier, L. Piálek, A. Krejčí, F. Karabüyük, K. Pužejová, and P. Kalushkov. 2018. Paternity and sperm competition following multiple mating in *Harmonia axyridis* (Coleoptera: Coccinellidae). IOBC-WPRS Bulletin 137: 17–24.

Nijhout, H. 2003. The control of body size in insects. Developmental Biology 261: 1–9.

Nóia, M., I. Borges, and A. O. Soares. 2008. Intraguild predation between the aphidophagous ladybird beetles *Harmonia axyridis* and *Coccinella undecimpunctata* (Coleoptera: Coccinellidae): the role of intra and extraguild prey densities. Biological Control 46: 140–146.

Obrycki, J. J., and T. J. Kring. 1998. Predaceous Coccinellidae in biological control. Annual Review of Entomology 43: 295–321.

Paul, S. C., J. K. Pell, and J. D. Blount. 2015. Reproduction in risky environments: the role of invasive egg predators in ladybird laying strategies (dataset). PLOS ONE 10(10): e0139404.

Polis, G. A., and R. D. Holt. 1992. Intraguild predation: the dynamics of complex trophic interactions. Trends in Ecology and Evolution 7: 151–154.

Powell, B. E., and J. Silverman. 2010. Impact of *Linepithema humile* and *Tapinoma sessile* (Hymenoptera: Formicidae) on three natural enemies of *Aphis gossypii* (Hemiptera: Aphididae). Biological Control 54: 285–291.

Purse, B. V., R. Comont, A. Butler, P. M. J. Brown, C. Kessel, and H. E. Roy. 2015. Landscape and climate determine patterns of spread for all colour morphs of the alien ladybird *Harmonia axyridis*. Journal of Biogeography 42: 575–588.

Raak-van den Berg, C. L., P. W. de Jong, G. Gort, B. F. J. Manly, and J. C. van Lenteren. 2018. Life histories of an invasive and native ladybird under field experimental conditions in a temperate climate. Entomologia Experimentalis et Applicata 166: 151–161.

Riddick, E. W., T. E. Cottrell, and K. A. Kidd. 2009. Natural enemies of the Coccinellidae: Parasites, pathogens, and parasitoids. Biological Control 51: 306–312.

Robertson, J. A., A. Ślipiński, M. Moulton, F. W. Shockley, A. Giorgi, N. P. Lord, D. D. Mckenna, W. Tomaszewska, J. Forrester, and K. B. Miller. 2015. Phylogeny and classification of Cucujoidea and the recognition of a new superfamily Coccinelloidea (Coleoptera: Cucujiformia). Systematic Entomology 40: 745–778.

Romanov, D. 2019. Parasitoids of the harlequin ladybeetle *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae). Russian Journal of Biological Invasions 10: 52–67.

Rondoni, G., F. Ielo, C. Ricci, and E. Conti. 2017. Behavioural and physiological responses to prey-related cues reflect higher competitiveness of invasive vs. native ladybirds. Scientific Reports 7: 9.

Rudolf, V. H. 2007. The interaction of cannibalism and omnivory: consequences for community dynamics. Ecology 88: 2697–2705.

Rudolf, V. H., and N. L. Rasmussen. 2013. Ontogenetic functional diversity: size structure of a keystone predator drives functioning of a complex ecosystem. Ecology 94: 1046–1056.

Saito, T., and S. Bjornson. 2008. Effects of a microsporidium from the convergent lady beetle, *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae), on three non-target coccinellids. Journal of Invertebrate Pathology 99: 294–301.

Sato, S., and A. F. G. Dixon. 2004. Effect of intraguild predation on the survival and development of three species of aphidophagous ladybirds: consequences for invasive species. Agricultural and Forest Entomology 6: 21–24.

Saxena, S., G. Mishra, and O. Omkar. 2019. Mating system drives the mate selection in aphidophagous ladybird, *Menochilus sexmaculatus*. Journal of Evolutionary Biology 33: 384-384.

Scorsetti, A. C., S. A. Pelizza, M. N. Fogel, M. F. Vianna, and M. I. Schneider. 2017. Interactions between the entomopathogenic fungus *Beauveria bassiana* and the Neotropical predator *Eriopis connexa* (Coleoptera: Coccinellidae): Implications in biological control of pest. Journal of Plant Protection Research 57:389-395.

Seago, A. E., J. A. Giorgi, J. Li, and A. Ślipiński. 2011. Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. Molecular Phylogenetics and Evolution 60: 137–151.

Seko, T., K.-i. Yamashita, and K. Miura. 2008. Residence period of a flightless strain of the ladybird beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in open fields. Biological Control 47: 194–198.

Seko, T., A. Sumi, A. Nakano, M. Kameshiro, T. Kaneda, and K. Miura. 2014. Suppression of aphids by augmentative release of larvae of flightless *Harmonia axyridis*. Journal of Applied Entomology 138: 326–337.

Singh, S., G. Mishra, and Omkar. 2018. Plasticity in reproductive output and development in response to thermal variation in ladybird beetle, *Menochilus sexmaculatus*. Journal of Thermal Biology 71: 180–188.

Ślipiński, A. 2007. *Australian ladybird beetles (Coleoptera: Coccinellidae): their biology and classification*, Australian Biological Resources Study, Canberra.

Ślipiński, S., R. Leschen, and J. Lawrence. 2011. Order Coleoptera Linnaeus, 1758. Animal biodiversity: an outline of higher-level classification and survey of taxonomic richness. Zootaxa 3148: 203–208.

Sloggett, J. J. 2005. Are we studying too few taxa? Insights from aphidophagous ladybird beetles (Coleoptera: Coccinellidae). European Journal of Entomology 102: 391.

Smith, C. A., and M. M. Gardiner. 2013. Biodiversity loss following the introduction of exotic competitors: does intraguild predation explain the decline of native lady beetles? PLOS ONE 8: e84448.

Snyder, W. E., and G. M. Clevenger. 2004. Negative dietary effects of Colorado potato beetle eggs for the larvae of native and introduced ladybird beetles. Biological Control 31: 353–361.

Snyder, W. E., G. M. Clevenger, and S. D. Eigenbrode. 2004. Intraguild predation and successful invasion by introduced ladybird beetles. Oecologia 140: 559–565.

Soares, A. O., and A. Serpa. 2007. Interference competition between ladybird beetle adults (Coleoptera: Coccinellidae): effects on growth and reproductive capacity. Population Ecology 49: 37–43.

Soares, A. O., I. Borges, P. A. Borges, G. Labrie, and E. Lucas. 2008. *Harmonia axyridis*: What will stop the invader?. BioControl 53: 127–145.

Soares, A. O., A. Honěk, Z. Martinkova, J. Skuhrovec, P. Cardoso, and I. Borges. 2017. *Harmonia axyridis* failed to establish in the Azores: the role of species richness, intraguild interactions and resource availability. BioControl 62: 423–434.

Steele, T., and S. Bjørnson. 2019. Effects of microsporidiosis and food availability on the two-spotted lady beetle, *Adalia bipunctata* L., and convergent lady beetle, *Hippodamia convergens* Guérin-Méneville. Journal of Invertebrate Pathology 161: 7–13.

Szawaryn, K., L. Bocak, A. Ślipiński, H. E. Escalona, and W. Tomaszewska. 2015. Phylogeny and evolution of phytophagous ladybird beetles (Coleoptera: Coccinellidae: Epilachnini), with recognition of new genera. Systematic Entomology 40: 547–569.

Takizawa, T., and H. Yasuda. 2006. The effects of attacks by the mutualistic ant, *Lasius japonicus* Santschi (Hymenoptera: Formicidae) on the foraging behavior of the two aphidophagous ladybirds, *Coccinella septempunctata brucki* Mulsant (Coleoptera: Coccinellidae) and *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae). Applied Entomology and Zoology 41: 161–169.

Tayeh, A., R. A. Hufbauer, A. Estoup, V. Ravigné, L. Frachon, and B. Facon. 2015. Biological invasion and biological control select for different life histories. Nature Communications 6: 1–5.

Tayeh, A., A. Estoup, G. Laugier, A. Loiseau, J. Turgeon, S. Toepfer, and B. Facon. 2012. Evolution in biocontrol strains: insight from the harlequin ladybird *Harmonia axyridis*. Evolutionary Applications 5: 481–488.

Togni, P. H., M. Venzon, C. A. Muniz, E. F. Martins, A. Pallini, and E. R. Sujii. 2016. Mechanisms underlying the innate attraction of an aphidophagous coccinellid to coriander plants: Implications for conservation biological control. Biological Control 92: 77–84.

Tscharntke, T., I. Steffan-Dewenter, A. Kruess, and C. Thies. 2002. Characteristics of insect populations on habitat fragments: A mini review. Ecological Research 17: 229–239.

Tumminello, G., T. A. Ugine, and J. E. Losey. 2015. Intraguild interactions of native and introduced coccinellids: the decline of a flagship species. Environmental Entomology 44: 64–72.

Turgeon, J., A. Tayeh, B. Facon, E. Lombaert, P. De Clercq, N. Berkvens, J. G. Lundgren, and A. Estoup. 2011. Experimental evidence for the phenotypic impact of admixture between wild and biocontrol Asian ladybird (*Harmonia axyridis*) involved in the European invasion. Journal of Evolutionary Biology 24: 1044–1052.

Turnipseed, R., T. A. Ugine, and J. E. Losey. 2015. Egg predation by the introduced lady beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae), lowers mortality but raises relative risk for the native lady beetle, *Coccinella novemnotata*. PLOS ONE 10: e0118493.

Veran, S., S. Piry, V. Ternois, C. N. Meynard, B. Facon, and A. Estoup. 2016. Modeling spatial expansion of invasive alien species: relative contributions of environmental and anthropogenic factors to the spreading of the harlequin ladybird in France. Ecography 39: 665–675.

Wallach, A. D., A. H. Dekker, M. Lurgi, J. M. Montoya, D. A. Fordham, and E. G. Ritchie. 2017. Trophic cascades in 3D: network analysis reveals how apex predators structure ecosystems. Methods in Ecology and Evolution 8: 135–142.

Werf, V. W., E. W. Evans, and J. Powell. 2000. Measuring and modelling the dispersal of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in alfalfa fields. European Journal of Entomology 97: 487–493.

Yasuda, H., E. W. Evans, Y. Kajita, K. Urakawa, and T. Takizawa. 2004. Asymmetric larval interactions between introduced and indigenous ladybirds in North America. Oecologia 141: 722–731.

Zenni, R. D., and M. A. Nunez. 2013. The elephant in the room: the role of failed invasions in understanding invasion biology. Oikos 122: 801–815.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annual Review of Entomology 42: 207–230.

Zerebecki, R. A., and C. J. Sorte. 2011. Temperature tolerance and stress proteins as mechanisms of invasive species success. PLOS ONE 6: e14806.

Zhang, G. F., G. L. Lovei, X. Wu, and F. H. Wan. 2016. Presence of native prey does not divert predation on exotic pests by *Harmonia axyridis* in its indigenous range. PLOS ONE 11(7): e0159048

Zhou, X., A. Honek, W. Powell, and N. Carter. 1995. Variations in body length, weight, fat content and survival in *Coccinella septempunctata* at different hibernation sites. Entomologia Experimentalis et Applicata 75: 99–107.

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION

DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Heshani Manula Edirisinghe
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In which Chapter is the Manuscript /Published work:	Chapter 2

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☐ The percentage of the manuscript/Published Work that was contributed by the candidate:	80%

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HME conducted the literature survey and led the design and writing of the manuscript.	
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Chapter 3

Inter- and intraspecific variation in ladybird morphology

Abstract

1. Only some introduced (i.e. non-native) species become successfully established into new environments. The level of phenotypic variation in a population can influence successful establishment in a new environment because higher variation in functionally important traits increases variation in individual fitness. Higher variability should therefore increase the probability that some individuals in the population will establish. However, studies investigating how variation in functionally important traits affects establishment success are limited.
2. Ladybird beetles (Coleoptera: Coccinellidae) are an excellent model to study establishment success because they are commonly introduced as biocontrol agents against pests of agricultural crops.
3. In this study, we compared levels of inter- and intraspecific variation in body size, wing shape and colour between five native (*Adoxellus flavihirtus, Harmonia antipoda, Hoangus venustus, Rhyzobius consors* and *Veronicobius aucklandiae*) and five introduced (*Halmus chalybeus, Illeis galbula, Rhyzobius forestieri, Epilachna vigintioctopunctata* and *Serangium maculigerum*) ladybird species in New Zealand.
4. We expected higher levels of morphological variation in widely distributed introduced species such as *Halmus chalybeus* than native species. We found that native ladybirds had narrower bodies than introduced ladybirds. Intraspecific variation in size is greater in introduced species compared to native species of ladybird. Further, we found that the level of intraspecific variation in size and colour is greater in a locally widespread introduced species (*H. chalybeus*) compared to other introduced species and native species of ladybirds. We discuss what role intraspecific variation in size and colour may have on the establishment success of introduced species.

Keywords: body size, wing shape, colour variation, principal component analysis, geometric morphometrics

3.1 Introduction

Species are introduced for many reasons: for conservation, for entertainment (i.e. ornamental plants, hunting), and for biocontrol agents in agriculture and aquaculture (e.g., fish, insects and plants) (Berger-Tal *et al.* 2020; Ellender and Weyl 2014; Kundoo and Khan 2017; Novoa *et al.* 2015). In addition, many species are introduced accidentally in plant exports and other cargo. However, there is considerable variation in the establishment success of introduced species: some fail to establish entirely, while other species become invasive.

Phenotypic diversity is expected to influence the establishment success of introduced species (Forsman, 2014). Higher levels of phenotypic diversity in functionally important traits increases the probability some individuals in a population will be pre-adapted to conditions in the new environment which then enables them to successfully establish (Forsman 2014). A functional trait (morphological, physiological or phenological) influences fitness and is measurable at an individual level (Griffiths *et al.* 2016). Comparing the functional traits of native and introduced species will improve our understanding of how phenotypic differences influence establishment success. However, currently there are few comparisons of phenotypic diversity between native and introduced animals, particularly in invertebrates (Forsman and Wennersten 2016; Gamfeldt *et al.* 2005; Hanley *et al.* 2016).

Ladybirds are an excellent system to investigate the factors affecting establishment success because they are often introduced as biocontrol agents or accidentally via plant exports. Furthermore, invasive ladybirds such as *Harmonia axyridis* have become a concern for agricultural and urban management authorities (Kopf *et al.* 2017). In New Zealand, there are about 19 introduced species including *H. axyridis*, and 25 described native species (Szawaryn and Leschen 2019; Maddison 2010). Most native species are dull-coloured and small, apart from the relatively large *Coccinella leonina* (Li *et al.* 2019), *Hoangus venustus* (Szawaryn and Leschen 2019), and *Harmonia antipoda* (Klimaszewski and Watt 1997; Li *et al.* 2019).

Here, we compare the morphology of native and introduced ladybirds in order to resolve how morphological traits influence the successful establishment of introduced species. Morphology (body size, colour and wing shape and wing venation) is associated with fitness related traits such as fecundity (Honěk 1993), predator-avoidance strategies (Briolat *et al.* 2019), dispersal ability (Hosseinzadeh *et al.* 2019; Ospina-Garcés *et al.* 2018) and survival (Sokolovska *et al.* 2000) in insects. For example, adult body size influences sexual selection, the number of eggs laid (Hemptinne *et al.* 2012) and development rates of offspring in the ladybird *Menochilus sexmaculatus* (Ankita and Geetanjali 2016). Body colour is also used in ladybird communication (Bahlai *et al.* 2008). Ladybirds show aposematic (warning) colouration - an association between prey appearance and its unprofitability to predators (Bahlai *et al.* 2008; Llaurens *et al.* 2014; Poulton 1890). The body colour of ladybirds is also associated with reproductive success, intraspecific recognition (Cummings and Crothers 2013) and Batesian mimicry – the mimicry of more toxic species to gain the advantages of prior predator learning (Llaurens *et al.* 2014). Wing polymorphism and wing shape are associated with dispersal, foraging behaviour and reproduction in beetles (Ospina-Garcés *et al.* 2018) including Carabidae, Staphylinidae and Curculionidae (Canovai 2016). While ladybirds are known to have winged and wingless morphs (Lommen 2013), wingless morphs are not as common as in other insect groups (Canovai 2016).

Populations with high levels of morphological variation are more likely to contain some individuals who are pre-adapted to a new environment and therefore better able to survive in the recipient ecosystem (Forsman 2014; Michie *et al.* 2010). For example in bumblebees, higher intraspecific variation in body size is associated with an increase in relative abundance (Austin and Dunlap 2019). However, our understanding of how intraspecific variation affects establishment success is limited. For instance, even though differences in size between ladybird species have been studied extensively (Ankita and Geetanjali 2016; Honěk 1993; Sokolovska *et al.* 2000), few have examined intraspecific variation in size (Bista 2013). Additionally, studies on colouration are mainly focused on underlying mechanisms. For example, in ladybirds, intraspecific variation in body colour is regulated by complex interactions between genetic mechanisms, predator behaviour, multiple selection pressures and environmental conditions during development (i.e. *Harmonia axyridis* Briolat *et al.* (2019)). Studies on wing venation in insects are mainly for the identification and taxonomic assignment of species (Comstock and Needham 1899; Li and Zhang 2017), sibling species (Villemant *et al.* 2007), fossil species (De Meulemeester *et al.* 2012) and the investigation of phylogenetic relationships (Rossa *et al.* 2016). However, Pitchers *et al.* (2013) showed that variation in wing venation is influenced by temperature but regulation of wing venation is correlated with other environmental factors. Therefore, studies of intraspecific variation and comparing the level of variation between native and successfully established introduced species can provide insights into how phenotypic variation influences the establishment success of introduced ladybirds.

In this study, we compared a) body size, colour and shape of the hind wings (via wing venation), and b) levels of intraspecific variation in morphology between native and introduced ladybirds. Having a high level of variation in morphology will enable introduced species to adapt to a wide range of environmental conditions increasing the fitness potential of introduced ladybirds. We predicted that widespread introduced species will have higher levels of intraspecific variation in morphology than native ladybird species.

3.2 Methodology

3.2.1 Ladybird collection

We used both fresh and museum specimens in this study. We collected ladybirds from eight field sites from the greater Auckland region, New Zealand (Appendix B: supplementary figure 3.1) from October 2017 to August 2018. Ladybirds were collected by hand or using beat sheets. We sampled three habitat types (Appendix B: supplementary table 3.1), including scrub, native and exotic vegetation. Most introduced species were found in habitats disturbed by the public (i.e. within botanical gardens, beside walking tracks or in car parks), and most native species were found in relatively pristine habitats (HME, personal observation). Therefore, sampling both disturbed and pristine habitats ensured that we captured the full range of morphological variation present in each species across different habitat types. Sampling sites were randomly picked with ArcMap GIS software (Version 10.4.1, ESRI Inc.) with a minimum distance of 3 km between each site (GPS locations and details of sampling sites are in Appendix B: supplementary table 3.1). As a result, there were unequal numbers of sampling sites for each habitat type because some habitat types have restricted distributions within the Auckland region. Finally, because native ladybirds were not found in sufficient sample sizes (n=2) during collections, we used dried and pinned native specimens from the New Zealand Arthropod Collection (NZAC, Manaaki-Whenua, Auckland).

We measured five native species available in sufficient numbers for the analysis (figure 3.1): a) *Veronicobius aucklandiae* (n=15), b) *Hoangus venustus* (n=11), c) *Harmonia antipoda* (n=10), d) *Adoxellus flavihirtus* (n=15) and e) *Rhyzobius consors* (n=15) randomly selected from the NZAC at Manaaki Whenua. We also measured five introduced ladybird species (figure 3.1): f) *Epilachna vigintioctopunctata* (n=34 present in 1 site) g) *Halmus chalybeus* (n=150 present in 8 sites) h) *Illeis galbula* (n=21 present in 1 field site) i) *Rhyzobius forestieri* (n=35 present in 3 sites) and j) *Serangium maculigerum* (n=59 present in 7 sites) (see Appendix B: supplementary table 3.1 for sampling sites).

The collected ladybirds and museum specimens from NZAC Manaaki Whenua were photographed using a Nikon DS-U3-Ri1 (Nikon Inc.) camera connected to a Leica M205A stereomicroscope. These images were used for both size and colour variation analyses and were photographed under constant standardised settings with a colour standard next to the specimen. We photographed and scored a subset (n=10) of these ladybirds independently twice to assess measurement repeatability. Fresh specimens were then boiled in PBS in a 1.5ml tube (Lommen *et al.* 2009), to facilitate the removal of hind wings for measurement. We removed the right hind wings (to avoid potential effects of asymmetry (Fink 1990)) and washed them with water before mounting. The wings were labelled and photographed using a Canon EOS 700D (Canon Inc., Japan) camera connected to a microscope (SMZ 168, Motic microscopes).

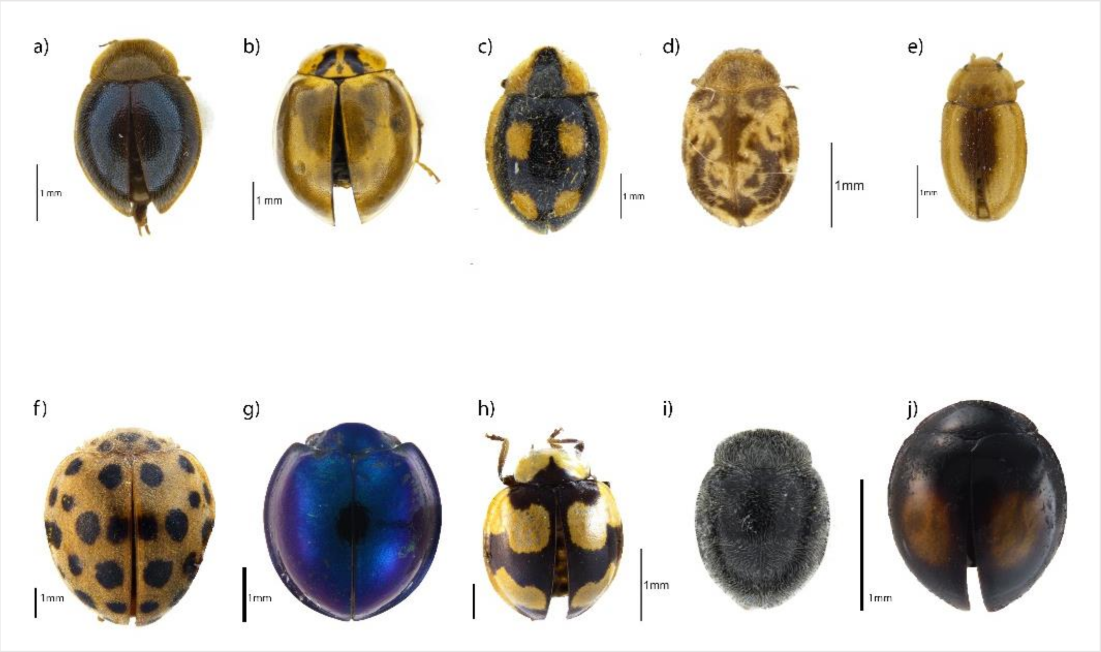

Figure 3.1 Ladybird species used in the study (scale bars indicate 1mm).
Native ladybirds are in the top row (a-e) and introduced ladybirds are in the bottom row (f-j). a) *Adoxellus flavihirtus,* b) *Harmonia antipoda,* c) *Hoangus venustus*, d) *Rhyzobius consors*, e) *Veronicobius aucklandiae*, f) *Epilachna vigintioctopunctata*, g) *Halmus chalybeus*, h) *Illeis galbula,* i) *Rhyzobius forestieri* and j) *Serangium maculigerum.*

3.2.1.1 Measurements

We measured five morphological measurements related to the length and width of the body when the elytra were closed as shown in figure 3.2a (Evans 2000; Seo *et al.* 2008) to the nearest 0.01mm using ImageJ 1.50i (Wayne Rasband, National Institute of Health USA). We selected six landmarks on the right hind wing (figure 3.2b), homologous between all species and which were clear in the images (Abdolahi Mesbah *et al.* 2015). We selected landmarks previously used by Bai *et al.* (2011), Bai *et al.* (2012), Ren *et al.* (2017) and Abdolahi Mesbah *et al.* (2015). Landmarks were defined as: 1- fork of radius anterior and media posterior 1+2 (RA and MP_{1+2}) veins (Ren *et al.* 2017), 2- fork of radius anterior and radius anterior 3+4 (RA and RA_{3+4}) veins (Abdolahi Mesbah *et al.* 2015; Bai *et al.* 2012), 3- distal corner of radial pigmentation (Abdolahi Mesbah *et al.* 2015), 4- fork of media posterior 1+2 and media posterior 1 (MP_{1+2} and MP_1) veins (Abdolahi Mesbah *et al.* 2015), 5- end of MP_1 vein (Abdolahi Mesbah *et al.* 2015) and 6- end of radius posterior 1 (RP_1) vein (Bai *et al.* 2011).

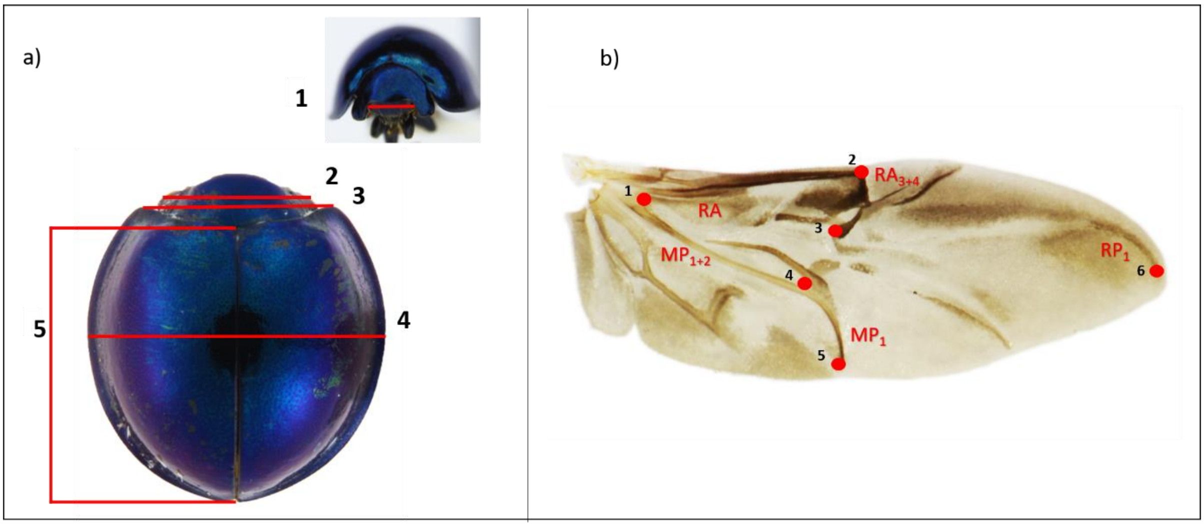

Figure 3.2 Morphometric measurements of ladybirds.
a) body size of ladybirds (example using *Halmus chalybeus*), 1 –width of the head, 2- width of the pronotum, 3- distance of the anterior edge of the elytra while the elytra are closed, 4- width of the elytra and 5- length of the elytral suture when elytra are closed; b) landmark locations to measure the shape of right hind wing (example using *H. chalybeus* wing), landmarks 1- fork of RA and MP$_{1+2}$ veins, 2- fork of RA and RA$_{3+4}$ veins, 3- distal corner of radial pigmentation, 4- fork of MP$_{1+2}$ and MP$_1$ veins, 5- end of MP$_1$ vein and 6- end of RP$_1$ vein indicated in red dots. Terminology follows (Kukalová-Peck and Lawrence 1993).

To score colour, we selected four colour patches on the elytra of the ladybird species (illustration in Appendix B: supplementary figure 3.2) to assess the RGB (Red, Green and Blue) scores of the colours using the methods of Valcu and Dale, 2014. Ladybirds had either one (*H. chalybeus* – blue, *R. forestieri* – black, *V. aucklandiae* – brown) or two colour patches (*I. galbula, H. antipoda, S. maculigerum, E. vigintioctopunctata* and *R. consors* – black and yellow). The patches were selected so that each colour was scored at least twice. The RGB scores were then averaged to obtain the mean RGB score for each colour patch using the 'CZdefine' function in the 'colorZapper' package (Valcu and Dale 2014). To obtain a final RGB score for each colour for every individual we then calculated the mean RGB scores for similar colour patches. The colour standard which was photographed side by side with the specimen included the most common colours in the ladybird elytra (black, white, red, yellow, blue and green) and was also scored using the 'colorZapper' package. Although ladybirds use the UV-visible light spectrum in their visual system, similar to other insects and birds, we used an RGB scoring system to assess variability. However, the RGB scoring system strongly correlates with the variation explained when a UV-visible light spectrum system is used to assess colour variation (Dale *et al.* 2015), and we assume here that variability measured in RGB would correlate to colour variation measured that included the UV component.

3.2.2 Statistical analyses

3.2.2.1 Morphometrics

The body size parameters are highly correlated. Therefore, we reduced the number of variables to orthogonal axes using principal component analysis (PCA). The principal components extracted provide a generalised representation of size variation. We used a Non-linear Iterative Partial Least Squares (NIPALS) algorithm to generate the principal components (PCs) as it allows missing data (interocular distance and width of the head of some ladybirds could not be measured). We used the package 'ade4' (Dray et al. 2017) in R statistical software (v3.4, R core Team, 2017) for the analyses.

Wing shape was analysed using geometric morphometric analysis of the right hind wings. Wing shape analysis only contained data for fresh specimens (the five introduced species - *H. chalybeus*, *R. foresteiri*, *I. galbula, S. maculigerum and E. vigintioctopunctata*) as we were not able to photograph the hind wings of native specimens without damaging museum specimens. Landmarks for geometric morphometric analysis were selected using the 'digitize2d' function in the package 'geomorph' (Adams *et al.* 2014). We created wing principal components using landmark coordinates (Lawing and Polly 2010). These principal components retain information about the shape of the hind wing, but are uncorrelated with one another and have appropriate degrees of freedom (Lawing and Polly 2010; Rohlf and Marcus 1993).

3.2.2.2 Repeatability

To measure repeatability, we sampled a subset of individuals (n = 10) and scored individuals three times. using the methods described above for both size and colour measurements. The repeatability scores (intraclass correlation coefficients, ICC) for size parameters included repeated measures of the width of the head, width of the pronotum, distance between elytra points, length of the elytra and width of the body. Repeatability scores for wing shape analysis included repeated measures of the six landmarks using a subset (n=10) of individuals taken at different times and by different scorers.

Repeatability scores for colour measurements were calculated by taking repeated measures of the colour standards for the same individual (two repeated measures of each colour) and different individuals ($n \leq 5$) to test whether the light settings were standardised. Further, we took repeated measurements of each of the colour patches (two measures) of the same individual to measure the effects of a) placement of the specimen and b) selecting the polygon for the colour patch. We estimated the intraclass correlation coefficient for size, shape and colour scores using the 'ICCest' function in the package 'ICC' (Wolak and Wolak 2015).

3.2.2.3 Colour variation between fresh and museum specimens

Museum specimens had mainly yellow and black colours due to the species selected. Therefore, we carried out a comparison of yellow and black colours between fresh and museum specimens to evaluate whether the museum specimens can be used to compare the level of variation between species. We randomly selected a subset of 10 individuals of *I. galbula* from both fresh and museum specimens and compared their variation in RGB scores between populations using a one-way Anova, using the package 'mass' (Ripley *et al.* 2013).

3.2.2.4 Variation in body size and wing shape

We fitted linear and linear mixed models for size and wing shape using species as a fixed effect and collecting site and specimen identity as random effects. We selected the best fitting models based on the lowest AIC values. We then calculated the interquartile distances of body size measurements and compared the level of variation between species based on their origin (native or introduced) to assess whether introduced ladybirds have higher levels of variation than native ladybirds. We used Fisher's F test using the 'var.test()' function in R (Pace, 2012) to compare size variation between native and introduced ladybirds.

3.2.2.5 Variation in colour

We calculated the mean RGB scores for each colour on each individual and plotted the distribution of these scores in 3D plots. We then calculated for each species the patch specific centroid of the distribution of each colour. We calculated the distance between each point (individual RGB score) to the patch specific centroid which estimates the level of colour variation within the species (Appendix B: Supplementary figure 3.3) (Delhey and Peters 2008; Delhey *et al.* 2017). We then fitted linear and generalised linear models for colour variation (distance to patch specific centroid) using species and colour as fixed effects and individual identity as a random effect. We selected the best fitting models based on the lowest AIC values.

3.3 Results

3.3.1 Repeatability

Size measurements (ICC ≥ 0.96; table 3.1) and coordinates for the wing shape measurements (ICC ≥ 0.99; table 3.2) were highly repeatable. Repeatability of the colour patches (table 3.3) and colour standards were calculated using mean RGB values. The values of the colour standard were highly repeatable (ICC for R: 0.99, G: 0.98, B:0.99). Scores for RGB of colour patches on elytra were highly repeatable except for the black of *I. galbula,* and yellow and black of *S. maculigerum* (ICCs < 0.90). Therefore, the black of *I. galbula* and black and yellow of *S. maculigerum* were excluded from later colour analysis.

Table 3.1 ICC repeatability results from ladybird size parameters

Measurement	ICC	LowerCI	UpperCI	N	k	varw	vara
Width of the pronotum	0.96	0.85	0.99	6.00	3.00	0.00	0.01
Points between elytra	1.00	0.99	1.00	10.00	3.00	0.00	0.23
Width of the head	0.97	0.93	0.99	10.00	3.00	0.00	0.02
Width of the elytra	1.00	1.00	1.00	10.00	3.00	0.00	0.78
Length of the elytra	1.00	1.00	1.00	10.00	3.00	0.00	0.37

Abbreviations stand for: ICC - intraclass correlation coefficients, LowerCI – lower confidence intervals, UpperCI – upper confidence intervals, N- number of samples, k- the number of measurements per individual, varw- the within individual variance, vara- the among individual variance

Table 3.2 ICC repeatability results from ladybird wing landmark coordinates

Measurement	ICC	LowerCI	UpperCI	N	k	varw	vara
Coordinate x	1.00	1.00	1.00	60	2.00	493	1.16E+06
Coordinate y	1.00	0.99	1.00	60	2.00	1.34E+03	3.38E+05

Abbreviations stand for: ICC - intraclass correlation coefficients, LowerCI – lower confidence intervals, UpperCI – upper confidence intervals, N- number of samples, k- the number of measurements per individual, varw- the within individual variance, vara- the among individual variance

Table 3.3 ICC repeatability results for the colour patches

Species	Patch	Colour	Red	Green	Blue
Illeis galbula	Left	Yellow	0.92	0.88	0.98
	Right	Yellow	0.91	0.92	0.97
	Front	**Black**	**0.45**	**0.07**	**0.00**
	Back	Black	0.97	0.90	0.82
Halmus chalybeus	Left	Blue	0.99	0.98	0.81
	Right	Blue	0.92	0.98	0.75
	Front	Blue	0.96	0.98	0.77
	Back	Blue	0.98	0.97	0.86
Serangium maculigerum	**Left**	**Yellow**	**-0.08**	**-0.26**	**0.30**
	Right	**Yellow**	**0.87**	**0.75**	**0.27**
	Front	**Black**	**-0.06**	**-0.68**	**-0.90**
	Back	**Black**	**0.68**	**-0.29**	**-0.89**
Epilachna vigintioctopunctata	Left	Yellow	0.89	0.85	0.74
	Right	Yellow	0.96	0.92	0.67
	Front	Black	0.74	0.78	0.72
	Back	Black	0.98	0.98	0.99

Left, right, front and back refer to the colour patches on the elytra as indicated in Appendix B: Supplementary figure 3.2. Colour patches and species with ICC values lower than the threshold (0.60) for at least one of RGB scores were not used in the later analyses and are indicated in bold.

3.3.2 Colour variation between fresh and museum specimens

We compared colour variation between museum specimens (n=6) and a subset (n=10) of fresh specimens of *I. galbula* species by comparing the log distance between individual RGB colour points in a 3D system to its patch specific centroid. Museum specimens had a higher level of variation in black than fresh specimens ($F_{1,15} = 13.9$, $p < 0.01$). Therefore, black cannot be compared between fresh and museum specimens. However, there was no difference in variation in yellow colour between museum and fresh specimens ($F_{1,15} = 3.9$, $p = 0.07$).

3.3.3 Variation in size

We extracted two principal components from the size NIPALS PCA analysis (table 3.4). All five size parameters contributed to the first principal component (PC1), which explained 98.72% of the total variance (eigenvalue = 4.89) and width of the elytra and head contributed to the second principal component (PC2) which explained 1.27% of the total variance (eigenvalue = 0.06). Together, PC1 and PC2 explained 99.99% of the total variance.

Table 3.4 Loading scores, eigenvalues and total variance explained from a NIPALS principal component analysis of size parameters.

Measurement	PC1	PC2
Width of the pronotum	0.45	0.38
Distance between elytra points	0.45	-0.01
Width of the elytra	0.44	-0.78
Length of the elytra	0.45	-0.05
Width of the head	0.45	0.49
Eigen value	**4.89**	**0.06**
Total variance explained	**98.72%**	**1.27%**

Loadings >0.4 were regarded as contributing to the principal component

The best fitting model to explain PC1, with the lowest AIC (487.92) included species identity as a fixed effect and collection site as a random effect. Native *A. flavihirtus* were larger than native *R. consors* and introduced *S. maculigerum,* and smaller than the introduced species *E. vigintioctopunctata, H. chalybeus, I. galbula* and native species *H. antipoda, H. venustus* and *V. aucklandiea,* except *R. forestieri*. (figure 3.3, table 3.5).

The best fitting model to explain PC2, with the lowest AIC (-171.23) included species identity as a fixed effect and collection site as a random effect. *Adoxellus flavihirtus* were narrower (lower mean width of the body) than *E. vigintioctopunctata, H. chalybeus, I. galbula, R. consors, R. forestieri* and *S. maculigerum* (figure 3.3, table 3.6).

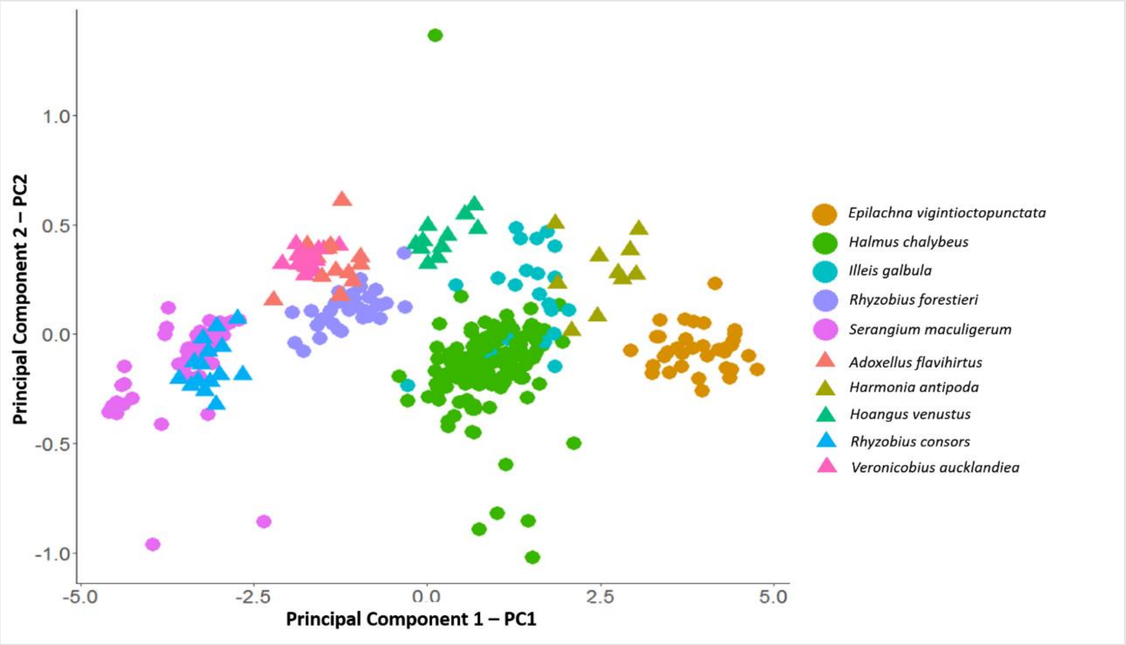

Figure 3.3 Distribution of ladybird species along size principal component 1 (PC1) and size principal component 2 (PC2)
Circles = introduced species, triangles = native species

Table 3.5 Results of generalised linear model comparing PC1 between native and introduced ladybirds

	Estimate	Std. Error	df	t value	p
(Intercept)	-1.39	0.18	7.01	-7.53	0.00
Epilachna vigintioctopunctata	**5.30**	**0.25**	**5.60**	**21.49**	**0.00**
Harmonia antipoda	**3.92**	**0.18**	**346.59**	**21.84**	**0.00**
Halmus chalybeus	**2.29**	**0.20**	**7.06**	**11.72**	**0.00**
Hoangus venustus	**1.60**	**0.17**	**346.59**	**9.17**	**0.00**
Illeis galbula	**2.76**	**0.22**	**11.11**	**12.32**	**0.00**
Rhyzobius consors	**-1.75**	**0.16**	**346.59**	**-10.91**	**0.00**
Rhyzobius forestieri	0.35	0.21	8.85	1.66	0.13
Serangium maculigerum	**-2.03**	**0.20**	**7.79**	**-10.10**	**0.00**
Veronicobius aucklandiea	**-0.33**	**0.16**	**346.59**	**-2.05**	**0.04**

Species comparisons were made with *Adoxellus flavihirtus.* Significant effects are indicated in bold.

Table 3.6 Results of generalised linear model comparing PC2 between native and introduced ladybirds

	Estimate	Std. Error	df	t value	p
(Intercept)	0.32	0.05	152.35	6.56	0.00
Epilachna vigintioctopunctata	**-0.39**	**0.07**	**24.52**	**-5.61**	**0.00**
Harmonia antipoda	-0.04	0.08	190.45	-0.47	0.64
Halmus chalybeus	**-0.47**	**0.05**	**91.87**	**-8.78**	**0.00**
Hoangus venustus	0.12	0.08	60.46	1.50	0.14
Illeis galbula	**-0.16**	**0.07**	**116.03**	**-2.36**	**0.02**
Rhyzobius consors	**-0.46**	**0.07**	**73.73**	**-6.36**	**0.00**
Rhyzobius forestieri	**-0.18**	**0.06**	**118.63**	**-3.00**	**0.00**
Serangium maculigerum	**-0.45**	**0.06**	**107.18**	**-7.99**	**0.00**
Veronicobius aucklandiea	0.03	0.07	142.27	0.45	0.66

Species comparisons were made with *Adoxellus flavihrtus.* Significant effects are indicated in bold.

We then compared the interquartile distances of the size PCs between native and introduced species to compare differences in intraspecific variation between native and introduced ladybirds. We found that introduced ladybirds have a higher level of variation in size than native ladybirds ($F_{1,4}$ = 36.67, p-value = 0.004).

3.3.4 Variation in wing shape

We extracted two principal components that described the shape of the right hind wing of the ladybirds using the six landmarks from geometric morphometric analysis (figure 3.2b and figure 3.4). The first principal component for wing shape (WPC1) explained 65.34% of the total variance and an increase in WPC1 reflects a decrease in wing breadth (decrease in the length of RP_1 vein) as landmark 6 moves towards the R-M loop (figure 3.4). Further, an increase in WPC1 (figure 3.4) suggests the fork of MP_{1+2} and MP_1 veins (landmark 4, figure 3.2b) becoming closer to the fork of RA and MP_{1+2} veins (landmark 1, figure 3.2b) than the distal corner of radial pigmentation (landmark 3, figure 3.2b). The second principal component for wing shape (WPC2) explained 12.13% of the total variance and an increase in WPC2 reflects (figure 3.4) the distal corner of radial pigmentation (landmark 3, figure 3.2b) and the fork of MP_{1+2} and MP_1 veins (landmark 4, figure 3.2b) moving proximally towards the base of the hind wing. Further, an increase in WPC2 (figure 3.4) also suggests the fork of MP_{1+2} and MP_1 veins (landmark 4, figure 3.2b) moving closer to the fork of RA and MP_{1+2} veins (landmark 1, figure 3.2b) and moving away from the end of MP_1 vein (landmark 5, figure 3.2b), creating a longer MP_1 vein in the medial region (figure 3.2b). Together, WPC1 and WPC2 explained 77.47% of the total variance in wing shape.

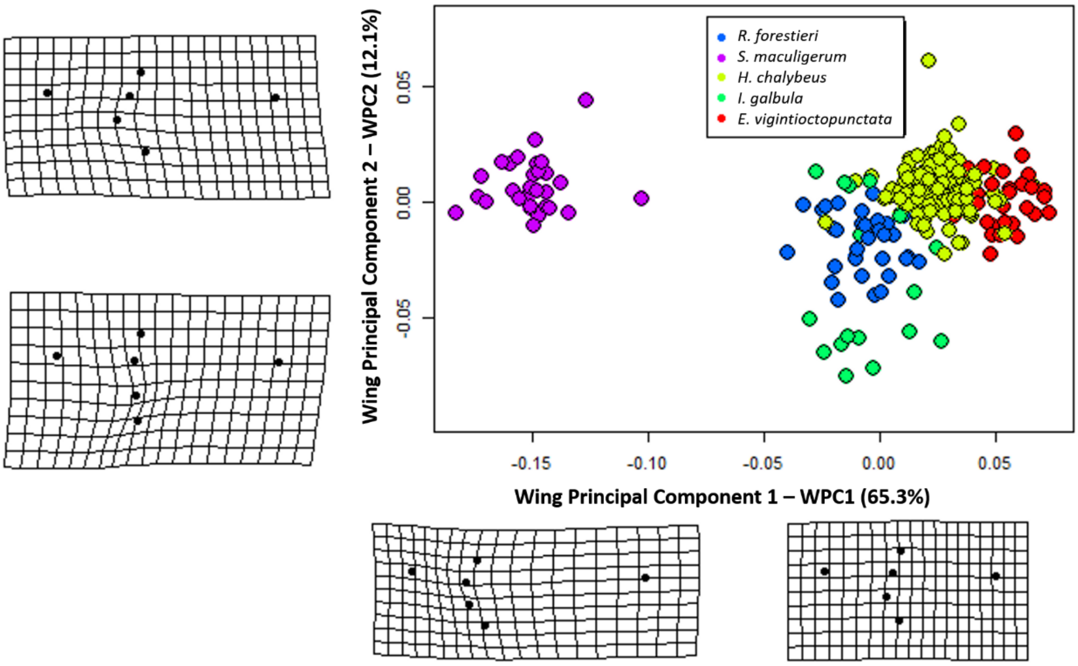

Figure 3.4 Distribution of ladybirds along geometric morphometric components WPC1 and WPC2.
Shape changes associated with WPC1 and WPC2 are indicated in the figures along the axes respectively (WPC1, WPC2 – wing principal components 1 and 2 respectively)

The best fitting model to explain WPC1 with the lowest AIC (-1322.70) included species identity as a fixed effect and collection site as a random effect. *Epilachna vigintioctopunctata* has the largest wing breadth, followed by *H. chalybeus. Illeis galbula* and *R. forestieri* have a larger wing breadth than *S. maculigerum* but smaller breadth than *H. chalybeus.* (p value < 0.01, table 3.7; figure 3.4).

Table 3.7 Results of generalised linear model comparing WPC1 between introduced ladybirds

	Estimate	Std. Error	df	t value	P
(Intercept)	0.05	0.00	233.03	22.42	0.00
Halmus chalybeus	**-0.03**	**0.00**	**233.03**	**-10.78**	**0.00**
Illeis galbula	**-0.06**	**0.00**	**233.03**	**-15.09**	**0.00**
Rhyzobius forestieri	**-0.06**	**0.00**	**233.03**	**-18.31**	**0.00**
Serangium maculigerum	**-0.20**	**0.00**	**233.03**	**-59.98**	**0.00**

Species comparisons were made with *Epilachna vigintioctopunctata.* Significant effects are indicated in bold.

The best fitting model to explain WPC2 with the lowest AIC (-1277.40) included species identity as a fixed effect and collection site as a random effect. *Epilachna vigintioctopunctata* has a longer MP_1 vein of the hind wings than *I. galbula* and *R. forestieri* (p value < 0.01, table 3.8; figure 3.4).

Table 3.8 Results of generalised linear model comparing WPC2 between introduced ladybirds

	Estimate	Std. Error	df	t value	p
(Intercept)	0.00	0.00	5.57	0.25	0.81
Halmus chalybeus	0.01	0.00	6.01	1.49	0.19
Illeis galbula	**-0.03**	**0.01**	**12.76**	**-5.99**	**0.00**
Rhyzobius forestieri	**-0.02**	**0.00**	**9.91**	**-4.34**	**0.00**
Serangium maculigerum	0.01	0.00	10.42	1.23	0.25

Species comparisons were made with *Epilachna vigintioctopunctata.* Significant effects are indicated in bold.

3.3.5 Variation in colour

The best fitting linear mixed effects model to explain colour variation (measured as the distance to the patch-specific centroid) included species identity and colour as fixed effects and individual identity as a random effect (AIC -738.3). *H. chalybeus* (blue) has a higher level of colour variation compared to the other ladybird species (p value < 0.01, table 3.9). Further, the black of *E. vigintioctopunctata* has a lower level of variation than the brown (p value = 0.04, table 3.9) and yellow colours across all species (p value < 0.01, table 3.9).

Table 3.9 Results of generalised linear model for colour between native and introduced ladybirds

	Estimate	Std. Error	df	t value	p
(Intercept)	0.04	0.02	181.80	2.07	0.04
Harmonia antipoda	0.03	0.02	183.10	1.24	0.22
Epilachna vigintioctopunctata	0.01	0.02	243.40	0.50	0.62
Halmus chalybeus	**0.07**	**0.02**	**195.80**	**3.12**	**0.00**
Hoangus venustus	-0.01	0.02	238.10	-0.30	0.77
Illeis galbula	0.00	0.02	249.90	0.09	0.93
Veronicobius aucklandiea	0.00	0.02	197.30	0.03	0.97
Colour: Brown	**0.03**	**0.01**	**64.66**	**2.06**	**0.04**
Colour: Yellow	**0.03**	**0.01**	**64.66**	**3.36**	**0.00**

Species comparisons were made with *Adoxellus flavihirtus* as the reference species and black as the reference colour. Significant effects are indicated in bold.

3.4 Discussion

We found that body size in New Zealand ladybirds varied considerably between and within species. Native *A. flavihirtus,* had a narrower body than the introduced species (*E. vigintioctopunctata, H. chalybeus, I. galbula, R. forestieri* and *S. maculigerum*). However, there was a significant difference between the size of native species as well, with *A. flavihirtus* being larger than *R. consors.* We also found that introduced ladybirds showed an overall higher level of intraspecific variation in size than native ladybirds. We couldn't compare the level of inter- and intraspecific variation in wing shape between native and introduced species as we could only measure introduced species. However, we found out that *E. vigintioctopunctata* had significantly longer MP_1 veins than *I. galbula* and *R. forestieri.* Finally, as predicted we found that the widely distributed *H. chalybeus* had a higher level of variation in colour than all the other species (although note *S. maculigerum* was excluded from this analysis). The level of variation in blue was higher than the other colours, and yellow and brown showed a higher level of variation than black. Overall, the level of morphological variation is higher in a widespread introduced ladybird (*H. chalybeus*) than less extensively distributed introduced (i.e*. I. galbula*, *E. vigintioctopunctata*) and native ladybirds (i.e*. A. flavihirtus*, *H. antipoda, V. aucklandiea, H. venustus*).

Having a high level of variation in body size may increase the prospects for establishment success of introduced ladybirds. Body size of ladybirds is correlated with the body size and mobility of their prey (Dixon and Hemptinne 2001), and the mean size differences observed between ladybird species may depend on their diet. For example, small ladybirds feed on mites and large ladybirds feed on caterpillars (Dixon and Hemptinne 2001). Therefore, the comparatively larger width (broad body sizes) of introduced ladybirds (i.e. *H. chalybeus*) may have influenced successful establishment by allowing them to exploit prey that cannot be accessed by native ladybirds due to size differences. Therefore, having a higher level of variation in body size may similarly increase the ability of introduced species (i.e. *H. chalybeus*) to disperse and establish across different habitats compared to less variable native and introduced ladybird populations. These patterns are similar to patterns of higher levels of intraspecific size variation in bumblebee species being associated with increased relative abundance in North America (Austin and Dunlap 2019).

A high level of colour variation may also influence the establishment of introduced ladybirds by increasing anti-predator defenses (Michie *et al.* 2010; Wheeler *et al.* 2015; Winters *et al.* 2014). Ladybirds show functional colouration such as aposematism for predator avoidance (Wheeler *et al.* 2015; Winters *et al.* 2014) via bright red and yellow morphs (as in *E. vigintioctopunctata, I. galbula, H. antipoda* and *H. venustus*). Recent studies on colouration in ladybirds show that carotenoid based colouration and melanism are both regulated by the ‘pannier’ gene and pigmentation genes, however the diversity of colour patterns are also regulated by environmental factors such as temperature and humidity during wing development (Ando and Niimi 2019). The level of interspecific variation in aposematic colouration is closely associated with their predators’ visual systems and memory (Arenas and Stevens 2017). Variation in redness does not reflect alkaloid content or toxicity – instead it is natural variation in aposematic signalling (Bezzerides *et al.* 2007). Therefore, aposematic colour morphs (such as *E. vigintioctopunctata, I.*

galbula, H. antipoda and *H. venustus*) may not be better chemically defended than other morphs.

We still have very little understanding of how effective ladybird visual communication is to their predators (Briolat *et al.* 2019). Increased visibility may increase the effectiveness of aposematic colouration (Arenas and Stevens 2017; Briolat *et al.* 2019). Therefore, having a higher level of variation in yellow (and red) colours may allow these species to contrast themselves visually from the natural environment increasing the effectiveness of aposematic colouration (i.e. *E. vigintioctopunctata, I. galbula, H. antipoda* and *H. venustus*) (Arenas and Stevens 2017). In our study, *Halmus chalybeus* (blue) shows higher levels of colour variation compared to all the other species (yellow, brown and black) and so far, this is the only study that has looked at iridescent colour variation in ladybirds. However, in morpho butterflies, iridescent blue in wings is associated with species recognition (Kinoshita *et al.* 2002) and in *Polyommatus icarus* butterflies it is predicted that structural blue colouration on the dorsal surface of their wings plays a role in cryptic predator avoidance rather than sexual communication (Kertész *et al.* 2019). This may suggest that cryptic colouration may be more effective than aposematic colouration to avoid predators. This could explain the higher level of colour variation and wider distribution of *H. chalybeus* in the Auckland region compared to the other species studied here. However, this hypothesis should be tested experimentally.

Melanism (black coloration) is also associated with fitness in ladybirds (i.e. *H. axyridis* - thermoregulation) (Michie *et al.* 2010) and melanism is influenced by temperature (Michie *et al.* 2010) and photoperiod (Yin *et al.* 2016). Therefore, having a higher level of variation in melanism may indicate increased variation in fitness within a population. However, the level of variation observed in black was lower than that seen in brown, yellow or blue colours. This may suggest that predator avoidance strategies may influence establishment and distribution of introduced ladybirds more than adaptations for thermoregulation (Honek *et al.* 2005; Kawakami *et al.* 2015; Michie *et al.* 2010). Again, however, it is important to conduct experimental studies that compare melanism, aposematic and cryptic colouration of ladybirds and their establishment success.

Hind wing features are associated with dispersal (Abdolahi Mesbah *et al.* 2015), migration (Johansson *et al.* 2009) and foraging behaviour (Abdolahi Mesbah *et al.* 2015). The proximal and distal parts of the hind wings function as separate functional modules at the R-M loop and evolution of the R-M loop influences the efficiency of wing folding in beetles (Bai *et al.* 2011; Ren *et al.* 2017). In our study, we observed that landmarks 2, 3 and 4 which lie along the R-M loop moved proximally in *E. vigintioctopunctata, H. chalybeus* and *S. maculigerum* compared to *I. galbula* and *R. foresteiri* producing a longer MP_1 vein in the medial region. Considering the wide distribution of *H. chalybeus* and *S. maculigerum* and limited distribution of *E. vigintioctopunctata* in the Auckland region, these differences in hind wing shape may have affected successful establishment into their current introduced areas by influencing their search rate for prey or anti-predator behaviour.

Inter- and intraspecific variation increases both establishment success and range expansions (Forsman 2014; González-Suárez *et al.* 2015). However, studies investigating intraspecific variation in animals are still limited, particularly in ladybirds (Gaudard *et al.* 2019; Griffiths *et al.* 2016). In this study, we observed that *H. chalybeus* has higher levels of size and colour variation compared to all the other introduced and native ladybirds measured. This may explain the wide distribution of *H. chalybeus* within the Auckland region compared to the other species studied. However, morphological variation is also influenced by environmental characteristics such as temperature, humidity and photoperiod (Dixon and Hemptinne 2001; Lommen *et al.* 2005; Perrard *et al.* 2014; Pope 1977; Reznik *et al.* 2015). Assessment of variation within and between introduced ladybird species was limited in our study as our sampling was restricted to the Auckland region. We suggest that future work quantifying intraspecific variation in ladybirds across geographical gradients will improve our understanding of a) how pre-existing morphological variation allows ladybirds to disperse widely, and b) how different habitats induce greater variation in established ladybirds.

3.5 Acknowledgments

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3.6 References

Abdolahi Mesbah, R., J. Nozari, and S. Dadgostar. 2015. A geometric morphometric study on geographical populations of *Hippodamia variegata* (Goeze, 1777)(Coleoptera: Coccinellidae) in some parts of Iran. Journal of Crop Protection 4: 207-215.

Adams, D., M. Collyer, E. Otarola-Castillo, E. Sherratt, and M. D. Adams. 2014. Package 'geomorph'. Software for geometric morphometric analysis. R package version 3.0.5. 2017. Available from: https://cran.r-project.org/web/packages/geomorph/index.html.

Ando, T., and T. Niimi. 2019. Development and evolution of color patterns in ladybird beetles: A case study in *Harmonia axyridis*. Development, Growth & Differentiation 61: 73-84.

Ankita, D., and M. Geetanjali. 2016. Adult body size drives sexual selection mutually in the ladybird, *Menochilus sexmaculatus* (Coleoptera: Coccinellidae). Acta Entomologica Sinica 59: 209-218.

Arenas, L. M., and M. Stevens. 2017. Diversity in warning coloration is easily recognized by avian predators. Journal of Evolutionary Biology 30: 1288-1302.

Austin, M. W., and A. S. Dunlap. 2019. Intraspecific variation in worker body size makes North American bumble bees (*Bombus* spp.) less susceptible to decline. The American Naturalist 194: 381-394.

Bahlai, C., J. Welsman, E. Macleod, A. Schaafsma, R. Hallett, and M. Sears. 2008. Role of visual and olfactory cues from agricultural hedgerows in the orientation behavior of multicolored Asian lady beetle (Coleoptera: Coccinellidae). Environmental Entomology 37: 973-979.

Bai, M., E. McCullough, K.-Q. Song, W.-G. Liu, and X.-K. Yang. 2011. Evolutionary constraints in hind wing shape in Chinese dung beetles (Coleoptera: Scarabaeinae). PLOS ONE 6: e21600.

Bai, M., R. G. Beutel, K.-Q. Song, W.-G. Liu, H. Malqin, S. Li, X.-Y. Hu, and X.-K. Yang. 2012. Evolutionary patterns of hind wing morphology in dung beetles (Coleoptera: Scarabaeinae). Arthropod Structure & Development 41: 505-513.

Berger-Tal, O., D. Blumstein, and R. Swaisgood. 2020. Conservation translocations: a review of common difficulties and promising directions. Animal Conservation 23: 121-131.

Bezzerides, A. L., K. J. McGraw, R. S. Parker, and J. Husseini. 2007. Elytra color as a signal of chemical defense in the Asian ladybird beetle *Harmonia axyridis*. Behavioral Ecology and Sociobiology 61: 1401-1408.

Bista, M. 2013. Effects of body size and prey quality on the reproductive attributes of two aphidophagous Coccinellidae (Coleoptera) species. The Canadian Entomologist 145: 566-576.

Briolat, E. S., E. R. Burdfield-Steel, S. C. Paul, K. H. Rönkä, B. M. Seymoure, T. Stankowich, and A. M. M. Stuckert. 2019. Diversity in warning coloration: selective paradox or the norm? Biological Reviews 94: 388-414.

Canovai, R. 2016. Wing dimorphism in Tuscan populations of *Rhyzobius Litura* (Fabricius) (Coleoptera Coccinellidae). Redia-Giornale Di Zoologia 99: 19-24.

Comstock, J. H., and J. G. Needham. 1899. The wings of insects. The American Naturalist 33: 845-860.

Cummings, M. E., and L. R. Crothers. 2013. Interacting selection diversifies warning signals in a polytypic frog: an examination with the strawberry poison frog. Evolutionary Ecology 27: 693-710.

Dale, J., C. J. Dey, K. Delhey, B. Kempenaers, and M. Valcu. 2015. The effects of life history and sexual selection on male and female plumage colouration. Nature 527: 367-370.

De Meulemeester, T., D. Michez, A. M. Aytekin, and B. N. Danforth. 2012. Taxonomic affinity of halictid bee fossils (Hymenoptera: Anthophila) based on geometric morphometrics analyses of wing shape. Journal of Systematic Palaeontology 10: 755-764.

Delhey, K., and A. Peters. 2008. Quantifying variability of avian colours: are signalling traits more variable? PLOS ONE 3: e1689.

Delhey, K., B. Szecsenyi, S. Nakagawa, and A. Peters. 2017. Conspicuous plumage colours are highly variable. Proceedings of the Royal Society B: Biological Sciences 284: 20162593.

Dixon, A., and J.-L. Hemptinne. 2001. Body size distribution in predatory ladybird beetles reflects that of their prey. Ecology 82: 1847-1856.

Ellender, B. R., and O. L. Weyl. 2014. A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. Aquatic Invasions 9(2): 117-132.

Evans, E. W. 2000. Morphology of invasion: body size patterns associated with establishment of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in western North America. European Journal of Entomology 97: 469-474.

Fink, W. L. 1990. Data acquisition for morphometric analysis in systematic biology. Proceedings of the Michigan Morphometrics Workshop: 9-19.

Forsman, A. 2014. Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. Proceedings of the National Academy of Sciences 111: 302-307.

Forsman, A., and L. Wennersten. 2016. Inter-individual variation promotes ecological success of populations and species: Evidence from experimental and comparative studies. Ecography 39: 630-648.

Gamfeldt, L., J. Wallén, P. R. Jonsson, K. M. Berntsson, and J. N. Havenhand. 2005. Increasing intraspecific diversity enhances settling success in a marine invertebrate. Ecology 86: 3219-3224.

Gaudard, C. A., M. P. Robertson, and T. R. Bishop. 2019. Low levels of intraspecific trait variation in a keystone invertebrate group. Oecologia 190: 725-735.

González-Suárez, M., S. Bacher, and J. M. Jeschke. 2015. Intraspecific trait variation is correlated with establishment success of alien mammals. The American Naturalist 185: 737-746.

Griffiths, H. M., J. Louzada, R. D. Bardgett, and J. Barlow. 2016. Assessing the importance of intraspecific variability in dung beetle functional traits. PLOS ONE 11: e0145598.

Hanley, T. C., A. R. Hughes, B. Williams, H. Garland, and D. L. Kimbro. 2016. Effects of intraspecific diversity on survivorship, growth, and recruitment of the eastern oyster across sites. Ecology 97: 1518-1529.

Hemptinne, J.-L., A. Magro, E. Evans, and A. Dixon. 2012. Body size and the rate of spread of invasive ladybird beetles in North America. Biological Invasions 14: 595-605.

Honek, A., Z. K. Martinkova, and S. Pekar. 2005. Temporal stability of morph frequency in central European populations of *Adalia bipunctata* and *A. decempunctata* (Coleoptera : Coccinellidae). European Journal of Entomology 102: 437-442.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects: A general relationship. Oikos 66: 483-492.

Hosseinzadeh, S., J. Ramsey, M. Mann, L. Bennett, W. B. Hunter, M. Shams-Bakhsh, D. G. Hall, and M. Heck. 2019. Color morphology of *Diaphorina citri* influences interactions with its bacterial endosymbionts and '*Candidatus Liberibacter asiaticus*'. PLOS ONE 14: e0216599.

Johansson, F., M. Söderquist, and F. Bokma. 2009. Insect wing shape evolution: independent effects of migratory and mate guarding flight on dragonfly wings. Biological Journal of the Linnean Society 97: 362-372.

Kawakami, Y., K. Yamazaki, and K. Ohashi. 2015. Increase in dark morphs and decrease in size during a range extension of *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae). European Journal of Entomology 112.

Kertész, K., G. Piszter, Z. Bálint, and L. P. Biró. 2019. Biogeographical patterns in the structural blue of male *Polyommatus icarus* butterflies. Scientific Reports 9: 2338.

Kinoshita, S., S. Yoshioka, Y. Fujii, and N. Okamoto. 2002. Photophysics of structural color in the Morpho butterflies. Forma-Tokyo- 17: 103-121.

Klimaszewski, J., and J. C. Watt. 1997. *Coleoptera: family-group review and keys to identification. Fauna of New Zealand No. 37*. Canterbury, N.Z.: Manaaki Whenua Press.

Kopf, R. K., D. G. Nimmo, P. Humphries, L. J. Baumgartner, M. Bode, N. R. Bond, A. E. Byrom, J. Cucherousset, R. P. Keller, and A. J. King. 2017. Confronting the risks of large-scale invasive species control. Nature Ecology & Evolution 1: 1-4.

Kukalová-Peck, J., and J. F. Lawrence. 1993. Evolution of the hind wing in Coleoptera. The Canadian Entomologist 125: 181-258.

Kundoo, A. A., and A. A. Khan. 2017. Coccinellids as biological control agents of soft bodied insects: a review. Journal of Entomology and Zoology Studies 5: 1362-1373.

Lawing, A. M., and P. D. Polly. 2010. Geometric morphometrics: recent applications to the study of evolution and development. Journal of Zoology 280: 1-7.

Li, J. P., S. A. Ślipiński, and H. Pang. 2019. *Ladybird beetles of the Australo-Pacific Region: Coleoptera: Coccinellidae: Coccinellini,* CSIRO Publishing.

Li, R.-R., and H.-F. Zhang. 2017. Geometric morphometric analysis of *Eysarcoris guttiger, E. annamita* and *E. ventralis* (Hemiptera: Pentatomidae). Zoological Systematics 42(1): 90-101.

Llaurens, V., M. Joron, and M. Théry. 2014. Cryptic differences in colour among Müllerian mimics: how can the visual capacities of predators and prey shape the evolution of wing colours? Journal of Evolutionary Biology 27: 531-540.

Lommen, S. T., S. V. Saenko, Y. Tomoyasu, and P. M. Brakefield. 2009. Development of a wingless morph in the ladybird beetle, *Adalia bipunctata*. Evolution & Development 11: 278-289.

Lommen, S. T. E. 2013. *Exploring and exploiting natural variation in the wings of a predatory ladybird beetle for biological control,* Department of Evolutionary Biology, Institute of Biology Leiden (IBL), Faculty of Science, Leiden University.

Lommen, S. T. E., P. W. De Jong, and P. M. Brakefield. 2005. Phenotypic plasticity of elytron length in wingless two-spot ladybird beetles, *Adalia bipunctata* (Coleoptera : Coceinellidae). European Journal of Entomology 102: 553-556.

Maddison, P.M. 2010. Checklist of New Zealand Hexapoda. Order Coleoptera. [Chapter] NINE: Phylum Arthropoda: Subphylum Hexapoda: Protura, springtails, Diplura, and insects. *In* Gordon, D. (Ed.), New Zealand Inventory of Biodiversity. Kingdom

Animalia. Chaetognatha, Ecdysozoa, ich-nofossils Vol. 2. Canterbury University Press, Christchurch, New Zealand: 233–467.

Michie, L. J., F. Mallard, M. E. N. Majerus, and F. M. Jiggins. 2010. Melanic through nature or nurture: genetic polymorphism and phenotypic plasticity in *Harmonia axyridis*. Journal of Evolutionary Biology 23: 1699-1707.

Novoa, A., J. J. Le Roux, M. P. Robertson, J. R. Wilson, and D. M. Richardson. 2015. Introduced and invasive cactus species: a global review. AoB Plants 7.

Ospina-Garcés, S. M., F. Escobar, M. L. Baena, A. L. V. Davis, and C. H. Scholtz. 2018. Do dung beetles show interrelated evolutionary trends in wing morphology, flight biomechanics and habitat preference? Evolutionary Ecology 32: 663-682.

Pace, L. 2012. One-Way Analysis of Variance. In Beginning R. Springer: Berlin/Heidelberg, Germany: 139–147.

Perrard, A., M. Baylac, J. M. Carpenter, and C. Villemant. 2014. Evolution of wing shape in hornets: why is the wing venation efficient for species identification? Journal of Evolutionary Biology 27: 2665-2675.

Pitchers, W., J. E. Pool, and I. Dworkin. 2013. Altitudinal clinal variation in wing size and shape in African *Drosophila melanogaster*: One cline or many?. Evolution 67: 438-452.

Pope, R. D. 1977. Brachyp tery and wing-polymorphism among the Coccinellidae (Coleoptera). Systematic Entomology 2: 59-66.

Poulton, E. B. 1890. *The colours of animals: their meaning and use, especially considered in the case of insects*, Kegan Paul, Trench & Trübner, London.

Ren, J., M. Bai, X.-K. Yang, R.-Z. Zhang, and S.-Q. Ge. 2017. Geometric morphometrics analysis of the hind wing of leaf beetles: proximal and distal parts are separate modules. ZooKeys 685: 131–149.

Reznik, S. Y., M. Y. Dolgovskaya, and A. N. Ovchinnikov. 2015. Effect of photoperiod on adult size and weight in *Harmonia axyridis* (Coleoptera: Coccinellidae). European Journal of Entomology 112: 642–647.

Ripley, B., B. Venables, D. M. Bates, K. Hornik, A. Gebhardt, D. Firth, and M. B. Ripley. 2013. Package 'mass'. Cran R 538. Available from: http://www.cran.r-project.org/package =MASS

Rohlf, F. J., and L. F. Marcus. 1993. A revolution morphometrics. Trends in Ecology & Evolution 8: 129-132.

Rossa, R., J. Goczał, and A. Tofilski. 2016. Within- and between-species variation of wing venation in genus Monochamus (Coleoptera: Cerambycidae). Journal of Insect Science 16(5), 1– 7.

Seo, M., G. Kim, and Y. Youn. 2008. Differences in biological and behavioural characteristics of *Harmonia axyridis* (Coleoptera: Coccinellidae) according to colour patterns of elytra. Journal of Applied Entomology 132: 239-247.

Sokolovska, N., L. Rowe, and F. Johansson. 2000. Fitness and body size in mature odonates. Ecological Entomology 25: 239-248.

Szawaryn, K., and R. A. Leschen. 2019. Redescription and notes on the New Zealand ladybird species *Hoangus venustus* (Pascoe, 1875)(Coleoptera: Coccinellidae). Journal of Asia-Pacific Entomology 22: 226-232.

Valcu, M., and J. Dale. 2014. colorZapper: Color extraction utilities. R package version 1.0. Available from: https://github.com/valcu/colorZapper.

Villemant, C., G. Simbolotti, and M. Kenis. 2007. Discrimination of *Eubazus* (Hymenoptera, Braconidae) sibling species using geometric morphometrics analysis of wing venation. Systematic Entomology 32: 625-634.

Wheeler, C. A., J. G. Millar, and R. T. Carde. 2015. Multimodal signal interactions in the ladybeetle, *Hippodamia convergens*, aposematic system. Chemoecology 25: 123-133.

Winters, A. E., M. Stevens, C. Mitchell, S. P. Blomberg, and J. D. Blount. 2014. Maternal effects and warning signal honesty in eggs and offspring of an aposematic ladybird beetle. Functional Ecology 28: 1187-1196.

Wolak, M., and M. M. Wolak. 2015. Package 'ICC'. Facilitating estimation of the Intraclass Correlation Coefficient. Available from: https://cran.r-project.org/web/packages/ICC/ICC.pdf.

Yin, H. C., Q. H. Shi, M. Shakeel, J. Kuang, and J. H. Li. 2016. The environmental plasticity of diverse body color caused by extremely long photoperiods and high temperature in *Saccharosydne procerus* (Homoptera: Delphacidae). Frontiers in Physiology 7: 401.

DRC 16

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STATEMENT OF CONTRIBUTION
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Chapter 4

Inter- and intraspecific variation in ladybird activity

This chapter is in review at the Journal of Insect Behaviour (submitted 9/12/2020).

Abstract

Ladybird beetles (Coleoptera: Coccinellidae) are often introduced into new ecosystems as biological control agents, but some of these introductions have resulted in rapid range expansions. To better understand the expansion and distribution of introduced ladybirds, it is essential to resolve the factors that affect dispersal ability. It is difficult to measure the dispersal ability of insects such as ladybirds directly. However, because dispersal ability is affected by general activity levels (speed and distances travelled) and responses to disturbances such as predation, these traits can be used as proxies of dispersal ability. To understand differences in dispersal ability in populations of introduced ladybirds, we compared inter- and intraspecific patterns of general activity levels, and responses to a simulated predator attack. We made video recordings in a controlled setting of the activity of individuals of four introduced species of ladybirds in New Zealand before and after a simulated predator attack. We found that *Illeis galbula* had a shorter response duration to the simulated predator attack and moved faster than *Halmus chalybeus.* However, there was no difference in intraspecific variation between species in total distances travelled, mean velocities and the duration of responses to the simulated predator attack. We observed strong positive correlations between overall body size and general activity levels in each species. We discuss how increased investment in general activity levels and anti-predator responses may increase the dispersal ability of introduced ladybirds.

Keywords: general activity, velocities, distances, intraspecific variation, interspecific variation, introduced species, ladybirds, pathtrackR, anti-predator behaviour, dispersal

4.1 Introduction

Dispersal is a critical factor that influences the structure and dynamics of populations and communities, diversity and gene flow in ecosystems, the evolution of populations in changing environments, range sizes, and individual fitness (Bowler and Benton 2005; Nathan *et al.* 2008). Dispersal patterns can be influenced by both internal and external factors (Le Galliard *et al.* 2012). Internal factors include body size, age, sex and cohort characteristics (Le Galliard *et al.* 2012). External factors include landscape changes, heterospecific interactions and habitat quality (including disturbance, predation, climate change and introduced species). External factors can additionally influence dispersal directly or indirectly, prior to or during movement from one habitat to another (Le Galliard *et al.* 2012). For example, predation can indirectly induce dispersal through the presence of predation cues such as non-fatal predator attacks that trigger the production of winged offspring in aphids (Poethke *et al.* 2010). Because the presence or absence of predators may affect general activity levels (Kohler and McPeek 1989; Parigi *et al.* 2019), changes in general activity levels in response to a simulated predator attack may indicate inter- and intraspecific variation (Parigi *et al.* 2019) in the dispersal behaviour of ladybirds.

Nathan *et al.* (2008) proposed a unified framework to understand the movement ecology of a species. Their framework includes 1) internal factors such as internal state (e.g. moving due to changes in body size or age), navigation (i.e. where to move) and motion (i.e. how to move) capacities; 2) external factors (i.e. changes in the environment) and 3) movement path (which path they move along, i.e. a sequential collection of steps and stops of movement) (Nathan *et al.* 2008). Any variation in the above can explain why some species disperse more than others (Nathan *et al.* 2008). For instance, general activity levels and the ability to withstand disturbances are major determinants of dispersal ability because they correlate with how fast or far a species can move (Hui and Pinter-Wollman 2014; Le Galliard *et al.* 2012). General activity levels are variable and differ both between and within species (Hui and Pinter-Wollman 2014; Van Beest *et al.* 2013). Therefore, comparing general activity levels between species will help us understand differences in constraints and potential of movement (Nathan *et al.* 2008). Further, quantifying responses to disturbances caused by anthropogenic or natural factors (including predators) will also help us understand movement ecology (Le Galliard *et al.* 2012; Nathan *et al.* 2008). The cohesive framework of Nathan *et al.* (2008) provides a better understanding of the overall causes, mechanisms and consequences of differences in dispersal abilities between species (Nathan *et al.* 2008), and in this study, we focus on general activity levels, responses to disturbances (i.e. simulated predator attacks) and how they may influence dispersal (i.e. changes in activity levels as a response to simulated predator attacks) in ladybirds.

Dispersal is usually assumed to be a fixed trait and only a few studies have investigated intraspecific variation in dispersal ability (Le Galliard *et al.* 2012; Nathan *et al.* 2008). Variation in dispersal behaviour occurs through differences in body size, reproductive rate and mating systems (Beaudoin-Ollivier *et al.* 2003; Le Galliard *et al.* 2012; Liebgold *et al.* 2018; Trochet *et al.* 2016). For example, body size is positively associated with dispersal potential (Hui and Pinter-Wollman 2014; Soares *et al.* 2018). A study evaluating the performance (dispersal and survival) of mass reared *Ceratitis capitata* (Wiedemann) showed that fruit flies with large body sizes showed greater dispersal abilities over small and medium sized flies (Bloem *et al.* 1994).

Variation in dispersal ability may increase the probability of individuals in a population that are pre-adapted to expand and explore which then enables them to successfully establish in a new environment (Forsman, 2014). Strong selection pressures (i.e. spatial selection and competition) may drive variation in dispersal ability. Therefore, behavioural traits such as dispersal ability may evolve rapidly given spatial selection pressure (Alford *et al.* 2009). For instance, in invasive species (i.e. the ladybird *Harmonia axyridis*), spatial selection may drive the evolution of phenotypes that increase dispersal ability (Phillips *et al.* 2010). Strong selection pressures such as competition in native habitats can drive higher levels of variation in speed in species such as Argentine ants (Hui and Pinter-Wollman 2014).

Ladybirds have a wide range of dietary specificities (Hodek, 1973). Coccinellines, however are mostly generalist predators on soft-bodied arthropods and have been introduced around the world for the biological control of agricultural pests such as aphids and whiteflies (Escalona *et al.* 2017; Giorgi *et al.* 2009; Seago *et al.* 2011).

There have been several successful and unsuccessful introductions (both intentional and accidental), and a few successful introductions have resulted in aggressive invasions and range expansions (Kajita *et al.* 2000; Lombaert *et al.* 2010; Snyder and Clevenger 2004). What accounts for the successful establishment of ladybird species? First, Soares *et al.* (2018) predicted that the dispersal ability of ladybirds may vary between species based on their potential reproductive capacity and adult body sizes (i.e. the larger *Harmonia axyridis* may have a greater dispersal ability than the smaller *H. conformis* and *H. variegata*). Secondly, other morphological features such as wing polymorphisms in *Adalia bipunctata* and *Harmonia axyridis* (Lommen *et al.* 2019; Lommen *et al.* 2009; Ueno *et al.* 2004), variation in wing shape, size of the wings and flight muscles (Zera and Denno 1997) may influence dispersal ability, however these factors have been rarely studied. Finally, having a higher level of variation in functionally important traits such as dispersal ability may increase the establishment success of introduced species (Forsman 2014). Therefore, the level of intraspecific variation in dispersal ability may be higher in widespread introduced ladybird species than less distributed ladybird species.

In this study, we compared inter- and intraspecific variation in activity levels and responses to simulated predator attacks in ladybirds as indicators of dispersal ability. We characterised activity levels by mean velocity and total distance travelled. We predicted a) significant interspecific variation in dispersal ability in ladybirds, b) that differences in dispersal ability between species correlates with adult body size and shape of the hind wings and c) that the level of intraspecific variation in dispersal ability between these species differs.

4.2 Methods

4.2.1 Sampling sites

From October 2017 to May 2018, we collected introduced ladybirds from seven field sites in the greater Auckland region, New Zealand (Appendix B: Supplementary figure 3.1). We sampled three habitat types: scrub, native and exotic vegetation (Appendix B: Supplementary table 3.1) to capture the full range of morphological and behavioural variation present in each species across the three different habitat types. Sampling sites were randomly picked with ArcMap GIS software (Version 10.4.1, ESRI Inc) with a minimum distance of 3 km between each site. Because some habitat types have a restricted distribution in the Auckland region, there are unequal numbers of sampling sites for each habitat type.

We collected four ladybird species introduced to New Zealand (figure 4.1); a) *Rhyzobius forestieri* introduced in 1889 (Kuschel 1990; Lo and Chapman 2001) (n=18 present in four sites), b) *Illeis galbula* introduced in 1985 (Kuschel 1990) (n=9 present in one site), c) *Halmus chalybeus* introduced in 1899 for agricultural bio-control (Kuschel 1990; Lo and Chapman 2001) (n=28 present in seven sites), and d) *Serangium maculigerum* self-introduced in 2005 (See Pyle and Jamieson (2015)) (n=19 present in six sites). Ladybirds were housed in a climate-controlled lab (19C ± 1°C, 50-70%RH) with natural and artificial light.

Figure 4.1 Ladybird species used in the study (scales indicate- 1mm)
a) *Rhyzobius forestieri,* b) *Illeis galbula,* c) *Halmus chalybeus,* d) *Serangium maculigerum.*

4.2.2 Experimental procedure

After collection, we kept ladybirds in the laboratory for 3-4 hours to acclimatise to the lab environment before starting the experimental assay. We used mean velocity and total distances travelled to quantify individual activity level. We introduced each individual to a petri dish (5cm diameter) in an inverted 5ml vial, giving them three minutes to acclimatise before the vial was removed. In cases where the ladybird did not enter the petri dish voluntarily, we gently transferred the individual into the petri dish using a paintbrush (n = 53). We video recorded the assays using a Canon XA20 HD camcorder (Canon Inc., Japan) while the ladybird explored the petri dish and activity levels were measured for eight minutes (one minute after time of release to one minute before the simulated predator attack). After 10 minutes, we simulated a non-fatal predator attack by using a coloured cotton bud to gently tap the ladybird on the elytra. The cotton buds were coloured using black nail polish to keep track of it easily in the video. One minute after the simulated predator attack, the ladybird could explore the petri dish for another eight minutes during which activity levels were measured. After each trial, we washed the petri dishes and the cotton bud with ethanol.

We used the ‘PathtrackR’ package (Harmer and Thomas 2019) in R software (v3.4, R Core Team, 2017) to score videos. This package automatically provides the mean velocity and total distances travelled by the ladybird before and after the simulated predator attack. In addition, we manually scored the videos to measure the response of ladybirds to the simulated predator attack (whether ladybird behaviour changed in response to the simulated predator attack was recorded as a binary yes/no state, and the duration of the behavioural change was timed). We observed four different behaviour sequences in response to the simulated predator attacks (table 4.1).

Table 4.1 Classification of changes in behaviour after the simulated predator attack

Response to the simulated predator attack		**Change in behaviour (Yes - Y/No - N)**
Before simulation	**After simulation**	
Moving	Continue moving	N
Moving	Stop moving	Y
Stationary	Continue stationary	N
Stationary	Start moving	Y

4.2.3 Morphometric measurements

After the experiment, we stored ladybirds at -20^{0}C and photographed them using a Nikon DS-U3-Ri1 (Nikon Inc.) camera connected to a Leica M205A stereomicroscope. We later measured five morphological features related to the length and width of the body when the elytra were closed as shown in figure 4.2a (Evans 2000; Seo *et al.* 2008). The measurements were taken to the nearest 0.01mm using ImageJ 1.50i (Wayne Rasband, National Institute of Health USA).

Fresh specimens were then frozen in -8O^{0}C and then transferred to ethanol before removing hind wings to measure wing shape. We removed the right hind wing of each ladybird (to avoid potential effects of asymmetry) and washed it with water before mounting. We labelled and photographed the wings using a Canon EOS 700D (Canon Inc., Japan) camera connected to a microscope (SMZ 168, Motic Microscopes). To get accurate and consistent measurements, we selected six landmarks (figure 4.2b) homologous between all individuals and which were clearly visible in the images (Abdolahi Mesbah *et al.* 2015).

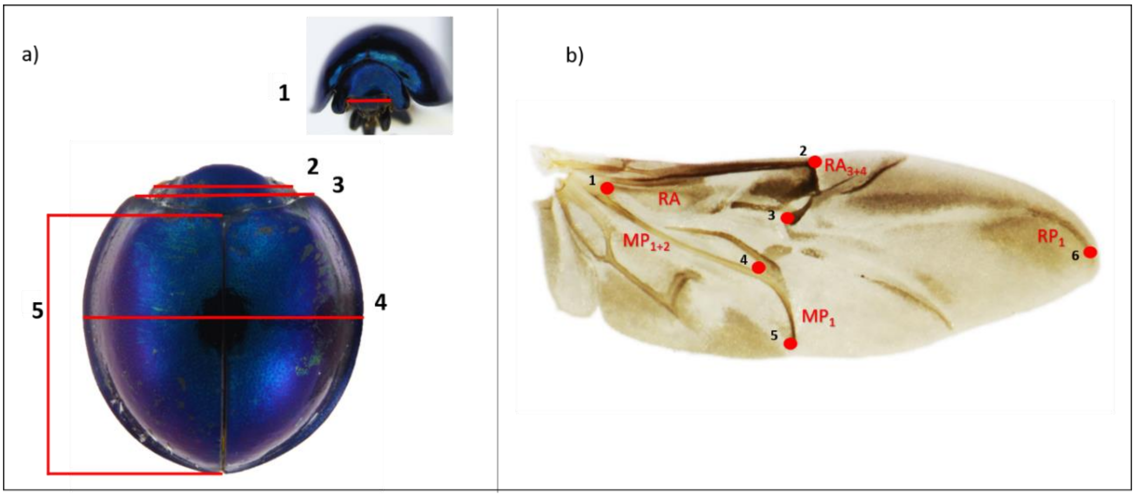

Figure 4.2 Morphometric measurements of ladybirds
a) body size of ladybirds (example using *Halmus chalybeus*), 1 –width of the head, 2- width of the pronotum, 3- distance of the anterior edge of the elytra while the elytra are closed, 4- width of the body and 5- length of the elytral suture when elytra are closed; b) landmark locations to measure the shape of right hind wing (example using *H. chalybeus* wing), landmarks 1- fork of radius anterior and media posterior 1+2 (RA and MP1+2) veins, 2- fork of radius anterior and radius anterior 3+4 (RA and RA3+4) veins, 3- distal corner of radial pigmentation, 4- fork of media posterior 1+2 and media posterior 1 (MP1+2 and MP1) veins, 5- end of MP1 vein and 6- end of radius posterior 1 (RP1) vein. Terminology follows (Kukalová-Peck and Lawrence 1993).

We selected landmarks representing areas where morphological variation occurred most obviously including the base of the hind wing and landmarks previously used by Bai *et al.* (2011), (Bai *et al.* 2012) and Abdolahi Mesbah *et al.* (2015). Landmarks were defined as; 1- fork of radius anterior and media posterior 1+2 (RA and MP_{1+2}) veins (Ren *et al.* 2017), 2- fork of radius anterior and radius anterior 3+4 (RA and RA_{3+4}) veins (Abdolahi Mesbah *et al.* 2015; Bai *et al.* 2012), 3- distal corner of radial pigmentation (Abdolahi Mesbah *et al.* 2015), 4- fork of media posterior 1+2 and media posterior 1 (MP_{1+2} and MP_1) veins (Abdolahi Mesbah *et al.* 2015), 5- end of MP_1 vein (Abdolahi Mesbah *et al.* 2015) and 6- end of radius posterior 1 (RP_1) vein (Bai *et al.* 2011).

4.3 Statistical analyses

4.3.1 Morphometrics

We used R statistical software (v3.4, R Core Team, 2017) for the statistical analyses. Because the body size parameters were highly correlated, we used principal component analysis (PCA) to reduce the number of variables to orthogonal axes. We generated the principal components (PCs) using Non-linear Iterative Partial Least Squares (NIPALS) algorithm as it allows missing data (width of the head of some ladybirds could not be measured), using the package 'ade4' (Dray et al. 2017). We compared wing shape via geometric morphometric analysis using the 'geomorph' package (Adams *et al.* 2013). We computed landmark coordinates and standardized them using generalized procrustes analysis (Lawing and Polly 2010). We then created shape variables using principal component scores, retaining the information about shape of the hind wing (Lawing and Polly 2010).

4.3.2 Activity levels

We fitted linear and generalised linear mixed models for mean velocity and total distance. We selected the best fitting models based on the lowest AIC values. We used treatment (before and after the simulated predator attack), species, size PC variable 1 (PC1) and wing shape PC variable 2 (WPC2) as fixed effects and individual identity as random effects in both models. We then compared the level of variation in total distances and mean velocity within the ladybird species with the asymptotic test using the "cvequality" package (Marwick and Krishnamoorthy 2016).

4.3.3 Response to a simulated predator attack

To assess the best model fit for our data, we fitted a gamma hurdle models for the change in behaviour as a response to the simulated predator attack and the duration of the changed behaviour. We used species, PCs for overall body size (PC1 and PC2) and the PCs for wing shape (WPC1 and WPC2) as predictor variables. The models were designed to assess whether there was any difference between species in response to the simulated predator attack, as well as the duration of any responses. We identified the best model by comparing models fitted with different distributions and predictors, while checking for over- and under-dispersion. The best model for the change in behaviour as a response to the simulated predator attack was a GLM with species identity, wing PC variable 1 and size PC variable 1 as fixed parameters. The best model for the duration of changed behaviour was a GLM with species identity, wing PC variable 1 and size PC variable 1 as fixed parameters. We then computed the coefficients of variation of ladybird species using an asymptotic test using the “cvequality” package (Marwick and Krishnamoorthy 2016) to compare the level of intraspecific variation in the duration of responses to a simulated predator attack.

4.4 Results

4.4.1 Morphometrics

We extracted two principal components from the size PCA analysis (Appendix C: Supplementary table 4.1). All five size parameters contributed cumulatively to the first factor (PC1), which explained 98.72% of the total variance (eigenvalue = 4.89) and width of the body contributed to the second factor (PC2) which explained 1.27% of the total variance (eigenvalue = 0.06). Together, PC1 and PC2 explained 99.99% of the total variance.

We extracted two principal components that described the shape of the right hind wing of the ladybirds using the six landmarks from geo-morphometric analysis (figure 4.3). The first component for wing shape (WPC1) explained 65.34% of the total variance and an increase in WPC1 suggests an increase in wing breadth (i.e. landmark 6 located more distally away from the R-M loop - figure 4.3). The second component for wing shape (WPC2) explained 12.13% of the total variance and an increase in WPC2 moves landmarks 3 and 4 more proximally towards the base of the hind wing. Further, an increase in WPC2 also moves landmark 4 closer to landmark 1 and away from landmark 5, creating a longer MP_1 vein in the media region (figure 4.3). Together, WPC1 and WPC2 explained 77.47% of the total variance in ladybird wing shape.

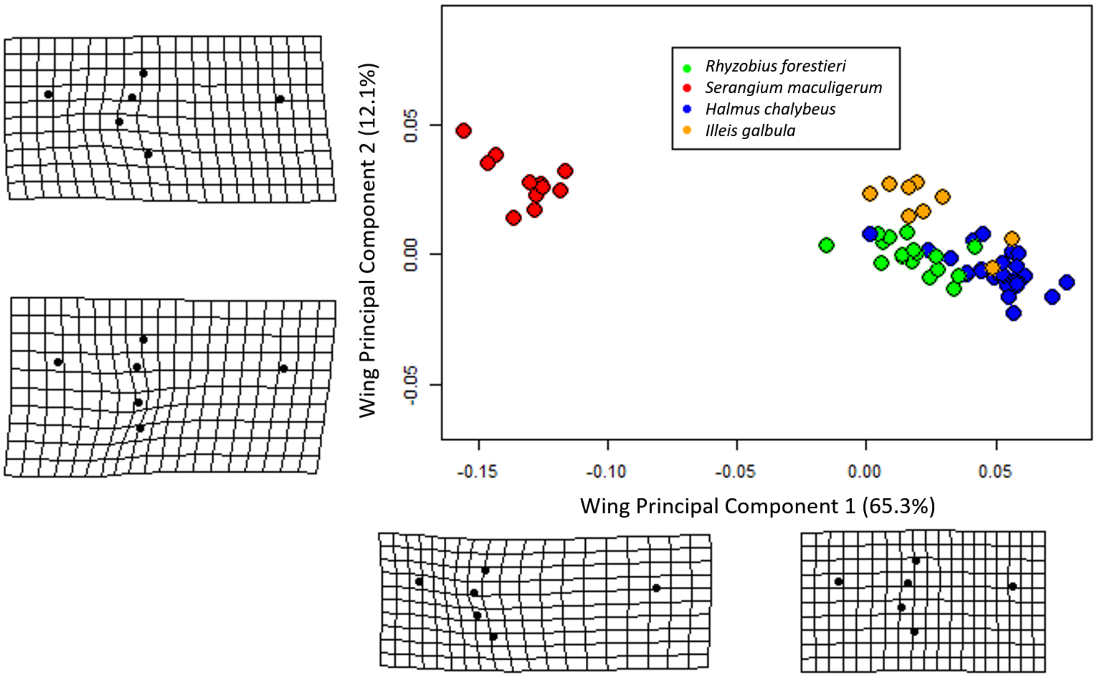

Figure 4.3 Distribution of ladybirds along geometric morphometric components WPC1 and WPC2.
Shape changes associated with the WPC1 and WPC2 are indicated in the grids along the axes respectively (WPC1, WPC2 – Wing principal components 1 and 2)

4.4.2 General activity

Halmus chalybeus had lower mean velocities than *I. galbula* ($p = 0.05$). There was a positive correlation between PC1 representing overall body size and the mean velocities of ladybirds ($p = 0.04$, table 4.2) and there was a weak positive correlation between mean velocities and the length of the MP_1 vein ($p = 0.06$). Mean velocity of the ladybirds did not change after a simulated predator attack ($p = 0.56$, table 4.2). Further, there was no difference in the level of intraspecific variation in mean velocity between the four ladybird species (all $p > 0.05$, Appendix C: Supplementary table 4.2).

Table 4.2 Summary statistics of linear mixed model for mean velocity of ladybirds using *H. chalybeus* as the base species

Predictor	**Estimate**	**Std. Error**	**t**	**p**
(Intercept)	1.09	0.23	4.64	0.00
treatment	-0.02	0.03	-0.58	0.56
Illeis galbula	-0.81	0.42	-1.94	0.05
Rhyzobius foresteri	0.75	0.44	1.69	0.09
Serangium maculigerum	0.86	0.85	1.01	0.31
WPC2	21.17	11.14	1.90	0.06
PC1	0.39	0.19	2.05	**0.04**

The total distances travelled by ladybirds did not change after a simulated predator attack ($p = 0.67$) and there was no difference between species in the total distances they travelled (all species $p > 0.05$, table 4.3). However, there was a weak positive correlation between total distances travelled by ladybirds and the length of the MP_1 vein of the hind wings ($p = 0.07$) and their overall body sizes ($p = 0.07$).

Table 4.3 Summary statistics of linear mixed model for total distances travelled by ladybirds using *H. chalybeus* as the base species

Predictor	**Estimate**	**Std. Error**	**df**	**t**	**p**
(Intercept)	1689.58	473.78	81.84	3.57	0.01
treatment	48.33	111.55	62.75	0.43	0.67
Illeis galbula	-1052.24	810.89	64.00	-1.30	0.20
Rhyzobius foresteri	1004.80	871.04	64.00	1.15	0.25
Serangium maculigerum	769.67	1661.81	64.00	0.46	0.64
WPC2	40884.96	22078.50	64.00	1.85	0.07
PC1	694.26	375.00	64.00	1.85	0.07

4.4.3 Response to a simulated predator attack

There was no difference between species in the change in behaviour (Appendix C: Supplementary table 4.3) or the duration of changed behaviour (figure 4.4) in response to the simulated predator attack ($p > 0.05$, table 4.4). There was no correlation between overall body size (as described by size PC1) or shape of the wing (as described by WPC1) and duration of response to a simulated predator attack ($p = 0.95$, $p= 0.41$ respectively, table 4.4). There was no difference in the level of intraspecific variation between all four species in their duration of response to the simulated predator attack (all species $p > 0.05$; Appendix C: Supplementary table 4.4).

Table 4.4 Gamma hurdle model GLM for the duration of the response to the simulated predator attack using *H. chalybeus* as the base species

Predictor	Estimate	Std. Error	Z value	P
Intercept	2.47	0.89	2.77	0.01
PC1	0.03	0.40	0.07	0.95
WPC1	14.43	17.23	0.84	0.41
Species *Illeis galbula*	-1.41	0.93	-1.52	0.14
Species *Rhyzobius foresteri*	0.18	0.91	0.20	0.85
Species *Serangium maculigerum*	3.11	3.11	1.00	0.33

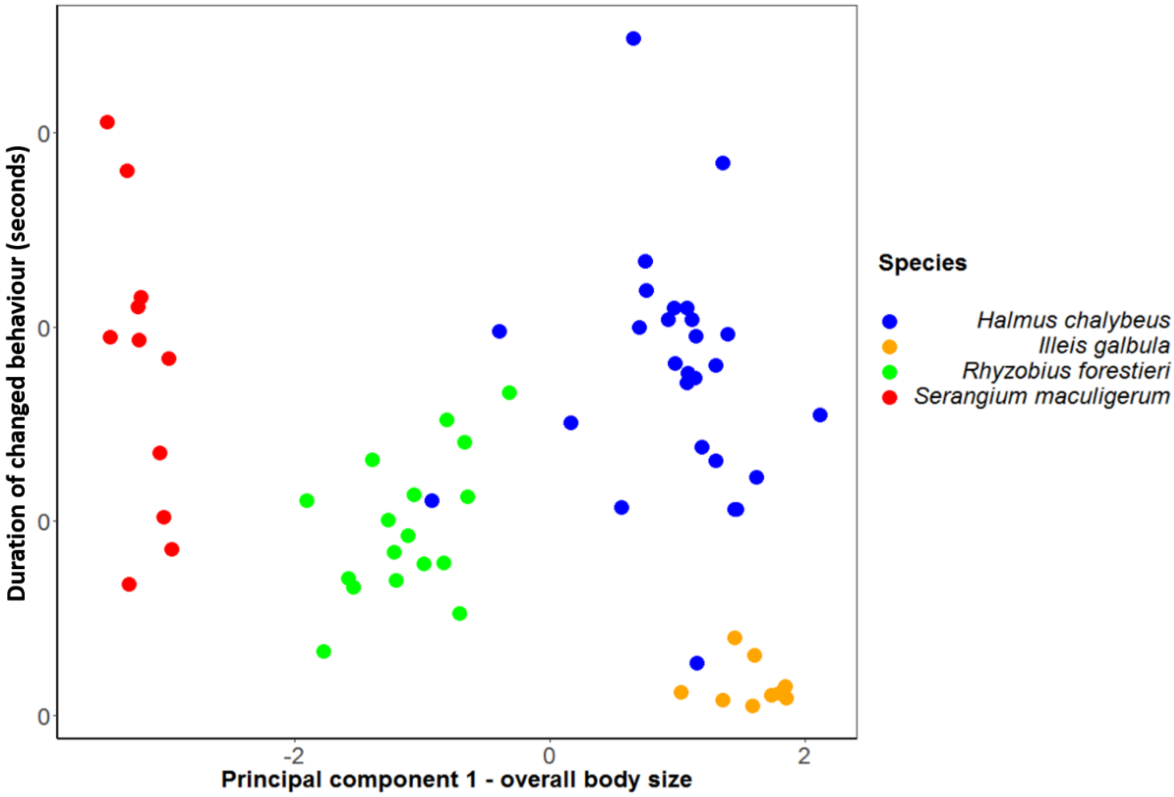

Figure 4.4 Relationship between duration of behavioural changes after a simulated predator attack and body size

4.5 Discussion and Conclusions

We compared the 1) activity levels and 2) responses to a simulated predator attack in four ladybird species introduced to New Zealand. We found that larger ladybirds travelled at higher velocities and slightly greater total distances. *Illeis galbula* had higher mean velocities than *H. chalybeus.* Furthermore, ladybirds with wings with long MP1 veins travelled faster and further than those with wings with short MP1 veins. Finally, and contrary to our predictions, the level of intraspecific variation in general activity was similar between the four ladybird species.

Contrary to our predictions, there was no effect of a simulated predator attack on mean velocity or total distances travelled. Additionally, we predicted that the duration of behavioural changes to a simulated predator attack would differ between species. However, there was no difference in the duration of responses to the simulated predator attack between species. Further, and also contrary to our predictions the level of intraspecific variation in the duration of responses to the simulated predator attack was similar between the four ladybird species.

Increased general activity suggests an increased ability to disperse (Hui and Pinter-Wollman 2014; Le Galliard *et al.* 2012). We predicted that species with higher activity levels (mean velocities and total distances travelled) have greater dispersal ability than species with lower activity levels. Therefore, *I. galbula* species from our data is predicted to be a stronger disperser than *H. chalybeus*. However, *I. galbula* (introduced in 1985), despite having high mean velocities, was limited to one field site in our collections in contrast to *H. chalybeus* (introduced in 1899), which was present in all seven field sites and *S. maculigerum* (introduced in 2005), which was present in six of seven field sites. Dispersers tend to be more thorough explorers than residents (Hui and Pinter-Wollman 2014; Le Galliard *et al.* 2012). Further, motivation to disperse may be driven by a myriad of selection pressures such as finding better resources such as food, suitable habitats, finding mates or to avoid predators (Lombaert *et al.* 2014). In this case, *I. galbula* prey on powdery mildew fungi compared to *H. chalybeus*, which preys on both soft and armoured scales, and mites (Lo and Chapman 2001). Therefore, the activity levels of *I. galbula* are higher but they are less widely distributed (at least in the Auckland region) than *H. chalybeus*, perhaps due to dietary restrictions. Higher activity levels may increase the ability of *I. galbula* to find resources locally. *Halmus chalybeus* however, may not require high general activity levels as local resources are sufficient.

General activity may be influenced by internal factors such as body size (Ghosh and Agarwala 2018; Hemptinne *et al.* 2012), age (Dixon and Agarwala 2002) and melanic content (Jong *et al.* 1996) of the elytra in ladybirds. For example, speed of movement is positively correlated with body mass in *H. axyridis* and *C. septempunctata* (Hemptinne *et al.* 2012). Interestingly, our results show that the overall body size of ladybirds may affect their general activity levels with a strong positive correlation with mean velocities. Therefore, our results support the hypothesis that larger body sizes increase the dispersal ability of ladybirds by increasing mean travel velocities (Soares *et al.* 2018). This may be because an increase in body size suggests increased food storage and energy for dispersal (Bowler and Benton 2005). However, body size may in turn limit dispersal due to lack of suitable habitats and food resources (Soares *et al.* 2017).

Understanding how dispersal ability and its intraspecific variability interact with other fitness related traits can clarify why some species disperse and expand their ranges widely while other species do not (Nathan *et al.* 2008; Rehage *et al.* 2016; Sweeney *et al.* 2013). Dispersal and fitness are connected in an evolutionary and ecological feedback loop (Bowler and Benton 2005). Species with increased investment in general activity levels (i.e. *I. galbula*) may have greater dispersal ability and thereby increased fitness in populations of introduced ladybirds (Clark and Schaefer 2016; Lind and Cresswell 2005). We need to incorporate demographic parameters (such as patch size, presence/absence data, population sizes, death rates or predation rates) and movement paths of ladybirds into our study systems to understand the movement ecology of introduced species. However, to date there are very few studies that have directly tracked the movement of ladybirds (Agustí *et al.* 2019; Ameixa *et al.* 2019). This is mainly because of difficulties in getting reliable data due to size restrictions on tracking devices. However, with technological advancements such as genetic assignment and high resolution spatio-temporal movement data, future studies of dispersal will allow us to better understand the movement ecology of introduced species.

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4.7 References

Abdolahi Mesbah, R., J. Nozari, and S. Dadgostar. 2015. A geometric morphometric study on geographical populations of *Hippodamia variegata* (Goeze, 1777)(Coleoptera: Coccinellidae) in some parts of Iran. Journal of Crop Protection 4: 207-215.

Adams, D. C., F. J. Rohlf, and D. E. Slice. 2013. A field comes of age: geometric morphometrics in the 21st century. Hystrix 24: 7.

Agustí, N., C. Castañé, I. Fraile, and O. Alomar. 2019. Development of a PCR-based method to monitor arthropod dispersal in agroecosystems: *Macrolophus pygmaeus* (Hemiptera: Miridae) from banker plants to tomato crops. Insect Science 27: 1125-1134.

Alford, R. A., G. P. Brown, L. Schwarzkopf, B. L. Phillips, and R. Shine. 2009. Comparisons through time and space suggest rapid evolution of dispersal behaviour in an invasive species. Wildlife Research 36: 23-28.

Ameixa, O. M., J. Šipoš, M. Burda, A. M. Soares, and A. O. Soares. 2019. Factors influencing the introduction and spread of *Harmonia axyridis* in the Iberian Peninsula. Biological Invasions 21: 323-331.

Bai, M., E. McCullough, K.-Q. Song, W.-G. Liu, and X.-K. Yang. 2011. Evolutionary constraints in hind wing shape in Chinese dung beetles (Coleoptera: Scarabaeinae). PLOS ONE 6: e21600.

Bai, M., R. G. Beutel, K.-Q. Song, W.-G. Liu, H. Malqin, S. Li, X.-Y. Hu, and X.-K. Yang. 2012. Evolutionary patterns of hind wing morphology in dung beetles (Coleoptera: Scarabaeinae). Arthropod Structure & Development 41: 505-513.

Beaudoin-Ollivier, L., F. Bonaccorso, M. Aloysius, and M. Kasiki. 2003. Flight movement of *Scapanes australis australis* (Boisduval)(Coleoptera: Scarabaeidae: Dynastinae) in Papua New Guinea: a radiotelemetry study. Australian Journal of Entomology 42: 367-372.

Bloem, K., S. Bloem, and D. Chambers. 1994. Field assessment of quality: release–recapture of mass-reared Mediterranean fruit flies (Diptera: Tephritidae) of different sizes. Environmental Entomology 23: 629-633.

Bowler, D. E., and T. G. Benton. 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biological Reviews 80: 205-225.

Clark, S. R., and J. F. Schaefer. 2016. Ecological influences on the local movement dynamics of the blackspotted topminnow, *Fundulus olivaceus*. Behavioral Ecology and Sociobiology 70: 557-567.

Dixon, A. F. G., and B. K. Agarwala. 2002. Triangular fecundity function and ageing in ladybird beetles. Ecological Entomology 27: 433-440.

Escalona, H. E., A. Zwick, H.-S. Li, J. Li, X. Wang, H. Pang, D. Hartley, L. S. Jermiin, O. Nedvěd, and B. Misof. 2017. Molecular phylogeny reveals food plasticity in the evolution of true ladybird beetles (Coleoptera: Coccinellidae: Coccinellini). BMC Evolutionary Biology 17: 151.

Evans, E. W. 2000. Morphology of invasion: body size patterns associated with establishment of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in western North America. European Journal of Entomology 97: 469-474.

Forsman, A. 2014. Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. Proceedings of the National Academy of Sciences 111: 302-307.

Ghosh, S., and B. Agarwala. 2018. Inter-specific relationship of size and walking speed in predaceous ladybirds (Insecta: Coleoptera: Coccinellidae). Current Science 115: 141-146.

Giorgi, J. A., N. J. Vandenberg, J. V. McHugh, J. A. Forrester, S. A. Ślipiński, K. B. Miller, L. R. Shapiro, and M. F. Whiting. 2009. The evolution of food preferences in Coccinellidae. Biological Control 51: 215-231.

Harmer, A. M., and D. B. Thomas. 2019. pathtrackr: An R package for video tracking and analysing animal movement. Methods in Ecology and Evolution 10: 1196-1202.

Hemptinne, J.-L., A. Magro, E. Evans, and A. Dixon. 2012. Body size and the rate of spread of invasive ladybird beetles in North America. Biological Invasions 14: 595-605.

Hodek, I. 1973. *Biology of coccinellidae*. Acad. Science. Prague.

Hui, A., and N. Pinter-Wollman. 2014. Individual variation in exploratory behaviour improves speed and accuracy of collective nest selection by Argentine ants. Animal Behaviour 93: 261-266.

Jong, P., S. Gussekloo, and P. Brakefield. 1996. Differences in thermal balance, body temperature and activity between non-melanic and melanic two-spot ladybird beetles (*Adalia bipunctata*) under controlled conditions. Journal of Experimental Biology 199: 2655-2666.

Kajita, Y., F. Takano, H. Yasuda, and B. K. Agarwala. 2000. Effects of indigenous ladybird species (Coleoptera: Coccinellidae) on the survival of an exotic species in relation to prey abundance. Applied Entomology and Zoology 35: 473-479.

Kohler, S. L., and M. A. McPeek. 1989. Predation risk and the foraging behavior of competing stream insects. Ecology 70: 1811-1825.

Kukalová-Peck, J., and J. F. Lawrence. 1993. Evolution of the hind wing in Coleoptera. The Canadian Entomologist 125: 181-258.

Kuschel, G. 1990. Beetles in a suburban environment: a New Zealand case study. The identity and status of Coleoptera in the natural and modified habitats of Lynfield, Auckland (1974-1989). DSIR Plant Protection Report 3: 119..

Lawing, A. M., and P. D. Polly. 2010. Geometric morphometrics: recent applications to the study of evolution and development. Journal of Zoology 280: 1-7.

Le Galliard, J. F., A. Remy, R. A. Ims, and X. Lambin. 2012. Patterns and processes of dispersal behaviour in arvicoline rodents. Molecular Ecology 21: 505-523.

Levri, E. P., R. Luft, and X. Li. 2019. Predator detection and a possible dispersal behavior of the invasive New Zealand mud snail, *Potamopyrgus antipodarum* (Gray, 1843). Aquatic Invasions 14: 417–432.

Liebgold, E. B., C. F. Kramer, T. C. Roomian, G. M. Dolezar, and P. R. Cabe. 2018. Heterozygosity–behavior and heterozygosity–fitness correlations in a salamander with limited dispersal. Population Ecology 60: 251-260.

Lind, J., and W. Cresswell. 2005. Determining the fitness consequences of antipredation behavior. Behavioral Ecology 16: 945-956.

Lo, P., and R. Chapman. 2001. Predation by *Halmus chalybeus* (Coleoptera: Coccinellidae) on *Ceroplastes destructor* and *C. sinensis* (Hemiptera: Coccidae: Ceroplastinae) infesting citrus in Northland, New Zealand. Biocontrol Science and Technology 11: 57-66.

Lombaert, E., T. Guillemaud, J.-M. Cornuet, T. Malausa, B. Facon, and A. Estoup. 2010. Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. PLOS ONE 5: e9743.

Lombaert, E., A. Estoup, B. Facon, B. Joubard, J. C. Grégoire, A. Jannin, A. Blin, and T. Guillemaud. 2014. Rapid increase in dispersal during range expansion in the invasive ladybird *Harmonia axyridis*. Journal of Evolutionary Biology 27: 508-517.

Lommen, S. T., S. V. Saenko, Y. Tomoyasu, and P. M. Brakefield. 2009. Development of a wingless morph in the ladybird beetle, *Adalia bipunctata*. Evolution & Development 11: 278-289.

Lommen, S. T., K. G. Koops, B. A. Cornelder, P. W. de Jong, and P. M. Brakefield. 2019. Genetics and selective breeding of variation in wing truncation in a flightless aphid control agent. Entomologia Experimentalis et Applicata 167: 636-645.

Marwick, B., and K. Krishnamoorthy. 2016. Cvequality: Tests for the equality of coefficients of variation from multiple groups. R package version 0.1.1. Available from: https://CRAN.R-project.org/package=cvequality.

Nathan, R., W. M. Getz, E. Revilla, M. Holyoak, R. Kadmon, D. Saltz, and P. E. Smouse. 2008. A movement ecology paradigm for unifying organismal movement research. Proceedings of the National Academy of Sciences 105: 19052-19059.

Parigi, A., C. Porter, M. Cermak, W. R. Pitchers, and I. Dworkin. 2019. The behavioral repertoire of *Drosophila melanogaster* in the presence of two predator species that differ in hunting mode. PLOS ONE 14: e0216860.

Phillips, B., G. Brown, and R. Shine. 2010. Evolutionarily accelerated invasions: the rate of dispersal evolves upwards during the range advance of cane toads. Journal of Evolutionary Biology 23: 2595-2601.

Poethke, Hans J., Wolfgang W. Weisser, and T. Hovestadt. 2010. Predator-induced dispersal and the evolution of conditional dispersal in correlated environments. The American Naturalist 175: 577-586.

Purandare, S. R., B. Tenhumberg, and J. A. Brisson. 2014. Comparison of the wing polyphenic response of pea aphids (*Acyrthosiphon pisum*) to crowding and predator cues. Ecological Entomology 39: 263-266.

Pyle, K. R., and L. E. Jamieson. 2015. Integrated pest and disease management in New Zealand: Progress, changes and challenges since 2004. Acta Horticulturae: 1105-1116.

Rehage, J. S., J. Cote, and A. Sih. 2016. The role of dispersal behaviour and personality in post-establishment spread. Biological Invasions and Animal Behaviour: 96-116.

Ren, J., M. Bai, X.-K. Yang, R.-Z. Zhang, and S.-Q. Ge. 2017. Geometric morphometrics analysis of the hind wing of leaf beetles: proximal and distal parts are separate modules. ZooKeys 685: 131–149.

Seago, A. E., J. A. Giorgi, J. Li, and A. Ślipiński. 2011. Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. Molecular Phylogenetics and Evolution 60: 137-151.

Seo, M., G. Kim, and Y. Youn. 2008. Differences in biological and behavioural characteristics of *Harmonia axyridis* (Coleoptera: Coccinellidae) according to colour patterns of elytra. Journal of Applied Entomology 132: 239-247.

Snyder, W. E., and G. M. Clevenger. 2004. Negative dietary effects of Colorado potato beetle eggs for the larvae of native and introduced ladybird beetles. Biological Control 31: 353-361.

Soares, A. O., A. Honěk, Z. Martinkova, P. M. Brown, and I. Borges. 2018. Can native geographical range, dispersal ability and development rates predict the successful establishment of alien ladybird (Coleoptera: Coccinellidae) species in Europe? Frontiers in Ecology and Evolution 6: 57.

Soares, A. O., A. Honěk, Z. Martinkova, J. Skuhrovec, P. Cardoso, and I. Borges. 2017. *Harmonia axyridis* failed to establish in the Azores: the role of species richness, intraguild interactions and resource availability. BioControl 62: 423-434.

Sweeney, K., B. Cusack, F. Armagost, T. O'Brien, C. N. Keiser, and J. N. Pruitt. 2013. Predator and prey activity levels jointly influence the outcome of long-term foraging bouts. Behavioral Ecology 24: 1205-1210.

Trochet, A., E. A. Courtois, V. M. Stevens, M. Baguette, A. Chaine, D. S. Schmeller, J. Clobert, and J. J. Wiens. 2016. Evolution of sex-biased dispersal. The Quarterly Review of Biology 91: 297-320.

Ueno, H., P. De Jong, and P. Brakefield. 2004. Genetic basis and fitness consequences of winglessness in the two-spot ladybird beetle, *Adalia bipunctata*. Heredity 93: 283.

Van Beest, F. M., E. Vander Wal, A. V. Stronen, and R. K. Brook. 2013. Factors driving variation in movement rate and seasonality of sympatric ungulates. Journal of Mammalogy 94: 691-701.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annual Review of Entomology 42: 207-230.

DRC 16

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Heshani Manula Edirisinghe
Name/title of Primary Supervisor:	Dr Anne Wignall/ Senior lecturer
Name of Research Output and full reference:	
Edirisinghe, H. M., Leschen, R., Dale, J. and Wignall, A.E. Inter- and intraspecific variation in ladybird activity. Journal of Insect behaviour. Ethology. In preparation.	
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GRS Version 4– January 2019

Chapter 5

Inter- and intraspecific variation in desiccation resistance in ladybirds

Abstract

Ladybirds are an important generalist predator introduced around the world and include several species classified as invasive. Ladybirds therefore serve as an excellent model system to study how physiological hardiness influences the establishment and dispersal success of introduced species. Desiccation resistance assays can be used to assess physiological hardiness. Differences in inter- and intraspecific variation in desiccation resistance (i.e. physiological hardiness) may indicate differences in survival, establishment success and the invasive potential of introduced species. In this study, we compared the physiological hardiness of three introduced species of ladybird in New Zealand (*Halmus chalybeus, Serangium maculigerum* and *Illeis galbula*) using desiccation resistance assays where individuals underwent a starvation (no food) or desiccation (no food or water) assay. The survival time was recorded in hours and compared using Cox proportional models. *Halmus chalybeus* survived the longest, compared to *I. galbula* and *S. maculigerum* in both the desiccation and starvation assays. During starvation assays, the smaller *S. maculigerum* have a higher level of desiccation resistance and greater variation in resistance than the larger *H. chalybeus*. This may be due to *S. maculigerum's* more compact body shape. Further, the size of the ladybirds positively correlates with survival time, with larger individuals surviving longer than smaller individuals. Therefore, the size of the ladybirds may contribute to survival time during the establishment of introduced species.

Keywords: dehydration tolerance, survival time, introduced species, establishment, starvation and desiccation assay

5.1 Introduction

Physiological hardiness and its variation between and within species influences species richness, abundance, and dispersal (Block 1996; Bykova and Blatt 2019; Walther *et al.* 2009). Physiological hardiness can be measured in terms of the ability to prevent water loss (desiccation resistance) and thermoregulation or cold tolerance (Gibbs *et al.* 1997). The ability to control water balance and thermoregulate may influence differences in distribution and abundance between and within species (Baranovská *et al.* 2019; De Smedt *et al.* 2018; Weldon *et al.* 2013a). For example, individuals acclimated to wet habitats are more sensitive to desiccation resistance (Baranovská *et al.* 2019) and increased desiccation resistance can be found in individuals adapted to dry conditions (Gibbs *et al.* 1997; Mogi *et al.* 1996).

Insects are extremely vulnerable to body water loss as they have a large surface area to volume ratio (Addo-Bediako *et al.* 2001). However, insects also show diverse adaptations to control water balance and cold hardiness. For example, water-proofing hydrocarbons in the cuticle to limit transpiration of water through the cuticle (Krupp *et al.* 2020; Rourke 2000) and to control of faecal water loss and the subelytral cavity of tenebrionid beetles in desert habitats (Duncan 2003) and rapid cold-hardening and mitochondrial degradation or seasonal changes in mitochondria in the moth *Gynaephora groenlandica* (Wocke) (Levin *et al.* 2003) for cold hardiness. In beetles, the body is comprised of three body segments which fit tightly together and limit water loss by concealing the spiracles and folding the hind wings beneath hardened elytra (Lawrence and Slipinski 2013).

Ladybirds are an excellent system to study the links between physiological hardiness and establishment as they are introduced around the world as biocontrol agents (Snyder and Clevenger 2004) with some of these ladybirds becoming invasive (Lombaert *et al.* 2014; Snyder *et al.* 2004). Successful and failed introductions of some ladybird species may provide insights into how differences in physiological hardiness influences the establishment success of introduced species. While cold tolerance in ladybirds has been studied extensively (Facon *et al.* 2017; Maes *et al.* 2015; Samways *et al.* 1999; Zeng *et al.* 2020), desiccation resistance has rarely been studied either directly (Lee Jr 1980) or indirectly in thermoregulation studies (Hamedi and Moharramipour 2013; Schuder *et al.* 2004). We used desiccation resistance assays to assess the physiological hardiness of three ladybird species (*Halmus chalybeus, Serangium maculigerum* and *Illies galbula*) introduced to New Zealand. In addition to interspecific comparisons, we compared the level of intraspecific variation in desiccation resistance between species to gain insights into how variation in desiccation resistance may have influenced the establishment of introduced ladybird species.

Desiccation resistance assays deprive individuals of both food and water and are therefore a function of both starvation and desiccation resistance (Gibbs *et al.* 1997; Matzkin *et al.* 2009). In this experiment, we exposed ladybirds from each of the three species to starvation and desiccation (desiccation assay), and a control group to starvation alone (starvation assay). We predicted that a) desiccation resistance in ladybirds is different between species, b) larger individuals show better desiccation resistance than smaller individuals, and c) well-established introduced ladybird species (i.e. *Halmus chalybeus* and *Serangium maculigerum*) show higher levels of intraspecific variation in desiccation resistance than species with more restricted distributions (i.e. *Illies galbula*).

5.2 Materials and Methods

5.2.1 Ladybirds

We collected three species of ladybirds (*Halmus chalybeus, Serangium maculigerum* and *Illies galbula*) from four field sites in the Auckland region (Appendix D: Supplementary table 5.1). On returning to the lab, we randomly assigned ladybirds to the desiccation and starvation assays (approximately one hour after collection). Mechanisms influencing desiccation resistance respond rapidly to environmental changes, therefore the acclimation period was limited to one hour (Fischer and Kirste 2018). The experiment was carried out under laboratory conditions (25^{0}C, and 60%-70% relative humidity under an LD 14:10h light photocycle). Temperature and humidity were constant throughout the experiment as changes in temperature and humidity may influence desiccation resistance (Bujan *et al.* 2016; Fischer and Kirste 2018; Weldon *et al.* 2019).

5.2.2 Desiccation resistance assay

Individuals [*H. chalybeus* (n=22)*, S. maculigerum* (n=12) and *I. galbula* (n=24)] were weighed (Ohaus EX125D semi-micro balance, to the nearest 0.01mg) and placed in individual vials (30ml) with 5mg of fresh Drierite (6 mesh; W.A. Hammond Drierite Co. Ltd, Xenia, Ohio) (10% relative humidity) desiccant, separated by cotton balls (methods described by Gibbs *et al.* (1997)). The vials were sealed with Parafilm (Bemis Company, Inc, USA), with the cap screwed on tightly. To determine starvation resistance, control individuals [*H. chalybeus* (n=21)*, S. maculigerum* (n=15) *and I. galbula* (n=16)] were placed in individual vials without desiccant, sealed with Parafilm with the cap screwed on tightly. Individuals in the starvation assay were provided with fresh water via a moistened ball of cotton wool (replaced every 48 hrs). This allows us to control for the water loss observed in individuals in the starvation assay by limiting water loss to that from metabolism rather than desiccation.

Vials were inspected every 2 hrs to record mortality. Individuals that did not move when the vial was tapped or inverted were declared dead. Individuals were weighed again at the time of death and stored in the freezer at -20^{0}C until the end of the experiment. All ladybirds were then dried in a standard incubator for 48 hours at 50^{0}C and weighed to obtain the dry weight.

Elytra length was measured as a proxy for body size (Edirisinghe *et. al.*, Chapter 3). *Serangium maculigerum* is the smallest species followed by *H. chalybeus,* then *I. galbula* (Appendix D: Supplementary figure 5.1, p < 0.01; Appendix D: Supplementary table 5.2) and the length of the elytra of the three species is significantly different between each species (F =885, p < 0.01; Appendix D: Supplementary table 5.3). We photographed the ladybirds using a Canon EOS 700D (Canon Inc., Japan) camera connected to a microscope (SMZ 168, Motic Microscopes) and the length of the elytra was measured to the nearest 0.01mm using ImageJ 1.50i (Wayne Rasband, National Institute of Health, USA).

5.2.3 Statistical analyses

Statistical analyses were performed using R statistical software (v3.4, R Core Team, 2017). We recorded survival time, body size (elytra length) and calculated the dehydration tolerance of ladybirds. Dehydration tolerance was calculated as the percentage of body water content at death (the difference between body mass at death and body mass after drying) over initial body water content (Gibbs *et al.* 1997). We used initial body mass as a proxy for initial body water content as there is a strong positive correlation between initial body mass and initial body water content (Gibbs *et al.* 1997).

We first computed a survival analysis model using the "survival" (Therneau and Lumley 2015) and "survminer" (Kassambara *et al.* 2017) packages in R. We did not have censored data as we continued the experiments until all individuals were dead. In this analysis, we initially used both the Cox proportional model and Weibull distribution to compute survival models as the Cox model assumes an increase in covariates multiplies the hazard by a constant while the Weibull distribution function assumes that the covariates accelerate or decelerate the hazard by a constant. However, the Cox proportional model returned the lowest AIC value and was used as the final model to describe desiccation resistance in the three ladybird species. Survival time was the dependent variable, treatment (starvation, desiccation), species identity, body size (length of the elytra) and desiccation resistance (calculated as dehydration tolerance) and the interaction of treatment and species were the fixed parameters. Proportional hazard models for survival consist of a baseline hazard function which describes how the risk of death per time unit changes at baseline levels of species, desiccation resistance and size.

We then computed a generalised linear model using the "lme4" package (Bates *et al.* 2007) to understand the relationships between desiccation resistance, species and size. We used desiccation resistance as the response variable, and included treatment, species identity and body size (length of the elytra) as fixed parameters and the interaction of treatment and species identity as the random effect. The best fitting model with the lowest AIC value was then selected as the final model. We compared differences in the level of variation in desiccation resistance between and within species in the desiccation and starvation assays, using an asymptotic test in the "cvequality" package (Marwick and Krishnamoorthy 2016).

5.3 Results

Survival of the three ladybird species is significantly affected by desiccation ($p < 0.001$; table 5.1). For all three ladybird species, survival of the individuals in the desiccation assay was reduced compared to individuals who were in the starvation assay (figure 5.1). According to hazard ratio analysis, the survival time of ladybirds in the starvation assay is increased by a factor of eight compared to ladybirds in the desiccation assay (figure 5.2; table 5.1). During starvation, *H. chalybeus* survived the longest followed by *I. galbula*, and then followed by *S. maculigerum* (figure 5.1b). In individuals who were in the desiccation assay, at the beginning (first 100 hours) *I. galbula* had a higher probability of survival compared to *H. chalybeus*, but after the first 100 hours, the probability of survival of *I. galbula* reduced drastically. Overall, under both starvation and desiccation, *H. chalybeus* survived the longest, followed by *I. galbula* then followed by *S. maculigerum* (figure 5.1a).

Table 5.1 Cox proportional model of survival in ladybird species (compared to *H. chalybeus*) when exposed to starvation or desiccation assays

	Co-efficient	Effect size	Z	p
Treatment: Starvation only assay	**-2.17**	**8.73**	**-5.87**	**0.00**
I. galbula	**1.30**	**3.67**	**3.59**	**0.00**
S. maculigerum	-0.52	1.68	-0.55	0.58
Length of the elytra	**-1.45**	**4.27**	**-3.40**	**0.00**
Desiccation resistance	**0.01**	**1.01**	**2.38**	**0.02**
Starvation: *I. galbula*	0.27	1.32	0.57	0.57
Starvation: *S. maculigerum*	-0.90	0.41	-1.57	0.12

Comparisons were made with 'Treatment: Desiccation assay' and *Halmus chalybeus.* Significant effects are indicated in bold.

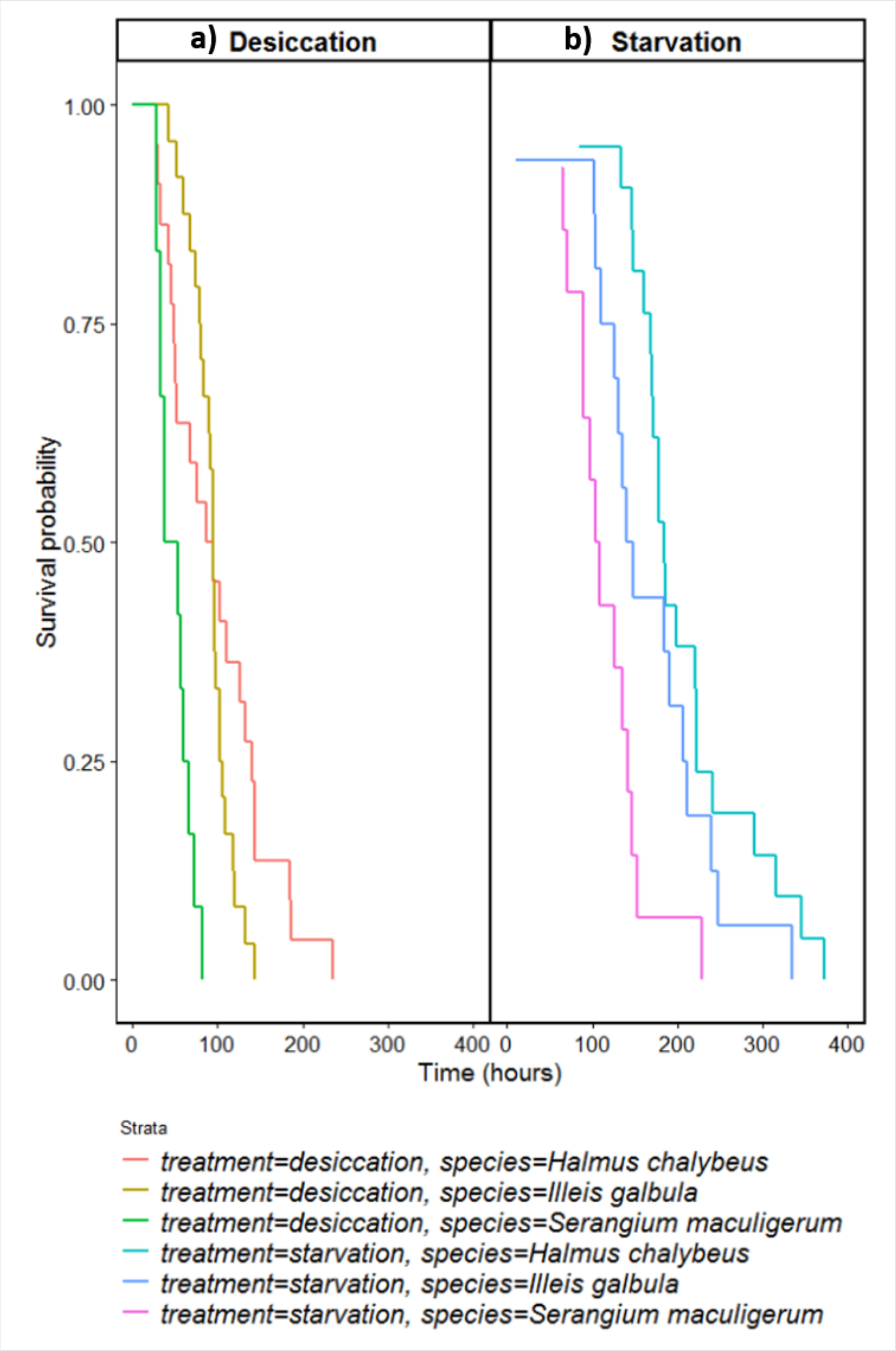

Figure 5.1 Survival curves for the three ladybird species in the desiccation resistance assay.
Survival probability is expressed as the proportion of individuals surviving since the commencement of the assay. Figure 5.1a) indicates survival during the desiccation assay and 5.1b) indicates survival during the starvation assay.

There was a strong negative correlation between body size ($p < 0.001$) and the risk of mortality in the three species. This means a unit increase in body size is associated with a decrease in the risk of mortality by a factor of four (coefficient: -1.452; figure 5.2).

The survival times of ladybirds was not significantly different in the starvation assay ($p > 0.01$; table 5.1). However, in the desiccation assay, the survival time of *I. galbula* was significantly lower than *H. chalybeus* ($p < 0.001$). There was no difference in the risk of mortality between *S. maculigerum* and *H. chalybeus* in the desiccation assay ($p > 0.01$; table 5.1; figure 5.2).

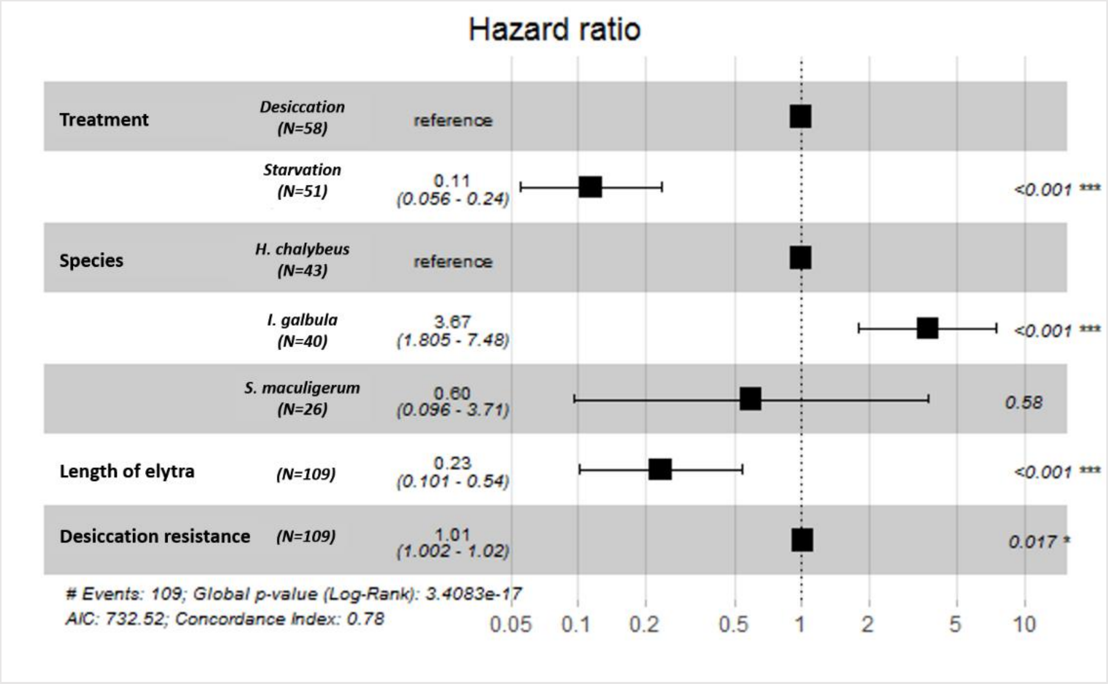

Figure 5.2 Hazard ratio of risk of mortality in ladybird species in the desiccation and starvation assays

The desiccation resistance of the three species was higher in the starvation assay than the desiccation assay ($p < 0.01$; table 5.2). Further, mean desiccation resistance was higher in *H. chalybeus* than *S. maculigerum* in the desiccation assay ($p < 0.01$; table 5.2).

Table 5.2 Generalised linear model for desiccation resistance in the desiccation assay

	Estimate	Std. Error	t value	p
(Intercept)	**0.03**	**0.00**	**10.01**	**0.00**
Treatment: Starvation only assay	**-0.01**	**0.00**	**-3.38**	**0.00**
I. galbula	0.01	0.00	1.37	0.17
S. maculigerum	**0.01**	**0.01**	**2.20**	**0.03**
Starvation: *I. galbula*	-0.01	0.01	-1.29	0.20
Starvation: *S. maculigerum*	**-0.02**	**0.01**	**-2.98**	**0.00**

Comparisons were made with 'Treatment: Desiccation assay' and *Halmus chalybeus.* Significant effects are indicated in bold.

There was no difference in the level of variation in survival time (figure 5.3b) between the three species in the starvation assay (p > 0.05; table 5.3). However, *H. chalybeus* had a higher level of variation in survival time (figure 5.3a) than *I. galbula* in the desiccation assay (p < 0.01; table 5.3). There was no significant difference in the level of variation in survival time between *H. chalybeus* and *S. maculigerum* and between *S. maculigerum* and *I. galbula* (p > 0.05; table 5.3).

Serangium maculigerum had a higher level of intraspecific variation in desiccation resistance than *I. galbula* (p < 0.01; table 5.3) in the desiccation assay (figure 5.3c). In the starvation assay, *S. maculigerum* had a higher level of intraspecific variation in desiccation resistance (figure 5.3d) than *I. galbula* (p < 0.01; table 5.3) and *H. chalybeus* (p = 0.02; table 5.3).

Table 5.3 Comparison of the level of variation between species in survival time and desiccation resistance during the starvation or desiccation assays using the asymptotic test

		Desiccation		Starvation	
		H. chalybeus	*I. galbula*	*H. chalybeus*	*I. galbula*
S. maculigerum	Survival time	0.21	0.13	0.76	0.54
	Desiccation resistance	0.10	**0.00**	**0.02**	**0.01**
H. chalybeus	Survival time		**0.00**		0.31
	Desiccation resistance		0.16		0.44

The level of variation in survival time in *I. galbula* was higher in the starvation assay (figure 5.3a) (p = 0.02; table 5.4), compared to the level of variation in the desiccation assay (figure 5.3b). The level of variation in desiccation resistance of each species was not significantly different in the starvation or desiccation assays (figure 5.3c and 5.3d; p > 0.01; table 5.4).

Table 5.4 Comparisons of the level of variation between survival time and desiccation resistance during the starvation and desiccation assays within species using asymptotic test

Species	Survival	Desiccation resistance
H. chalybeus	0.05	0.31
S. maculigerum	0.99	0.84
I. galbula	**0.02**	0.65

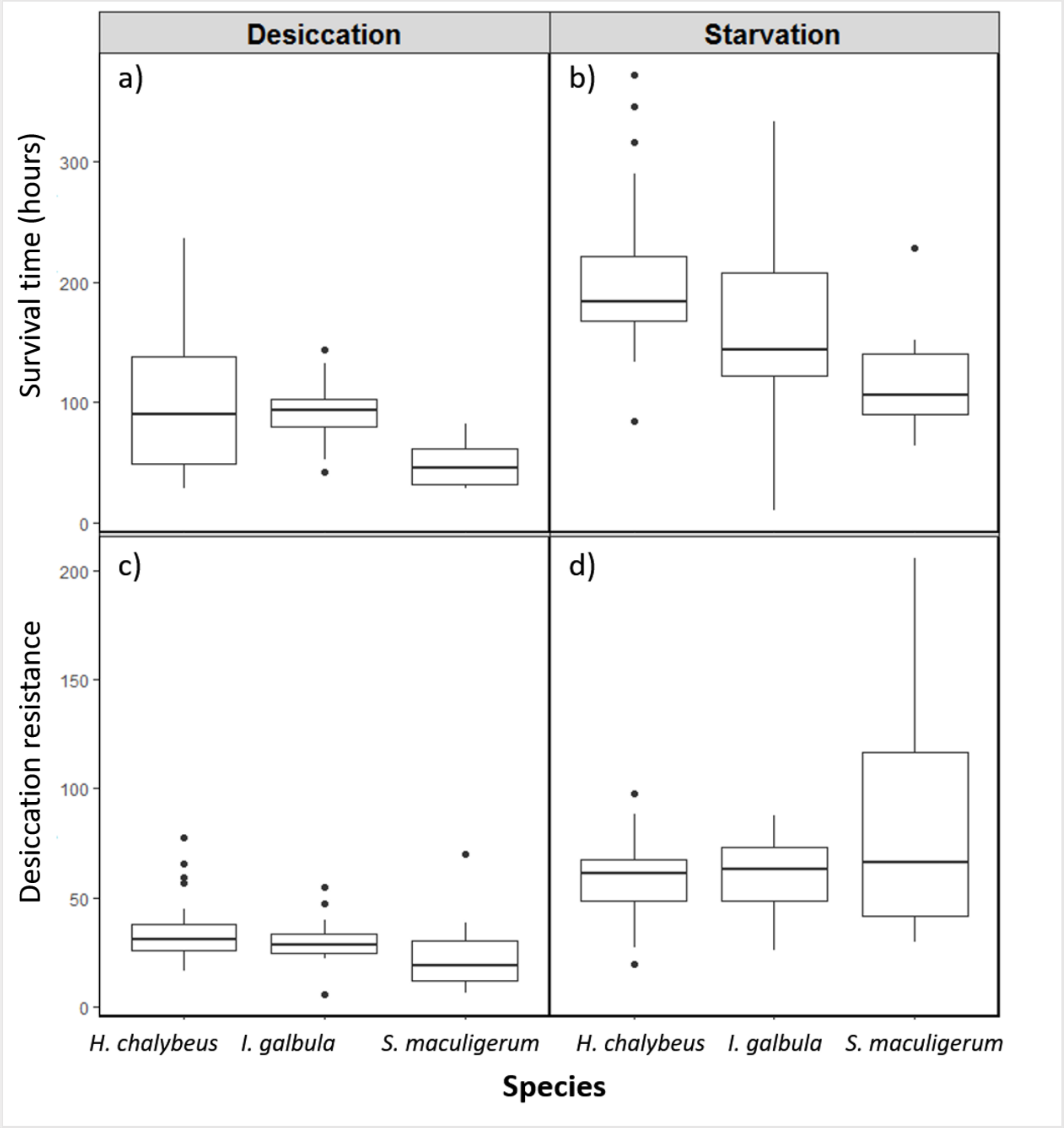

Figure 5.3 Variation between species in survival time and desiccation resistance in the starvation or desiccation assays
The figure represents differences in survival time during a) desiccation assay, b) starvation assay and differences in desiccation resistance during c) desiccation assay and d) starvation assay.

5.4 Discussion

We compared desiccation resistance between three ladybird species (*H. chalybeus, I. galbula* and *S. maculigerum*) using survival analysis and modelling differences in desiccation resistance. There was a significant difference between species in survival time and desiccation resistance between the starvation and desiccation assays. *Halmus chalybeus* survived the longest compared to *I. galbula* and *S. maculigerum* in both the starvation and desiccation assays. There is inter- and intraspecific variation in the level of desiccation resistance between the three introduced ladybird species. There is a strong negative correlation between body size and their survival time.

The body size of ladybirds affects desiccation resistance, with larger body sizes showing greater desiccation resistance as observed in Central European carabid species (Baranovská *et al.* 2019; Bujan *et al.* 2016) and southern African keratin beetles (Le Lagadec *et al.* 1998). However, selecting an accurate measurement of body size is important. For example, wing length in *Bactrocera tryoni* flies is not correlated with desiccation resistance (Weldon and Taylor 2010; Weldon *et al.* 2013a) while body mass is correlated with desiccation resistance (Weldon and Taylor 2010). However, in beetles such as carabid beetles elytra length is correlated with body size (Baranovská *et al.* 2019). In our study, we used length of the elytra which previous work has shown is an appropriate proxy for body size in ladybirds (Edirisinghe *et al.,* Chapter 3). According to our survival analysis, there is a strong negative relationship between body size and risk of mortality in ladybirds. For instance, ladybirds with larger body sizes such as *H. chalybeus* and *I. galbula* have a lower risk of mortality during starvation and desiccation assays than ladybirds with smaller body sizes such as *S. maculigerum*. Therefore, the size of the ladybirds may contribute to survival time during the establishment of introduced species, although this has yet to be tested experimentally. However, *S. maculigerum* had a higher desiccation resistance than *I. galbula* despite being smaller, which may be due to its more compact body shape compared to both *H. chalybeus* and *I. galbula* (Chapin 1940; Ślipiński and Burckhardt 2006).

Dehydration tolerance as calculated in (Gibbs *et al.* 1997), is an indication of body water content at death as a percentage of initial body mass. There was a weak positive relationship between dehydration tolerance (rate of water loss) and risk of mortality in the three species of ladybirds. According to the desiccation resistance model, water loss is lower during starvation assays than in the desiccation assays. This aligns with the fact that water was provided to ladybirds during the starvation assays and the longer survival times that we found when treated with starvation alone compared to the desiccation assay. However, during starvation assays, we noticed that the smaller *S. maculigerum* have a higher level of desiccation resistance than the larger *H. chalybeus.* Our model for desiccation resistance does not include elytra length, as it was not in the best fitting model and did not have a significant effect on desiccation resistance when included in the model. Therefore, differences in desiccation resistance between species may not relate to body size. Instead, differences in desiccation resistance may be due to differences in factors such as the amount of melanism in the cuticle (Parkash *et al.* 2011), metabolic rate, activity levels, and adaptations to reduce water loss through transpiration (Gibbs *et al.* 2003) that may contribute to desiccation resistance and thereby survival.

Variation in survival time describes differences in the ability of individuals to survive and disperse under stress (Baranovská *et al.* 2019; De Smedt *et al.* 2018; Weldon *et al.* 2013a). During starvation assays *S. maculigerum* has a higher level of variation in desiccation resistance than *H. chalybeus.* However, during desiccation assays the variability in desiccation resistance did not differ between these two species. *Serengium maculigerum* and *H. chalybeus* populations may have an increased ability to control water loss during starvation and desiccation compared to *I.galbula.* Desiccation resistance in insects is associated with increased fitness and geographical distribution (Bykova and Blatt 2019; Roy *et al.* 2016). We sampled *H. chalybeus, S. maculigerum* and *I. galbula* within the Auckland region across multiple habitat types in this study. *Ileis galbula* has a relatively restricted distribution despite being in New Zealand since 1985 (Kuschel 1990) compared to *Halmus chalybeus* (introduced in 1899 to Auckland (Kuschel 1990; Lo and Chapman 2001)) and *S. maculigerum* which have a wide distribution in Auckland region despite *S. maculigerum* being recently introduced in 2005 (Pyle and Jamieson 2015)

(Appendix D: Supplementary figure 5.2). Therefore, differences in the desiccation resistance of these species may influence their distribution in New Zealand. However, how desiccation resistance influences the distribution of introduced ladybirds needs to be tested over a broader geographical scale than the present study.

Recent findings show that acclimation may have a significant role in differences in desiccation resistance in some insects (Fischer and Kirste 2018; Terblanche and Kleynhans 2009; Weldon *et al.* 2018). Therefore, differences in the desiccation resistance levels between and within species may be explained by differences in metabolic rates, water loss through the cuticle or by differences in adaptations to acclimate to climatic conditions in the sites they were collected from (Fischer and Kirste 2018; Weldon *et al*. 2018). In addition, factors such as the age and sex of ladybirds may affect cuticular water loss (Fischer *et al.* 2005; Teets *et al.* 2012). For example, females of two cryptic species of velvet worms (*Opisthopatus* spp.) show higher rates of cuticular water loss than male velvet worms (Clusella-Trullas and Chown 2008; Weldon *et al.* 2013b). In the mosquito *Anopheles funestus* however, females were more desiccation resistant than males (Lyons *et al.* 2014). Further, age may decrease desiccation resistance in insects such as *A. funestus* (Lyons *et al.* 2014) and the fruit fly *Bactrocera tryoni* (Weldon and Taylor 2010). However, in our study, sexing or ageing was not possible as ladybirds were too brittle after the experiment to dissect. In addition, the temperature the experiment was carried out in may have affected cuticular water loss (Weldon *et al.* 2013b). For example, ladybirds which were acclimated to a colder environment than that of the laboratory temperature (25^0C) may show decreased desiccation resistance compared to those who may have been acclimated to warmer environments. However, temperature variations were minimised due to our collecting ladybirds within a limited region (Auckland) and within a small timeframe (2 weeks).

Cold tolerance has previously been studied in ladybirds to assess their invasive potential (Hamedi and Moharramipour 2013; Roy *et al.* 2016; Schuder *et al.* 2004). We propose that combining cold tolerance together with desiccation resistance in future studies will provide useful insights into the dispersal potential of ladybirds. Assessments of desiccation resistance in introduced species can be combined with the quantification of lethal temperature limits, desiccation resistance, metabolic rates and development times. These traits represent further aspects of physiological hardiness and would provide a strong foundation with which to understand how species respond to climatic and seasonal variability and thereby allow us to accurately predict the future dispersal of species.

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5.6 References

Addo-Bediako, A., S. L. Chown, and K. J. Gaston. 2001. Revisiting water loss in insects: a large scale view. Journal of Insect Physiology 47: 1377-1388.

Baranovská, E., P. Chajma, and M. Knapp. 2019. Desiccation resistance in Central European carabid species: effects of body size and habitat preferences. ARPHA Conference Abstracts 2: e38513.

Bates, D., D. Sarkar, M. D. Bates, and L. Matrix. 2007. The lme4 package. R package version 2.4.1. Available from: http://www.r-project.org.

Block, W. 1996. Cold or drought-The lesser of two evils for terrestrial arthropods? European Journal of Entomology 93: 325-340.

Bujan, J., S. P. Yanoviak, and M. Kaspari. 2016. Desiccation resistance in tropical insects: causes and mechanisms underlying variability in a Panama ant community. Ecology and Evolution 6: 6282-6291.

Bykova, O., and S. Blatt. 2019. Inclusion of physiological responses in insect pest risk analysis: evaluating species potential to achieve pest status. Journal of the Acadian Entomological Society 15: 1-13.

Chapin, E. A. 1940. New genera and species of lady-beetles related to *Serangium* Blackburn (Coleoptera: Coccinellidae). Journal of the Washington Academy of Sciences 30: 263-272.

Clusella-Trullas, S., and S. L. Chown. 2008. Investigating onychophoran gas exchange and water balance as a means to inform current controversies in arthropod physiology. Journal of Experimental Biology 211: 3139-3146.

De Smedt, P., L. Baeten, M. P. Berg, E. Gallet-Moron, J. Brunet, S. A. O. Cousins, G. Decocq, M. Diekmann, B. Giffard, P. De Frenne, M. Hermy, D. Bonte, and K. Verheyen. 2018. Desiccation resistance determines distribution of woodlice along forest edge-to-interior gradients. European Journal of Soil Biology 85: 1-3.

Duncan, F. 2003. The role of the subelytral cavity in respiration in a tenebrionid beetle, *Onymacris multistriata* (Tenebrionidae: Adesmiini). Journal of Insect Physiology 49: 339-346.

Facon, B., A. Estoup, R. A. Hufbauer, J. Foucaud, and A. Tayeh. 2017. Mating status influences cold tolerance and subsequent reproduction in the invasive ladybird *Harmonia axyridis*. Frontiers in Ecology and Evolution 5: 108.

Fischer, K., and M. Kirste. 2018. Temperature and humidity acclimation increase desiccation resistance in the butterfly *Bicyclus anynana*. Entomologia Experimentalis et Applicata 166: 289-297.

Fischer, K., I. Zeilstra, S. K. Hetz, and K. Fiedler. 2005. Physiological costs of growing fast: does accelerated growth reduce pay-off in adult fitness? Evolutionary Ecology 18: 343-353.

Gibbs, A. G., A. K. Chippindale, and M. R. Rose. 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. Journal of Experimental Biology 200: 1821-1832.

Gibbs, A. G., F. Fukuzato, and L. M. Matzkin. 2003. Evolution of water conservation mechanisms in *Drosophila*. Journal of Experimental Biology 206: 1183-1192.

Hamedi, N., and S. Moharramipour. 2013. Long-term cold response in overwintering adults of ladybird *Hippodamia variegata* (Coleoptera: Coccinellidae). Journal of Crop protection 2: 119-126.

Kassambara, A., M. Kosinski, P. Biecek, and S. Fabian. 2017. Package ‘survminer’. Drawing Survival Curves using ‘ggplot2’. R package version 3. 1. Available from: https://CRAN.R-project.org/package=survminer.

Krupp, J. J., K. Nayal, A. Wong, J. G. Millar, and J. D. Levine. 2020. Desiccation resistance is an adaptive life-history trait dependent upon cuticular hydrocarbons, and influenced by mating status and temperature in *D. melanogaster*. Journal of Insect Physiology 121: 103990.

Kuschel, G. 1990. Beetles in a suburban environment: a New Zealand case study. The identity and status of Coleoptera in the natural and modified habitats of Lynfield, Auckland (1974-1989). DSIR Plant Protection Report 3: 119.

Lawrence, J., and A. Slipinski. 2013. *Australian beetles volume 1: morphology, classification and keys*. Collingwood, Vic: CSIRO Publishing 1:576.

Le Lagadec, M. D., S. L. Chown, and C. H. Scholtz. 1998. Desiccation resistance and water balance in southern African keratin beetles (Coleoptera, Trogidae): the influence of body size and habitat. Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 168: 112-122.

Lee Jr, R. E. 1980. Physiological adaptations of Coccinellidae to supranivean and subnivean hibernacula. Journal of Insect Physiology 26: 135-138.

Levin, D., H. Danks, and S. Barber. 2003. Variations in mitochondrial DNA and gene transcription in freezing-tolerant larvae of *Eurosta solidaginis* (Diptera:

Tephritidae) and *Gynaephora groenlandica* (Lepidoptera: Lymantriidae). Insect Molecular Biology 12: 281-289.

Lo, P., and R. Chapman. 2001. Predation by *Halmus chalybeus* (Coleoptera: Coccinellidae) on *Ceroplastes destructor* and *C. sinensis* (Hemiptera: Coccidae: Ceroplastinae) infesting citrus in Northland, New Zealand. Biocontrol Science and Technology 11: 57-66.

Lombaert, E., A. Estoup, B. Facon, B. Joubard, J. C. Gregoire, A. Jannin, A. Blin, and T. Guillemaud. 2014. Rapid increase in dispersal during range expansion in the invasive ladybird *Harmonia axyridis*. Journal of Evolutionary Biology 27: 508-517.

Lyons, C. L., M. Coetzee, J. S. Terblanche, and S. L. Chown. 2014. Desiccation tolerance as a function of age, sex, humidity and temperature in adults of the African malaria vectors *Anopheles arabiensis* and *Anopheles funestus*. Journal of Experimental Biology 217: 3823-3833.

Maes, S., J.-C. Grégoire, and P. De Clercq. 2015. Cold tolerance of the predatory ladybird *Cryptolaemus montrouzieri*. BioControl 60: 199-207.

Marwick, B., and K. Krishnamoorthy. 2016. Cvequality: Tests for the equality of coefficients of variation from multiple groups. R package version 0.1.1. Available from: https://CRAN.R-project.org/package=cvequality.

Matzkin, L. M., T. D. Watts, and T. A. Markow. 2009. Evolution of stress resistance in *Drosophila*: interspecific variation in tolerance to desiccation and starvation. Functional Ecology 23: 521-527.

Mogi, M., I. Miyagi, K. Abadi, and syafruddin. 1996. Inter- and intraspecific variation in resistance to desiccation by Adult *Aedes* (Stegomyia) spp. (Diptera: Culicidae) from Indonesia. Journal of Medical Entomology 33: 53-57.

Parkash, R., D. D. Aggarwal, B. Kalra, and P. Ranga. 2011. Divergence of water balance mechanisms in two melanic *Drosophila* species from the western Himalayas. Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology 158: 531-541.

Pyle, K. R., and L. E. Jamieson. 2015. Integrated pest and disease management in New Zealand: progress, changes and challenges since 2004. Acta Horticulturae: 1105-1116.

Rourke, B. C. 2000. Geographic and altitudinal variation in water balance and metabolic rate in a California grasshopper, *Melanoplus sanguinipes*. Journal of Experimental Biology 203: 2699-2712.

Roy, H. E., P. M. J. Brown, T. Adriaens, N. Berkvens, I. Borges, S. Clusella-Trullas, R. F. Comont, P. De Clercq, R. Eschen, A. Estoup, E. W. Evans, B. Facon, M. M. Gardiner, A. Gil, A. A. Grez, T. Guillemaud, D. Haelewaters, A. Herz, A. Honek, A. G. Howe, C. Hui, W. D. Hutchison, M. Kenis, R. L. Koch, J. Kulfan, L. Lawson Handley, E. Lombaert, A. Loomans, J. Losey, A. O. Lukashuk, D. Maes, A. Magro, K. M. Murray, G. S. Martin, Z. Martinkova, I. A. Minnaar, O. Nedved, M. J. Orlova-Bienkowskaja, N. Osawa, W. Rabitsch, H. P. Ravn, G. Rondoni, S. L. Rorke, S. K. Ryndevich, M.-G. Saethre, J. J. Sloggett, A. O. Soares, R. Stals, M. C. Tinsley, A. Vandereycken, P. van Wielink, S. Viglášová, P. Zach, I. A. Zakharov, T. Zaviezo, and Z. Zhao. 2016. The harlequin ladybird, *Harmonia axyridis*: global perspectives on invasion history and ecology. Biological Invasions 18: 997-1044.

Samways, M. J., R. Osborn, H. Hastings, and V. Hattingh. 1999. Global climate change and accuracy of prediction of species' geographical ranges: establishment success of introduced ladybirds (Coccinellidae, *Chilocorus* spp.) worldwide. Journal of Biogeography 26: 795-812.

Schuder, I., M. Hommes, and O. Larink. 2004. The influence of temperature and food supply on the development of *Adalia bipunctata* (Coleoptera: Coccinellidae). European Journal of Entomology 101: 379-384.

Ślipiński, A., and D. Burckhardt. 2006. *Revision of the Australian Coccinellidae (Coleoptera). Part 5. Tribe Serangiini*. *In*, Annales Zoologici. Museum and Institute of Zoology, Polish Academy of Sciences. 37-58.

Snyder, W. E., and G. M. Clevenger. 2004. Negative dietary effects of Colorado potato beetle eggs for the larvae of native and introduced ladybird beetles. Biological Control 31: 353-361.

Snyder, W. E., G. M. Clevenger, and S. D. Eigenbrode. 2004. Intraguild predation and successful invasion by introduced ladybird beetles. Oecologia 140: 559-565.

Teets, N. M., Y. Kawarasaki, R. E. Lee, and D. L. Denlinger. 2012. Energetic consequences of repeated and prolonged dehydration in the Antarctic midge, *Belgica antarctica*. Journal of Insect Physiology 58: 498-505.

Terblanche, J. S., and E. Kleynhans. 2009. Phenotypic plasticity of desiccation resistance in *Glossina puparia*: are there ecotype constraints on acclimation responses? Journal of Evolutionary Biology 22: 1636-1648.

Therneau, T. M., and T. Lumley. 2015. Package 'survival'. R Top Doc 128. Available from:ftp://164.41.45.44/pub/plan/R/web/packages/survival/survival.pdf.

Walther, G.-R., A. Roques, P. E. Hulme, M. T. Sykes, P. Pyšek, I. Kühn, M. Zobel, S. Bacher, Z. Botta-Dukat, and H. Bugmann. 2009. Alien species in a warmer world: risks and opportunities. Trends in Ecology & Evolution 24: 686-693.

Weldon, C. W., and P. W. Taylor. 2010. Desiccation resistance of adult Queensland fruit flies *Bactrocera tryoni* decreases with age. Physiological Entomology 35: 385-390.

Weldon, C. W., S. Yap, and P. W. Taylor. 2013a. Desiccation resistance of wild and mass-reared *Bactrocera tryoni* (Diptera: Tephritidae). Bulletin of Entomological Research 103: 690-699.

Weldon, C. W., F. Díaz-Fleischer, and D. Pérez-Staples. 2019. Desiccation resistance of tephritid flies. Area-Wide Management of Fruit Fly Pests: 27.

Weldon, C. W., S. R. Daniels, S. Clusella-Trullas, and S. L. Chown. 2013b. Metabolic and water loss rates of two cryptic species in the African velvet worm genus *Opisthopatus* (Onychophora). Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology 183: 323-332.

Weldon, C. W., C. Nyamukondiwa, M. Karsten, S. L. Chown, and J. S. Terblanche. 2018. Geographic variation and plasticity in climate stress resistance among southern African populations of *Ceratitis capitata* (Wiedemann)(Diptera: Tephritidae). Scientific Reports 8: 9849.

Zeng, B., S. Wang, Y. Li, Z. Xiao, M. Zhou, S. Wang, and D. Zhang. 2020. Effect of long-term cold storage on trehalose metabolism of pre-wintering *Harmonia axyridis* adults and changes in morphological diversity before and after wintering. PLoS ONE 15: e0230435.

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HME co-designed the study, collected and analysed the data, and led the writing of the manuscript.	

For manuscripts intended for publication please indicate target journal:
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Chapter 6

Molecular phylogeny of New Zealand ladybirds

Abstract

Evolutionary relationships between native and introduced species has been argued to influence the successful establishment and dispersal of introduced species in novel environments. However, currently there is little data to test this hypothesis in arthropods. Ladybirds provide an excellent opportunity for testing whether evolutionary relationships influence establishment as they are introduced for the biocontrol of agricultural pests and some species are known invasives. Because we have little understanding of the phylogenetic relationships of New Zealand ladybirds, in this study, we constructed the first molecular phylogeny of New Zealand ladybirds (both native and introduced), based on mitochondrial (cytochrome oxidase subunit I gene) and nuclear (28S rRNA) gene data. We used 15 specimens of known origin (native/introduced) to assess the phylogenetic relationship between invasive (n=1), introduced (n=9) and native (n=5) ladybird species. We further included 15 ladybird species of which the origin is unknown. Our data strongly support the monophyly of Coccinellidae and the subfamilies Microweiseinae and Coccinellinae. Further, our data support the monophyly of the tribe Epilachnini and the paraphyly of tribes Coccinellini, Scymnini, Chilocorini and Coccidulini. Our data indicate that ecological factors rather than relatedness between introduced and native species influences the establishment of introduced ladybirds in New Zealand.

6.1 Introduction

6.1.1 Background

Introduced and invasive species can displace native species, change the pathways of food webs and cause biological homogenisation in recipient ecosystems (Mollot *et al.* 2017; Olden and Poff 2004). Understanding the factors that affect the establishment success of introduced species is important to predict the establishment and dispersal of introduced species and mitigate adverse effects of invasive species. Factors such as dietary range, competition, phenotypic diversity, dispersal ability, climate, propagule pressure and genetic variability can influence the successful establishment of an introduced population (Bomford *et al.* 2010; Dufour *et al.* 2018; Liang *et al.* 2020; Marchetti *et al.* 2004). Evolutionary relatedness (hereafter "relatedness") to native species may be an important factor enabling introduced species to establish in a new environment via two opposing hypotheses. Firstly, the invader's environmental fit may improve as relatedness to native species increases as they may be pre-adapted to similar environmental conditions (preadaptation hypothesis) (Cadotte *et al.* 2018; Curnutt 2000; Sheppard *et al.* 2018). Secondly, invader success may improve as the relatedness to native species decreases due to less competition as there will be less overlap in resource requirements between species (naturalization hypothesis) (Burns 2006; Cadotte *et al.* 2018; Sheppard *et al.* 2018; Strauss *et al.* 2006). Similar ecological functions are observed in phylogenetically related species and research on reptile species in California and Florida (USA) has shown that successfully introduced species are more distantly related to native species compared to failed introductions (Strauss *et al.* 2006; Van Wilgen and Richardson 2011) supporting the naturalisation hypothesis. Studies investigating the relatedness between native and introduced species in arthropods are limited, however research on other animals and plants provide mixed support for both hypotheses (Cadotte *et al.* 2018; Sheppard *et al.* 2018).

6.1.2 Ladybirds as a model system

Ladybirds (Coleoptera: Coccinellidae), are predators that play a complex role in community dynamics (Rudolf 2007; Rudolf and Rasmussen 2013; Wallach *et al.* 2017). There are approximately six thousand described species of ladybird (Ślipiński *et al.* 2011) - also called lady beetles, ladybugs and coccinellids (Hodek *et al.* 2012). Ladybirds are generally thought of as specialised predators of stenorrhynchan Homoptera, but their prey includes many other insects (including ladybirds) and arthropods (mainly mites) and they may also feed, often opportunistically, on honeydew, pollen, and some, like the Epiplachnini are plant feeding while still, some Coccinellini are specialists on fungi (Escalona *et al.* 2017; Giorgi *et al.* 2009; Seago *et al.* 2011). Many ladybirds are introduced around the world as biocontrol agents, although some are also accidentally introduced via plant exports and other cargo. Their broad dietary range can increase establishment success (Emiljanowicz *et al.* 2017) and makes them an ideal model group to study how phylogenetic relationships influence establishment success.

The ladybird (Coccinellidae) phylogeny has been recently revised and reconstructed with combined molecular data (Aruggoda *et al.* 2010; Katoh *et al.* 2014) and data on phenotypic traits such as morphology (Magro *et al.* 2010; Seago *et al.* 2011) and food preferences (Escalona *et al.* 2017; Giorgi *et al.* 2009; Yuan *et al.* 2020). The Coccinellidae phylogenies that have been developed support the monophyly of the group. However, formally the Coccinellidae belonged to the Coleoptera suborder Polyphaga of the superfamily Cucujoidea, and Coccinellidae was a member of the Cerylonid complex (Hodek *et al.* 2012). Recent research classifies the Coccinellidae as one of 15 families in the superfamily Coccinelloidea and is the most derived group and species-rich family of the superfamily with over 6,000 species in approximately 360 genera (Hunt *et al.* 2007; Li *et al.* 2019; Robertson *et al.* 2015; Vandenberg 2002). Coccinellidae is the largest lineage in the superfamily Coccinelloidea and is considered a sister group to the Eupsilobiidae (Robertson *et al.* 2015). The family Coccinellidae is currently divided into two subfamilies, the smaller Microweiseinae (Escalona and Slipinski 2012) and the larger Coccinellinae (Slipinski 2013). Microweiseinae consists of three tribes: Cariodulini, Serangini and Microweiseni (Robertson *et al.* 2015). Coccinellinae contains up to 20 tribes (Seago *et al*. 2011) whose relationships are poorly understood (Robertson *et al.* 2015). However, in Coccinellinae, monophyly of the tribes Coccinellini and Chilocorini which are known as sister taxa, are supported by (Magro *et al.* 2010; Robertson *et al.* 2015; Seago *et al.* 2011). In recent classifications, the genus *Monocoryna* was recently moved to Coccinellidae from Endomychidae (Robertson *et al.* 2015).

6.1.3 Molecular phylogeny

Phylogeny can be based on phenotypic data (morphology and behaviour), molecular data or both. The evolutionary relationships revealed through phylogenies based only on morphological data may be indicative of morphological synapomorphies that are highly homoplastic (Plotkin *et al.* 2017) and the limited number of unambiguous characters that can be used to assess homology is a key constraint in morphology-based phylogenies (Scotland *et al.* 2003). Therefore, to better understand the evolutionary relationships between taxa, it is important to develop molecular phylogenies.

There are different types of genomic data that can be used to develop molecular phylogenies, such as whole genome sequences, individual gene sequences and protein/amino acid sequences.

6.1.3.1 Molecular data

Gene sequences that are used in phylogenetic studies include mitochondrial genes such as cytochrome c oxidase subunit I (COI) (Escalona *et al.* 2017; Ghosh *et al.* 2017; Kobayashi *et al.* 2011; Szawaryn *et al.* 2015; Wang *et al.* 2019), carbamoylphosphate synthetase / aspartate transcarbamylase / dihydroorotase (CAD) (Escalona *et al.* 2017), topoisomerase I (TOPO) (Escalona *et al.* 2017), wingless genes (WGL) (Escalona *et al.* 2017), mitochondrial NADH dehydrogenase subunit 2 (ND2) (Katoh *et al.* 2014), 12S rDNA mitochondrial region (12S) (Magro *et al.* 2010) and nuclear rRNA genes such as 16S rRNA (16S) (Sayed 2016; Szawaryn *et al.* 2015), 18S rRNA (18S) (Giorgi *et al.* 2009; Marin *et al.* 2010; Robertson *et al.* 2008; Szawaryn *et al.* 2015) and 28S rRNA

(28S) (Katoh *et al.* 2014; Kobayashi *et al.* 2009; Li *et al.* 2019; Magro *et al.* 2010; Robertson *et al.* 2008) and internal transcribed spacer regions (ITS) (Kobayashi *et al.* 2011; Schulenburg *et al.* 2001; Wang *et al.* 2019). COI is an informative gene in the mitochondrial genome and can be a good candidate to assess taxonomic connections at family and subfamily level, however to assess relationships between close relatives at lower levels it is important to include highly conserved regions such as nuclear genes (18S, 28S or ITS) (Howland and Hewitt 1995). Even though nuclear 28S rRNA genes are not used as frequently as COI genes in phylogeny construction, they contain conserved and variable regions which could be useful in resolving the relationships between native and introduced ladybirds in New Zealand - as it has been in many insect groups (Philips *et al.* 2016). Therefore, in this study, we used both COI and 28S rRNA genes to develop the phylogeny of ladybirds in New Zealand.

In particular, the phylogenetic relationships between species of ladybirds in New Zealand are poorly understood. Therefore, in this chapter, we constructed a phylogeny of native and introduced ladybirds in New Zealand to understand their relatedness. In our phylogeny, we further included undescribed specimens of ladybird species to determine the taxonomy of these unknown species. In this study, we used Sanger sequencing to generate gene sequences over whole genome sequencing. This way we were able to control which regions were sequenced in order to compare our sanger data with published sequences.

6.2 Methods

6.2.1 Sampling and genotyping

We used specimens of ladybirds collected from eight field sites in the Auckland region (2016- 2018), vouchered museum specimens from the New Zealand Arthropod Collection (1920-2017) and DNA sequences obtained from online databases NCBI and BOLD to build the phylogeny. All specimens collected in New Zealand for the molecular phylogeny were collected in 95% ethanol or stored at -20^{0}C. All samples were identified taxonomically using established taxonomic keys for ladybirds introduced from

Australia as indicated in (Slipinski 2013). The remaining specimens including native ladybird species were identified by H. M. Edirisinghe and confirmed by R. A. D. Leschen (Appendix E: Supplementary table 6.1).

6.2.2 DNA extraction

DNA extraction comprises four steps (See Roe *et al.* (1996) and Zhou and Ling (2011)). Different DNA extraction toolkits have been developed based on cell type and age or the quality of samples. Standard protocols from the manufacturer's manual for DNA extraction from animal tissues were followed using industry standard toolkits (Qiagen DNeasy Blood and Tissue toolkit & X-tractor GeneTM etc.) for 30 specimens (details in Appendix E: Supplementary table 6.1). After extracting DNA from the samples, we cut the sequences corresponding to the genes COI and 28S from the DNA using primer pairs. Primers are short DNA sequences that bind onto single stranded DNA (Rowe *et al.* 2017). Partial sequences of mitochondrial gene cytochrome oxidase subunit I (COI) and nuclear gene 28S rRNA (Philips et al., 2016, Escalona et al., 2017) were amplified by polymerase chain reaction (PCR) with the primers Cl-J-1718/TL2-N-3014 and 28S ff/28S dd respectively (primer sequences; Appendix E: Supplementary table 6.2). We were not able to obtain specimens of all recorded native and introduced ladybird species recorded in New Zealand. However, for the introduced species we used sequenced data from GenBank from either the same species or same genus (Escalona *et al.* 2017). We obtained sequences of other ladybird species from published phylogenies (Appendix E: Supplementary table 6.3) and outgroup sequences from NCBI and BOLD databases using the 'regPhlyo' package (Eme *et al.* 2019) in R statistical software; Endomychidae (*Corynomalus* sp. and *Mycetina horni* Crotch), Discolomatidae (*Aphanocephalus* sp.), Latridiidae (*Latridius crenatus* Le Conte, 1855), Corylophidae (*Periptyctus* sp.) and Erotylidae (*Pselaphacus nigropunctatus* Percheron, 1835).

6.2.3 Sequencing and sequence alignment

We sequenced COI and 28S genes using Sanger's sequencing method to develop our phylogeny. In Sanger's method we follow the natural process of DNA polymerisation using artificially prepared dideoxyribose nucleotides (Saitou 2013). Dideoxyribose stops DNA polymerisation by DNA polymerase. PCR products for COI and 28S genes were cycle-sequenced with the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, EcoGene lab, Manaaki Whenua, Auckland, New Zealand) in a reaction volume of 10 µL consisting of 2 µL of 5X Sequencing buffer, 0.5 µL of BigDye, 0.5 µL (10 µM) of each primer, and 2.0 µL of PCR product. 50 cycle sequencing reactions were carried out at 96°C for 10 s, 50°C for 5 s, 60°C for 4 min and the products from cycle sequencing were precipitated using a standard ethanol precipitation method (Sambrook and Russell 2006). The PCR products of COI gene were visualised on 10% agarose gel. The PCR products of 28S genes were also visualised on 10% agarose gel and purified before cycle sequencing. The precipitated products were re-suspended in 10 µl of ABI Hi-Di formamide and denatured at 95°C for two minutes followed by 5 min on ice. Finally, the products were sequenced on an ABI Prism 3130xl automated genetic analyzer (Applied Biosystems, EcoGene lab, Manaaki Whenua, Auckland, New Zealand).

6.2.4 Phylogenetic analyses

There are many phylogeny building methods including Neighbour Joining (NJ), Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Inference (BI) methods (Saitou 2013). The maximum likelihood method is one of the most widely used methods in molecular phylogenies of ladybirds and other beetles (Arenas and Stevens 2017; Ghosh *et al.* 2017; Kobayashi *et al.* 2009; Magro *et al.* 2020; Szawaryn *et al.* 2015). This includes both nucleotide and protein data. In a comparison of the most commonly used methods ML, MP and BI; MP produces the best fit tree in the least number of steps without the ability to specify character evolution in the model as opposed to ML and BI methods (Felsenstein 2004). Bayesian methods are sometimes considered closely related to ML but use the relationship between prior and posterior probabilities in solving the phylogenetic relationships (Huelsenbeck *et al.* 2001). The goal of using BI methods is to find the tree which has the highest posterior probability for a given data set (Giorgi *et al.* 2009; Halim *et al.* 2017; Magro *et al.* 2010; Niu *et al.* 2016; Robertson *et al.* 2008; Szawaryn *et al.* 2015; Wang *et al.* 2019; Zhang *et al.* 2016). Therefore, the difference between ML and Bayesian methods is that the ML method assumes prior probabilities or the tree probability to be equal between taxonomic units (Felsenstein 2004; Saitou 1990). However, since we use different likelihood functions in different tree topologies, comparing different tree topologies is not practical when we use ML trees as different likelihood functions use different probability spaces. This confusion in prior probabilities is avoided in Bayesian methods and selecting the tree topology with the highest probability in the ML may produce the correct tree (Huelsenbeck *et al.* 2001). In this study, we selected the BI method as it allows an estimate of posterior probability data based on the observed data (adjusted for prior probabilities) based on the character evolution model designed. Further, the Markov Chain Monte Carlo sampling method which is used for sampling in BI methods makes it a less biased predictor of phylogenetic accuracy compared to other methods using traditional bootstrapping (Huelsenbeck *et al.* 2001; Saitou 2013).

6.2.4.1 Phylogenetic Analyses

All the phylogenetic analyses were conducted in Geneious Prime 2019.0.4 software (Kearse *et al.* 2012). We created the consensus sequences for each specimen from sequences in forward and reverse directions. The sequences were then translated from coding genes to protein sequences to confirm that there were no stop codons, frameshifts, inserts or deletions. Multiple sequence alignment was carried out using the built-in multiple align plugin; MAFFT version 7.388 (Katoh *et al.* 2002; Katoh and Standley 2013) in Geneious software using default parameters. All the analysed sequences were submitted to NCBI for accession numbers.

We used BI methods to reconstruct a phylogeny of ladybird beetles including New Zealand native ladybirds using COI (Appendix E: Supplementary figure 6.1) and 28S gene (Appendix E: Supplementary figure 6.2) data as well as concatenated alignments using both COI and 28S alignment data (figure 6.1 and Appendix E: Supplementary figure 6.3). Bayesian Inference analysis was performed using MrBayes version 3.2.6 (Ronquist *et al.* 2011) in the Geneious software. The analysis was run for 10 million cycles using four heated chains with a sampling frequency of 1/5,000 trees and burn-in at 100,000 samples. The result was visualized and edited using FigTree version 1.4.4 (Rambaut 2018). The confidence values of the BI tree were expressed as the Bayesian posterior probabilities (BPP).

6.3 Results

DNA extraction and sequencing were successful for most specimens collected in New Zealand for both COI and 28S rRNA genes. However, for some species, COI genes were unable to be sequenced and the 28S gene was not sequenced in two specimens (Table 6.1). Sequence length in COI genes ranged from 1134-1253bp and in 28S genes ranged from 330-687bp.

Table 6.1 Sequence lengths (seq. length) and guanine-cytosine (GC) content of COI and 28s sequences used in the phylogenetic analysis

Specimen	**Origin***	**COI**		**28s**	
		%GC	**Seq. length (bp)**	**%GC**	**Seq length (bp)**
Adoxellus_flavihirtus	Native	29.10%	1253	52.70%	659
Apolinus_lividigaster	Introduced	32.20%	1231	53.20%	669
Cleobora_mellyi	Introduced	32.30%	1248	52.80%	642
Coccinella_undecimpunctata	Introduced			51.80%	682
Coelophora_inaequdlis	Introduced	29.50%	1233	52.60%	642
Cryptolaemus_montrouzieri	Introduced	27.60%	1223	53.00%	643
Diomus_sp.	Introduced	28.80%	1245	53.10%	659
Epilachna_vigintioctopunctata	Introduced	29.40%	1250	54.20%	660
Halmus_chalybeus	Introduced	28.80%	1252	52.10%	664
Harmonia_antipoda	Native	28.30%	1238	52.10%	641
Harmonia_axyridis	Invasive	30.80%	1218	52.20%	687
Illeis_galbula	Introduced			52.30%	661
Rhyzobius_minutulus	Native	29.10%	1134	49.40%	330
*Rhyzobius*_pink				53.20%	687
Rhyzobius_rarus	Native	29.00%	1203	53.30%	660

*Rhyzobius_sp_*Albany		31.40%	1221	52.50%	642
*Rhyzobius_sp_*Craigieburn		32.50%	1246		
*Rhyzobius_sp_*Longbay1		30.30%	1239	53.10%	663
*Rhyzobius_sp_*Longbay2		31.60%	1232	51.90%	626
*Rhyzobius_sp_*MtIron				52.80%	668
*Rhyzobius_sp_*Threekings		28.60%	1249	53.40%	626
Rhyzobius_sp1		32.20%	1217	53.30%	672
Rhyzobius_sp18				53.30%	662
Rhyzobius_sp2				52.30%	671
Rhyzobius_sp20				52.90%	671
Rhyzobius_sp24				53.10%	674
Rhyzobius_sp25				53.20%	663
Rhyzobius_sp3		31.10%	1233	53.50%	673
Rhyzobius_sp4		31.80%	1227	52.80%	606
Rhyzobius_sp5				54.30%	663
Rhyzobius_sp8		32.30%	1207	53.20%	649
Serangium_maculigerum	Introduced	30.00%	1234	52.10%	666
Veronicobius_sp.	Native	32.50%	1135	53.60%	668

*Origin is left blank when unknown

These sequences were then aligned with sequences obtained from the NCBI and BOLD databases. They were then concatenated based on the specimen/sequence name to run the BI to create phylogenetic trees. The phylogenetic tree developed from BI showed monophyly of Coccinellidae with a clear separation from the outgroup species (PP = 1.000; figure 6.1). The BI phylogeny supports the recent classification of subfamily Microweiseinae as a sister group to Coccinellinae (PP = 0.9980; figure 6.1) (Robertson *et al.* 2015). However, our phylogeny highly supports the *Rodolia cardinalis* as the sister taxa for subfamily Microweiseinae. The data highly supports the clustering of Microweiseinae + *Rodolia cardinalis* as sister taxa for the rest of the Coccinellinea species (PP= 0.9520; figure 6.1). Our concatenated phylogeny does not support the monophyly of subfamilies Microweiseinae and Coccinellinae based on the classification of *Rodolia cardinalis* (PP= 0.9980; figure 6.1). However, the phylogeny constructed using only COI sequences moderately supports the monophyly of subfamilies Microweiseinae and Coccinellinae, and *Rodolia cardinalis* as the sister taxon for other taxa in Coccinellinae (PP=0.7502, Appendix E: Supplementary figure 6.1).

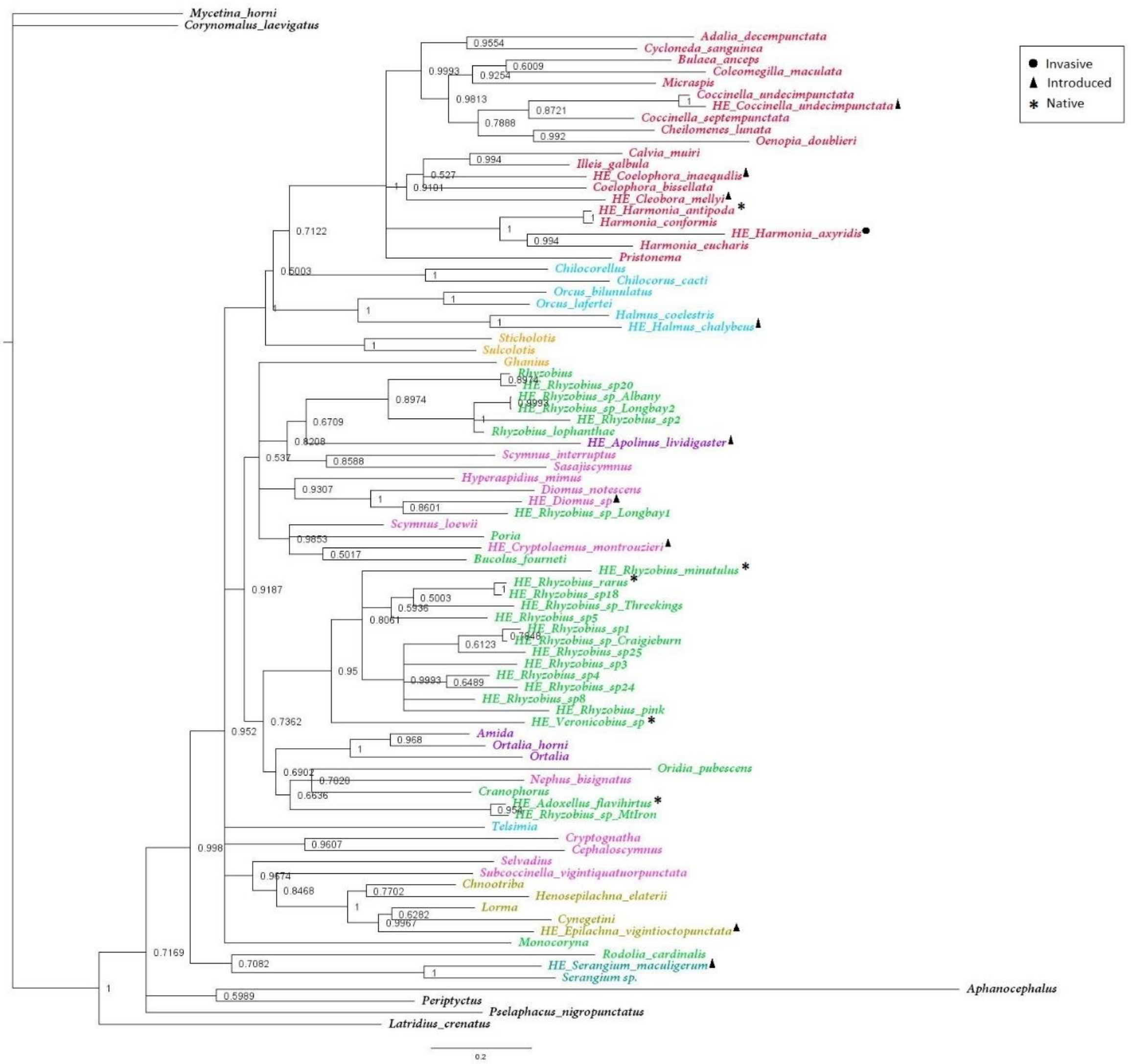

Figure 6.1 Phylogenetic relationships among 88 Coccinellidae species inferred from mitochondrial (COI) and nuclear (28s) sequences.

One invasive, nine introduced and five native species collected in New Zealand are included in this phylogenetic analysis. Numbers at nodes are Bayesian posterior probabilities (PP) of the dataset. Species collected in New Zealand has a prefix of 'HE' in the name displayed in the phylogeny. Species that belong to the same tribe as clustered in (Robertson et al. 2015) are indicated in similar text colours.

Serangium sp. was the only representative of the family Microweiseinae for the phylogeny. Within Coccinellinae, the monophyly of tribe Epilachnini (PP = 0.8468; figure 6.1) and paraphyly of Chilochorini (PP = 1.000; figure 6.1) were highly supported by the data. Further, within Coccinellinea, branches such as Sulcolotis + Sticholotis were recovered as monophylytic with high support from the data (PP = 1.000; figure 6.1). *Ghanius* however was recovered as a sister taxon for Coccidulini + Scymnini + *Apolinus* sp. with low support from the data (PP = 0.5370; figure 6.1). Tribes Chilochorini, Coccinellini and Sulcolotis + Sticholotis were recovered as sister tribes with high support from the data (PP= 1.000; figure 6.1). Tribes Coccidulini and Schymnini were also supported as paraphyletic groups (PP = 0.7169) where a) *Scymnus loewii* + *Poria* sp. + (*Chryptolaemus montrouzieri* [introduced to New Zealand] + *Bucolus* sp.) were clustered together (PP=0.9853; figure 6.1), b) *Diomus* sp. (introduced to New Zealand) clustered with *Rhyzobius* sp. (unknown origin) (PP= 0.8601; figure 6.1) and c) *Cranophorus* and *Oridia pubescens* clustered with *Nephus bisignatus* (PP= 0.7828; figure 6.1). In the concatenated sequences we removed the *Illeis galbula* (introduced to New Zealand) sample we collect from New Zealand as it was problematic. For example, due to the clustering of *Illeis galbula* with *Rhyzobius* species (PP=1.000; Appendix E: Supplementary figures 6.2 and 6.3), the tribe Coccinellini was classified as paraphyletic. Clustering of *I. galbula* with *Rhyzobius* species was supported by BLAST results where the 28S fragment of *I. galbula* extracted from the New Zealand specimen was revealed as 99% similar to *Rhyzobius lopanathae* (Appendix E: Supplementary table 6.4). Therefore, considering the similarity of 28S fragment to *Rhyzobius* sp. and the absence of COI sequence, we removed the *I. galbula* sample from the final phylogenetic analysis.

Coccinella undecimpunctata (introduced to New Zealand) collected from New Zealand was clustered with *C. undecimpunctata* sequences obtained from the NCBI database with high support from the data (PP = 1.000; figure 6.1). Data highly supported the clustering of *Cleobora melyi* (introduced to New Zealand) + (*Coelophora bissellata* + *Coelophora inaequdils* (introduced to New Zealand) + (*Calvia muiri* + *Illeis galbula*)) (PP = 0.9101; figure 6.1). *Harmonia axyridis* (invasive in New Zealand) collected from Auckland was clustered with *H. eucharis* and the clustering was highly supported by the data (PP = 0.9940; figure 6.1). Clustering of native *H. antipoda* (native to New Zealand) with *H. conformis* was highly supported by data (PP = 1.000; figure 6.1). *Halmus chalybeus* (introduced to New Zealand) collected from New Zealand was clustered together with *Halmus coelestris* and data highly supported the clustering (PP=1.000; figure 6.1). Three *Rhyzobius* specimens collected from New Zealand were clustered as sister taxa for *Rhyzobius lophanthae* and this clustering was highly supported by the data (PP = 1.000; figure 6.1). *Apolinus lividigaster* (introduced to

New Zealand) was clustered as a sister taxon for the above clustering of Rhyzobius species with high support from the data (PP=0.8208; figure 6.1). *Diomus* species (introduced to New Zealand) collected from New Zealand was clustered together with a *Rhyzobius* species (unknown origin) collected from New Zealand, and the clustering was highly supported from the data (PP=0.8601; figure 6.1). Further data showed high support for the clustering of *Rhyzobius* specimen (unknown origin) and *Diomus* sp. (introduced to New Zealand) as a sister taxon for *Diomus notescens* (PP=1.000; figure 6.1). *Cryptolaemus montrouzieri* (introduced to New Zealand) was clustered together with *Bucolus fourneti* with low support from the data (PP=0.5017; figure 6.1). *Veronicobius* sp. (native to New Zealand) was classified as a sister taxon for a cluster of 13 out of 19 *Rhyzobius* sp. (origin unknown) collected in New Zealand (PP = 0.9500; figure 6.1). There was high support for the clustering of *Rhyzobius* sp. specimen collected from New Zealand with native *Adoxellus flavirhtus* by the data (PP = 0.9540; figure 61). *Epilachna vigintioctopunctata* collected in New Zealand is clustered as a sister taxon for the cluster of Lorma + Cynegetini was supported highly by the data (PP = 0.9967; figure 6.1). *Serangium maculigerum* specimen collected from New Zealand clustered with *Serangium* sp. and the clustering was highly supported from the data (PP=1.000; figure 6.1).

6.4 Discussion

In this study, we used mitochondrial (COI) and nuclear (28S) data to develop a phylogeny of New Zealand ladybirds. Using both conserved mitochondrial regions together with nuclear data such as 28S rRNA increases the accuracy of predicting phylogenetic relationships in higher taxonomic levels as well as closely related taxa (Wang *et al.* 2019). Therefore, this molecular analysis produced the first phylogeny to include native ladybirds in New Zealand.

Our phylogeny supports the monophyly of Coccinellidae as has previous studies using both molecular and morphological data (Szawaryn and Szwedo 2018). Our concatenated phylogeny does not support the classification of two main subfamilies (Magro *et al.* 2010; Seago *et al.* 2011). However, our phylogeny based on COI sequences supports classification of the smaller Microweiseinae and the larger Coccinellinae similar to the recent reconstruction of a phylogeny for the Coccinelloidea (Robertson *et al.* 2015). Our COI data strongly supports *Monocoryna* and *Rodalia cardinails* as sister taxa to all other Coccinellinae tribes as in previous studies (Robertson *et al.* 2015). Further, our study aligns with the monophyly of Epilachnini as shown in previous studies (Halim *et al.* 2017; Robertson *et al.* 2008; Wang *et al.* 2019; Yuan *et al.* 2020). Clustering of *E. vigintioctopunctata* in our phylogeny aligns with the Australian *Epilachna* sp. phylogeny by (Katoh *et al.* 2014), which was developed considering biogeographical and host relationships between species in tribe Epilachnini.

Our results suggested a monophyly of Chilocorini as found in Li *et al.* (2019), but our results did not cluster *Telsimia* with Chilororini. Stichotolidini are clustered within the Chilocorini separating the larger cluster of Chilocorus + (Orcus + Halmus) from *Telsimia* species. In our phylogeny the monophyly of Chilocorus is supported as opposed to Li *et al.* (2019). The moderate support for the clustering of *H. chalybeus* collected from New Zealand suggests that there the two populations may have genetically diverged since its introduction to New Zealand in 1899. Further, *H. chalybeus* shows a wider local distribution than the native species including some areas that overlap (iNaturalistNZ data, 2020). Therefore, the establishment and distribution of introduced ladybirds may be affected by ecological factors rather than the relatedness between introduced and native species.

Our phylogeny highly supports Coccinellini as a sister group of Chilocorini as in previous studies (Escalona *et al.* 2017). Paraphyly of the Coccinellini is also supported by our data as shown by (Yuan *et al.* 2020) based on the diet of ladybird species. Both COI and 28S molecular data supports phylogeny of all species except *I. galbula* (supported by 28S only) and *Coccinella undecimpunctata* (supported by COI only). However, *I. galbula* was revealed to be more related to *R. lophanthae* than other *I. galbula* 28s sequences in the NCBI database. This may have resulted in the clustering of *I. galbula* with *Rhyzobius* sp. and separated from other species in the tribe Coccinellini in the phylogenies constructed using 28S sequences and concatenated sequences. Also, note that the quality of our 28S sequences (short sequence length) may have influenced misplacements that does not support recently accepted phylogenies. The clustering of the native *H. antipoda* and invasive *H. axyridis* was highly supported by our data, and the phylogeny suggested that *H. anitipoda* is more closely related to the introduced Australian *H. conformis* than *H. axyridis*. However, native *H. anitipoda* shows limited distribution in the South Island of New Zealand compared to introduced *H. conformis* and invasive *H. axyridis* which is distributed in both the North and South Islands of New Zealand (iNaturalistNZ data, 2020). This further suggests that the distribution and establishment of introduced species may be affected by ecological factors than the relatedness between native and introduced species (Chapin and Brou 1991; Ukrainsky and Orlova-Bienkowskaja 2014) in New Zealand ladybirds.

Our data support that tribes Scyminini and Coccidulini are paraphyletic as found in previous studies (Wang *et al.* 2019). Clustering of introduced *Diomus* sp. collected from New Zealand with *Diomus notescens* (Australian) suggests it is closely related to the Australian species. A specimen misidentified as *Rhyzobius* sp. was clustered with *Diomus* sp. in our phylogeny. Revisiting its classification using morphology, H. M. Edirisinghe and R. A. D. Leschen confirmed that the specimen was a *Diomus* species misidentified as a *Rhyzobius* species. One of the two large clusters of Coccidulini shows *Veronicobius* sp. as a sister taxon for 13 *Rhyzobius* spp. (including *R. minutulus*). Even though these *Rhyzobius* spp. were clustered with both *Veronicobius* sp. and *R. minulutus,* more morphological and molecular analysis should be carried out to determine their taxonomy and origin (figure 6.1). *Apolinus lividigaster* was identified as the sister taxon for the smaller cluster in tribe Coccidulini which consisted of four *Rhyzobius* specimens collected from New Zealand. However, it is difficult to infer the origin of the undescribed *Rhyzobius* sp. specimens from our data.

Our results do not support relatedness influencing the establishment success of introduced ladybirds in New Zealand. Instead we believe that other ecological factors as discussed in chapter 2, such as the invasiveness, invasibility and stochastic events following introduction affect the establishment success of introduced species. Therefore, we believe, while relatedness correlates with invasiveness (Cadotte *et al.* 2018; Sheppard *et al.* 2018; Strauss *et al.* 2006), it is likely that individual traits and invasibility of habitats drive establishment success more. For instance, if native and introduced ladybird species that are in New Zealand target the same resources and habitat niches, there will be competition between species. If the introduced ladybirds are more aggressive than native ladybirds, then they would have a competitive advantage increasing the probability they will establish in the new environment.

Alternatively, native ladybirds may be well adapted to the environment and therefore have a competitive advantage over introduced ladybirds. However, successfully introduced ladybirds often have a large capacity to adapt and are often harmful to native species, displacing or reducing native populations (Roy *et al.* 2012).

We need to understand and compare the relatedness between species with failed introductions with native and successfully established introduced species. This will help us identify if relatedness influence failed introductions. However, studies investigating phylogenetic relationships in unsuccessful introductions of ladybirds are limited (Cock *et al.* 2016; Soares *et al.* 2017). We can further improve our understanding on establishment successes and evolutionary relationships by including corresponding samples of introduced species from their countries of origin to understand how introduced populations have genetically diverged from the source populations.

In conclusion, our study shows relatedness may not play as large a role in the establishment success of introduced ladybird species as previously thought. Therefore, molecular phylogenies can be coupled with phenotypic traits and invasibility of introduced areas to understand the evolutionary relationships between native, established species and failed introductions to further understand the factors affecting establishment success.

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6.6 References

Arenas, L. M., and M. Stevens. 2017. Diversity in warning coloration is easily recognized by avian predators. Journal of Evolutionary Biology 30: 1288-1302.

Aruggoda, A. G. B., R. Shunxiang, and Q. Baoli. 2010. Molecular phylogeny of ladybird beetles (Coccinellidae: Coleoptera) inferred from mitochondrial 16s rDNA sequences. Tropical Agricultural Research 21: 209-217.

Bomford, M., S. C. Barry, and E. Lawrence. 2010. Predicting establishment success for introduced freshwater fishes: a role for climate matching. Biological Invasions 12: 2559-2571.

Burns, J. H. 2006. Relatedness and environment affect traits associated with invasive and noninvasive introduced Commelinaceae. Ecological Applications 16: 1367-1376.

Cadotte, M. W., S. E. Campbell, S.-p. Li, D. S. Sodhi, and N. E. Mandrak. 2018. Preadaptation and naturalization of nonnative species: Darwin's two fundamental insights into species invasion. Annual Review of Plant Biology 69: 661-684.

Chapin, J. B., and V. Brou. 1991. *Harmonia axyridis* (Pallas), the third species of the genus to be found in the United States (Coleoptera: Coccinellidae). Proceedings of the Entomological Society of Washington 93: 630-635.

Cock, M. J., S. T. Murphy, M. T. Kairo, E. Thompson, R. J. Murphy, and A. W. Francis. 2016. Trends in the classical biological control of insect pests by insects: an update of the BIOCAT database. BioControl 61: 349-363.

Curnutt, J. L. 2000. Host-area specific climatic-matching: similarity breeds exotics. Biological Conservation 94: 341-351.

Dufour, C. M., J. B. Losos, and A. Herrel. 2018. Do differences in bite force and head morphology between a native and an introduced species of anole influence the outcome of species interactions? Biological Journal of the Linnean Society 125: 576-585.

Eme, D., M. J. Anderson, C. D. Struthers, C. D. Roberts, and L. Liggins. 2019. An integrated pathway for building regional phylogenies for ecological studies. Global Ecology and Biogeography 28: 1899-1911.

Emiljanowicz, L. M., H. A. Hager, and J. A. Newman. 2017. Traits related to biological invasion: A note on the applicability of risk assessment tools across taxa. NeoBiota 32: 31-64.

Escalona, H. E., and A. Slipinski. 2012. Generic revision and phylogeny of Microweiseinae (Coleoptera: Coccinellidae). Systematic Entomology 37: 125-171.

Escalona, H. E., A. Zwick, H.-S. Li, J. Li, X. Wang, H. Pang, D. Hartley, L. S. Jermiin, O. Nedved, B. Misof, O. Niehuis, A. Slipinski, and W. Tomaszewska. 2017. Molecular phylogeny reveals food plasticity in the evolution of true ladybird beetles (Coleoptera: Coccinellidae: Coccinellini). BMC Evolutionary Biology 17.

Felsenstein, J. 2004. *Inferring phylogenies, volume 2*. Sinauer Associates Sunderland, MA.

Ghosh, S., G. T. Behere, and B. K. Agarwala. 2017. Molecular characterization of ladybird predators (Coleoptera: Coccinellidae) of aphid pests (Homoptera: Aphididae) in North East India. Current Science 113: 1755-1759.

Giorgi, J. A., N. J. Vandenberg, J. V. McHugh, J. A. Forrester, S. A. Silpinski, K. B. Miller, L. R. Shapiro, and M. F. Whiting. 2009. The evolution of food preferences in Coccinellidae. Biological Control 51: 215-231.

Halim, M., A. Aman-Zuki, M. A. Mohammed, and S. Yaakop. 2017. DNA barcoding and relationships of eight ladybugs species (Coleoptera: Coccinellidae) that infesting several crops from Peninsular Malaysia. Journal of Asia-Pacific Entomology 20: 814-820.

Hodek, I., A. Honek, and H. F. Van Emden. 2012. *Ecology and behaviour of the ladybird beetles (Coccinellidae)*, Wiley-Blackwell, Chichester.

Howland, D. E., and G. M. Hewitt. 1995. Phylogeny of the Coleoptera based on mitochondrial Cytochrome-Oxidase-I sequence data. Insect Molecular Biology 4: 203-215.

Huelsenbeck, J. P., F. Ronquist, R. Nielsen, and J. P. Bollback. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294: 2310-2314.

Hunt, T., J. Bergsten, Z. Levkanicova, A. Papadopoulou, O. S. John, R. Wild, P. M. Hammond, D. Ahrens, M. Balke, and M. S. Caterino. 2007. A comprehensive phylogeny of beetles reveals the evolutionary origins of a superradiation. Science 318: 1913-1916.

Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution 30: 772-780.

Katoh, K., K. Misawa, K. i. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30: 3059-3066.

Katoh, T., S. Koji, T. A. Ishida, K. W. Matsubayashi, S. Kahono, N. Kobayashi, K. Furukawa, V. Bui Tuan, J. Vasconcellos-Neto, C. N. Lange, G. Goergen, S. Nakano, N.-N. Li, G.-Y. Yu, and H. Katakura. 2014. Phylogeny of *Epilachna, Henosepilachna,* and some minor genera of phytophagous ladybird beetles (Coleoptera: Coccinellidae: Coccinellinae: Epilachnini), with an analysis of ancestral biogeography and host-plant utilization. Zoological Science 31: 820-830.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, and C. Duran. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647-1649.

Kobayashi, N., Y. Ohta, T. Katoh, S. Kahono, S. Hartini, and H. Katakura. 2009. Molecular phylogenetic analysis of three groups of Asian epilachnine ladybird beetles recognized by the female internal reproductive organs and modes of sperm transfer. Journal of Natural History 43: 1637-1649.

Kobayashi, N., M. Kumagai, D. Minegishi, K. Tamura, T. Aotsuka, and H. Katakura. 2011. Molecular population genetics of a host-associated sibling species complex of phytophagous ladybird beetles (Coleoptera: Coccinellidae: Epilachninae). Journal of Zoological Systematics and Evolutionary Research 49: 16-24.

Li, W., P. Laczynski, H. E. Escalona, J. Eberle, L. Huo, X. Chen, W. Huang, B. Chen, D. Ahrens, A. Slipinski, W. Tomaszewska, and X. Wang. 2019. Combined molecular and morphological data provide insights into the evolution and classification of Chilocorini ladybirds (Coleoptera: Coccinellidae). Systematic Entomology 45:447–463.

Liang, S.-H., B. A. Walther, and B.-S. Shieh. 2020. Determinants of establishment success: Comparing alien and native freshwater fishes in Taiwan. PLoS ONE 15: e0236427.

Magro, A., E. Lecompte, F. Magne, J. L. Hemptinne, and B. Crouau-Roy. 2010. Phylogeny of ladybirds (Coleoptera: Coccinellidae): Are the subfamilies monophyletic? Molecular Phylogenetics and Evolution 54: 833-848.

Magro, A., E. Lecompte, J.-L. Hemptinne, A. O. Soares, A.-M. Dutrillaux, J. Murienne, H. Fursch, and B. Dutrillaux. 2020. First case of parthenogenesis in ladybirds (Coleoptera: Coccinellidae) suggests new mechanisms for the evolution of asexual reproduction. Journal of Zoological Systematics and Evolutionary Research 58: 194-208.

Marchetti, M. P., P. B. Moyle, and R. Levine. 2004. Invasive species profiling? Exploring the characteristics of non-native fishes across invasion stages in California. Freshwater Biology 49: 646-661.

Marin, J., B. Crouau-Roy, J.-L. Hemptinne, E. Lecompte, and A. Magro. 2010. *Coccinella septempunctata* (Coleoptera, Coccinellidae): a species complex? Zoologica Scripta 39: 591-602.

Mollot, G., J. Pantel, and T. Romanuk. 2017. The effects of invasive species on the decline in species richness: a global meta-analysis. Advances in Ecological Research 56: 61-83.

Niu, F.-F., L. Zhu, S. Wang, and S.-J. Wei. 2016. The mitochondrial genome of the multicolored Asian lady beetle *Harmonia axyridis* (Pallas) and a phylogenetic analysis of the Polyphaga (Insecta: Coleoptera). Mitochondrial DNA Part A 27: 2725-2727.

Olden, J. D., and N. L. Poff. 2004. Ecological processes driving biotic homogenization: testing a mechanistic model using fish faunas. Ecology 85: 1867-1875.

Philips, T. K., M. Callahan, J. Orozco, and N. Rowland. 2016. Phylogenetic analysis of the North American beetle genus *Trichiotinus* (Coleoptera: Scarabaeidae: Trichiinae). Psyche: A Journal of Entomology 2016.

Plotkin, A., O. Voigt, E. Willassen, and H. T. Rapp. 2017. Molecular phylogenies challenge the classification of Polymastiidae (Porifera, Demospongiae) based on morphology. Organisms Diversity & Evolution 17: 45-66.

Rambaut, A. 2018. FigTree v1. 4.4, a Graphical Viewer of Phylogenetic Trees. 2014. Java.

Robertson, J. A., M. F. Whiting, and J. V. McHugh. 2008. Searching for natural lineages within the Cerylonid Series (Coleoptera : Cucuioidea). Molecular Phylogenetics and Evolution 46: 193-205.

Robertson, J. A., A. ŚLIPIŃSKI, M. Moulton, F. W. Shockley, A. Giorgi, N. P. Lord, D. D. Mckenna, W. Tomaszewska, J. Forrester, and K. B. Miller. 2015. Phylogeny and classification of Cucujoidea and the recognition of a new superfamily Coccinelloidea (Coleoptera: Cucujiformia). Systematic Entomology 40: 745-778.

Roe, B. A., J. S. Crabtree, and A. Khan. 1996. *DNA Isolation and Sequencing*. John Wiley, New York.

Ronquist, F., J. Huelsenbeck, and M. Teslenko. 2011. Draft MrBayes version 3.2 manual: tutorials and model summaries. Distributed with the software from http://brahms. biology. rochester. edu/software. html: 1-105.

Rowe, G., M. Sweet, and T. J. C. Beebee. 2017. *An introduction to molecular ecology*. Oxford: Oxford University Press: 346.

Roy, H. E., T. Adriaens, N. J. Isaac, M. Kenis, T. Onkelinx, G. S. Martin, P. M. Brown, L. Hautier, R. Poland, and D. B. Roy. 2012. Invasive alien predator causes rapid declines of native European ladybirds. Diversity and Distributions 18: 717-725.

Rudolf, V. H. W. 2007. The interaction of cannibalism and omnivory: consequences for community dynamics. Ecology 88: 2697-2705.

Rudolf, V. H. W., and N. L. Rasmussen. 2013. Ontogenetic functional diversity: Size structure of a keystone predator drives functioning of a complex ecosystem. Ecology 94: 1046-1056.

Saitou, N. 1990. Maximum likelihood methods. Methods in Enzymology 183: 584-598.

Saitou, N. 2013. *Introduction to evolutionary genomics.* Springer. London.

Sambrook, J., and D. W. Russell. 2006. Standard ethanol precipitation of DNA in microcentrifuge tubes. Cold Spring Harbor Protocols 2006: pdb. prot4456.

Sayed, S. M. 2016. Molecular diversity of the lady beetles, *Coccinella undecimpunctata* L. and *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) in Saudi Arabia. Egyptian Journal of Biological Pest Control 26: 351-355.

Schulenburg, J., J. M. Hancock, A. Pagnamenta, J. J. Sloggett, M. E. N. Majerus, and G. D. D. Hurst. 2001. Extreme length and length variation in the first ribosomal internal transcribed spacer of ladybird beetles (Coleoptera : Coccinellidae). Molecular Biology and Evolution 18: 648-660.

Scotland, R. W., R. G. Olmstead, and J. R. Bennett. 2003. Phylogeny Reconstruction: The Role of Morphology. Systematic Biology 52: 539-548.

Seago, A. E., J. A. Giorgi, J. Li, and A. Slipinski. 2011. Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. Molecular Phylogenetics and Evolution 60: 137-151.

Sheppard, C. S., M. Carboni, F. Essl, H. Seebens, D. Consortium, and W. Thuiller. 2018. It takes one to know one: Similarity to resident alien species increases establishment success of new invaders. Diversity and Distributions 24: 680-691.

Slipinski, A. 2013. *Australian ladybird beetles (Coleoptera: Coccinellidae): their biology and classification.* Csiro Publishing.

Ślipiński, S., R. Leschen, and J. Lawrence. 2011. Order coleoptera linnaeus, 1758. Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. Zootaxa 3148: 203-208.

Soares, A. O., A. Honěk, Z. Martinkova, J. Skuhrovec, P. Cardoso, and I. Borges. 2017. *Harmonia axyridis* failed to establish in the Azores: the role of species richness, intraguild interactions and resource availability. BioControl 62: 423-434.

Strauss, S. Y., C. O. Webb, and N. Salamin. 2006. Exotic taxa less related to native species are more invasive. Proceedings of the National Academy of Sciences 103: 5841-5845.

Szawaryn, K., and J. Szwedo. 2018. Have ladybird beetlesand whiteflies co-existed for at least 40 Mya? Palz 92: 593-603.

Szawaryn, K., L. Bocak, A. Slipinski, H. E. Escalona, and W. Tomaszewska. 2015. Phylogeny and evolution of phytophagous ladybird beetles (Coleoptera: Coccinellidae: Epilachnini), with recognition of new genera. Systematic Entomology 40: 547-569.

Ukrainsky, A. S., and M. J. Orlova-Bienkowskaja. 2014. Expansion of *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) to European Russia and adjacent regions. Biological Invasions 16: 1003-1008.

Van Wilgen, N. J., and D. M. Richardson. 2011. Is phylogenetic relatedness to native species important for the establishment of reptiles introduced to California and Florida? Diversity and Distributions 17: 172-181.

Vandenberg, N. J. 2002. Coccinellidae Latreille 1807. *In* American Beetles. (Eds. Arnett, RH jr.(†), Thomas, MC, Skelly, PE & Frank, JH: 371-389.

Wallach, A. D., A. H. Dekker, M. Lurgi, J. M. Montoya, D. A. Fordham, and E. G. Ritchie. 2017. Trophic cascades in 3D: network analysis reveals how apex predators structure ecosystems. Methods in Ecology and Evolution 8: 135-142.

Wang, Z.-L., T.-Z. Wang, H.-F. Zhu, Z.-Y. Wang, and X.-P. Yu. 2019. DNA barcoding evaluation and implications for phylogenetic relationships in ladybird beetles (Coleoptera: Coccinellidae). Mitochondrial DNA Part A 30: 1-8.

Yuan, M.-L., L.-J. Zhang, Q.-L. Zhang, L. Zhang, M. Li, X.-T. Wang, R.-Q. Feng, and P.-A. Tang. 2020. Mitogenome evolution in ladybirds: Potential association with dietary adaptation. Ecology and Evolution 10: 1042–1053.

Zhang, H., N. Liu, Z. Han, and J. Liu. 2016. Phylogenetic analyses and evolutionary timescale of Coleoptera based on mitochondrial sequence. Biochemical Systematics and Ecology 66: 229-238.

Zhou, C., and X. Ling. 2011. *DNA binding and DNA extraction: methods, applications, and limitations*. Nova Science Publishers Inc. New York.

Chapter 7

Concluding remarks

Species are introduced deliberately for conservation, agriculture and fisheries, and also inadvertently (typically via plant exports, cargo, travelling and wind currents) around the world (Hulme 2009; Meyerson and Mooney 2007; Renner 2004). However, the rate of successful establishment in introduced species is very low (Cock *et al.* 2016), creating challenges for conservation and biological control programmes.

Research into the establishment success of introduced species has focused on a few ecologically beneficial (i.e. biocontrol agents), harmful or invasive species (Blackburn *et al.* 2011; Simberloff *et al.* 2005). Further, literature has mostly considered how introduced populations go from the establishment to the spreading stages, with much less attention given to factors driving the initial establishment – this is a problem as this is the stage where most introductions fail (Blackburn *et al.* 2011). It is crucial to identify the underlying mechanisms that enable the successful establishment of introduced species in order to comprehend and mitigate the invasive potential of introduced species. However, Forsman (2014) proposed that greater genotypic and phenotypic variation in functionally important traits may increase the successful establishment of introduced species.

Ladybirds are insect predators with diverse dietary ranges and are extensively introduced as biocontrol agents for agricultural purposes and inadvertently via plant exports and other cargo (Michaud 2012). While many introductions have been successful, there are a few invasive ladybird species (i.e. *Harmonia axyridis* and *Coccinella septempunctata*), in addition to other species which have failed to establish despite multiple introduction attempts (Samways *et al.* 1999).

In my thesis, I used New Zealand ladybird beetles (Coleoptera: Coccinellidae) as a model system to study factors affecting the successful establishment of introduced species. I compared levels of phenotypic variation between native and introduced ladybird populations to better understand the traits that lead to successful establishment and developed a phylogeny of New Zealand ladybirds. My research shows that phenotypic variation particularly in body size is more likely to aid the establishment of introduced species than evolutionary relatedness between introduced and native species. In this chapter, I will discuss the key findings of my thesis research, limitations and future directions.

7.1 A framework to examine establishment success

I proposed a framework consisting of three categories of research foci to understand establishment success: invasibility, invasiveness and stochastic events (*sensu* Abrams and Moffat 2018) (chapter 2, figure 2.2). This framework provides a checklist of assessment to be carried out when introducing a new species for biocontrol and conservation. Further, this framework can be used as a foundation to assess the invasiveness of already established species and thereby to predict their future distribution and mitigate the effects of invasive species. This framework is applicable across taxa, but ladybirds provide an ideal case study to test and understand establishment processes.

7.2 Factors that influence establishment success

Chapter 2 highlights that there are many factors that may influence the establishment success of introduced species. There are three main factors that should be prioritised within the research framework I proposed (Ch 2):

1) Differences in phenotypic and genetic variation between successfully introduced/widely distributed species and less distributed/ unsuccessfully introduced species. Current research in ecology mainly focuses on mean trait differences between species (González-Suárez et al. 2015; Moser and Obrycki 2009), however, understanding differences in intraspecific trait variation may improve the predictability of establishment success.

   For example, variation in body size may affect establishment success in ladybirds. Body size in ladybirds is associated with food and water storage, energy for dispersal, and with the body size and mobility of their prey (Baranovská *et al.* 2019; Bowler and Benton 2005; Dixon and Hemptinne 2001). I compared body sizes between native and introduced species (Ch 3) and the effect of body size on general activity levels, responses to simulated predator attacks (Ch 4) and on desiccation resistance (Ch 5). I found that the native ladybird *A. flavihirtus* was narrower than introduced species (*H. chalybeus, I. galbula, R. forestieri, E. vigintioctopunctata* and *S. maculigerum*) (Ch 3). While the number of species comparisons are limited to date, it is indicative of differences in potential prey ranges between native and introduced ladybirds due to differences in body size. For instance, ladybirds with larger body sizes may be able to exploit prey that cannot be accessed by narrower native ladybirds. Therefore, future studies may be conducted to compare the effect of body sizes between native and introduced ladybirds in more species to examine these relationships further.

   Further, I found that there is a strong positive correlation between body size and the general activity levels of ladybirds (Ch 4). Larger body sizes may increase the dispersal ability of ladybirds by increasing mean travel velocities (Soares *et al.* 2018). Larger body sizes in ladybirds (i.e. *H. chalybeus* and *I. galbula*) also reduced the risk of mortality during desiccation and starvation assays compared to smaller body sizes (i.e. *S. maculigerum*) (Ch 5). Large body size may therefore contribute to establishment success by increasing survival time until sufficient resources are located.

Having a higher level of variation in body size is associated with increased relative abundance (Austin and Dunlap 2019). *Halmus chalybeus* showed higher levels of intraspecific variation in body size compared to native New Zealand (*A. flavihirtus, H. antipoda, H. venustus, R. consors* and *V. aucklandiae*) and introduced (*I. galbula, R. forestieri, E. vigintioctopunctata* and *S. maculigerum*) ladybirds. *Halmus chalybeus* has a wider distribution than other introduced (Appendix D: Supplementary figure 5.2, ch 5) and native ladybird species (personal observation) in the greater Auckland region. Therefore, this provides some support for our hypothesis that greater intraspecific of variation in size increases the distribution of introduced ladybird species (and by extension, establishment success).

Similarly, high colour variation may increase establishment success. Body colour in ladybirds is used in anti-predator behaviour (i.e. aposematism and Batesian mimicry), intraspecific communication and reproduction (Bahlai *et al.* 2008; Cummings and Crothers 2013; Llaurens *et al.* 2014). Structural colours such as blue and carotenoid based colours such as yellow and red are associated with predator avoidance strategies (Briolat *et al.* 2019; Kertész *et al.* 2019a) and melanism (black) is associated with immunity and thermo-regulation (Kawakami *et al.* 2015; Michie *et al.* 2010) in insects including ladybird species. I found that the introduced *H. chalybeus* (iridescent structural colour: blue) has higher levels of colour variation compared to all of the other species tested, native and introduced (yellow and black) (Ch 3). Further, blue showed the highest level of variation, followed by yellow and black respectively (Ch 3). Even though iridescence in *H. chalybeus* is not experimentally studied yet, iridescent blue in butterflies is known to influence cryptic defense against predators (Kertész *et al.* 2019b). Therefore, high levels of intraspecific variation in blue and the wider distribution of *H. chalybeus* compared to other species suggest that cryptic colouration may be more effective than aposematic colouration to avoid predators. In Chapter 4, I found that *H. chalybeus* had longer predator responses than the introduced *I. galbula* (yellow and black), indicating greater investment in predator avoidance strategies corresponding to their higher levels of colour variation. Further, black colouration (melanism) is associated with thermoregulation in ladybirds (Michie *et al.* 2010). Therefore, lower levels of variation in black may limit distribution by limiting adaptability to different climate conditions. Future experimental studies that investigate how melanism, cryptic and aposematic colouration influence predator avoidance may further improve our understanding of how their variation influences the establishment success of introduced ladybirds. Lastly as hypothesised, a widespread, introduced species (i.e. *H. chalybeus*) had higher levels of colour variation than native and less distributed ladybird species (Ch 3). Similarly, high variation in colour aligns with high variation in size and a wide distribution in *H. chalybeus*. Species with more restricted distributions showed lower levels of variation in colour and size.

2) Investigation of the role of climatic parameters on species establishment and distribution. While temperature and habitat match are studied extensively in biocontrol research, the role of climate on establishment and the role of climate change on range expansions are limited (Samways *et al.* 1999). In particular, we need recognition of how the invasiveness of the species may interact with invasibility parameters of the habitat (Ch 2). For example, interspecific differences in physiological hardiness (invasiveness, e.g. desiccation resistance) should be examined with respect to habitat parameters (invasibility, i.e. fluctuations in temperature and average rainfall).

   Overall, I found a strong negative correlation between body size and the risk of mortality in desiccation assays in all three species (Ch 5). This suggests larger ladybirds may survive longer than smaller ladybirds during desiccation. Future studies should therefore experimentally assess whether species with larger body sizes and higher intraspecific variation in body sizes are more likely to successfully establish than species with smaller body sizes and lower variation in body sizes.

Further, I found mixed results in the level of variation in survival times in the desiccation resistance experiment (Ch 5). *Serangium maculigerum* had higher intraspecific variation in desiccation resistance than *H. chalybeus* and *I. galbula* in the desiccation assays. But there was no difference in intraspecific variation in survival time between ladybird species during starvation assays. Physiological hardiness during times of climatic extremes may influence establishment success (Block 1996; Bykova and Blatt 2019; Walther *et al.* 2009). Therefore, variation in desiccation resistance and survival time may influence the ability of ladybirds to survive and disperse under stressful conditions, although this is yet to be experimentally tested.

Given the current predictions for climate change (Knapp and Řeřicha 2020), future work on the distribution of ladybirds should investigate the role of climate change (invasibility and stochastic events) and its interaction with physiological hardiness (invasiveness) on potential range expansions. This could be facilitated in many ways. Firstly, studying ladybird migrations. However, to date, very few studies have tracked the movement of ladybirds (Agustí *et al.* 2019; Ameixa *et al.* 2019) due to size restrictions on tracking devices. Technological advancements such as genetic assignment and high resolution spatio-temporal movement data can solve these restrictions in the future. Secondly, field experiments comparing life history traits between introduced and native ladybirds while also considering invasibility parameters of the environment (Raak-van den Berg *et al.* 2018) will provide insights into how ladybird traits interact with habitat parameters. Finally, further investigations into desiccation resistance concentrating on dehydration tolerance and metabolic rates of invasive/introduced species, species with failed introductions and native species will provide a strong foundation to understand how species respond to climatic and seasonal variability. Overall, these investigations will improve predictability of the establishment and dispersal of ladybirds under future climate scenarios.

Greater intraspecific variation in dispersal ability may increase the probability of individuals in a population that are pre-adapted to expand their range which then enables them to successfully establish in a new environment (Forsman, 2014). Examining dispersal ability in ladybirds is logistically challenging and therefore has not been directly compared between introduced species to date (Firebaugh and Haynes 2016; Gutierrez and Menendez 1997). I compared dispersal ability between introduced species of ladybirds indirectly using activity level assays (Ch 4). I found that *I. galbula* was faster than *H. chalybeus*. Increased general activity levels would therefore suggest that *I. galbula* would have a greater dispersal ability than *H. chalybeus.* However, contrary to my prediction, *I. galbula* had a more limited distribution along my field sites than *H. chalybeus* and *S. maculigerum* despite having greater mean velocities. Also contrary to predictions, ladybirds did not show differences in the level of intraspecific variation in general activity levels or responses to simulated predator attacks (Ch 4).

In contrast to behavioural responses, I found that ladybirds with high levels of morphological (body size and colour) and physiological variation (desiccation resistance) were widely distributed, introduced species (Ch's 3 and 5). Hind wing features are also associated with dispersal, migration and foraging behaviour (Abdolahi Mesbah et al., 2015; Ren et al., 2017; Johansson et al., 2009). Ladybird species with long MP1 veins (*H. chalybeus,* and *S. maculigerum*) travelled faster and further than ladybird species with shorter MP1 veins (*I. galbula*, ch's 3 and 4). Therefore, having longer MP1 veins may have increased dispersal ability by improving flight capabilities in those introduced ladybirds with wide distributions such as *H. chalybeus* and *S. maculigerum*. Alternatively, distributions of ladybirds can be restricted due to the influence of prey availability or competition which were not assessed in this thesis.

3) Identifying the phylogenetic relationships between native and introduced species to determine the influence of relatedness on the establishment success of introduced species. As shown in chapter 6, the first phylogeny of ladybirds containing native New Zealand species shows that the introduced *H. chalybeus* (clustered in Chilocorini) is not closely related to most of the native species (clustered in Coccidulini and Coccinellini) included in the phylogeny. Therefore, it may be more likely that having close relatives in the

habitat increases the competition between species (Darwin's naturalization hypothesis, Darwin (1859)), limiting the establishment success and dispersal of introduced species, although this is yet to be tested directly in ladybirds. However, Darwin's naturalization hypothesis has been demonstrated in invasive plants (Strauss *et al.* 2006) and reptiles (Van Wilgen and Richardson 2011) where successfully established species are distantly related to native species in the recipient ecosystem. Further, the introduced species *Harmonia conformis* was found to be more related to the native species *Harmonia antipoda* than its invasive cogener *Harmonia axyridis* (Ch 6). However, both introduced *H. conformis* and invasive *H. axyridis* show wider distribution in the South Island of New Zealand than native *H. antipoda*. This may therefore suggest that the establishment of introduced species maybe more affected by ecological factors than relatedness to the native species in the introduced area. However, my phylogeny has only five native New Zealand ladybird species due to limited availability of molecular grade specimens, meaning that there are limited inferences that can be made to date. Therefore, future molecular phylogenies of New Zealand ladybirds may a) improve robustness by including more species of native and introduced New Zealand ladybirds, b) include specimens from the native ranges of species of introduced ladybirds and c) compare the level of genetic variation between native and introduced ladybirds in New Zealand.

7.3 Limited understanding of ladybird biology

Ladybirds consist of more than 6000 species in about 360 genera in two sub families and 30 tribes. Our knowledge of ladybird biology is however mostly limited to just a few ladybird species (*H. axyridis, C. septempunctata, A. bipunctata, H. chalybeus, C. montrouzieri* and *Rodolia cardinalis*) who are either introduced as biocontrol agents or have become invasive (Ch 2). This has limited our understanding of the factors that influence the distribution of ladybirds. For instance, in addition to alien species, there are about 483 native species in North America (Gordon 1985), 253 native species in Europe (Roy and Migeon 2010), 46 native species in Great Britain (Roy and Migeon 2010), 86 native species in Netherlands (Roy and Migeon 2010), 550 species on the Indian subcontinent (Ramani *et al.* 2019) and about 500 species from Australia (Slipinski and Tomaszewska 2010). However, we know very little about these native species.

In New Zealand, there are 25 native species (Szawaryn and Leschen 2019; Maddison 2010). Ladybirds are one of the most species rich and diverse taxa and many species are important predators (Escalona *et al.* 2017; Giorgi *et al.* 2009; Seago *et al.* 2011). Therefore, comparisons of the biology of native and introduced ladybirds will provide valuable insights into their distribution, and in turn help us to understand how they become invasive. Future research in New Zealand should initially focus on the native ladybird species *A. flavihirtus, H. antipoda, H. venustus, R. consors* and *V. aucklandiae* as they are more readily accessible than other native species. One of the main limitations I faced during my PhD was sourcing sufficient numbers of native ladybirds. Future efforts on monitoring and recording native ladybirds can be helped via citizen science programmes (Losey *et al.* 2007).

Failed introductions will also provide particularly valuable insights into the factors that drive establishment success. Few studies have examined failed introductions of the invasive *H. axyridis* (Evans *et al.* 2011; Soares *et al.* 2008; Soares *et al.* 2017; Zenni and Nunez 2013). Comparison of failed introductions of *H. axyridis* in Azores, United States showed a weak effect of photoperiod on diapause induction that prevented its establishment (Reznik *et al.* 2015; Reznik *et al.* 2017; Soares *et al.* 2008). Future work on ladybirds should concentrate on investigating the factors that differ between successful and unsuccessful introductions.

7.4 Contradictory evidence between laboratory and field studies

Most ladybirds are important generalist predators, therefore, investigating ladybird ecology will improve our understanding of ecosystem functioning (Sloggett 2005). There is contradictory evidence about the interactions between introduced and native ladybird populations in laboratory and field studies. For instance, most laboratory research has found that intra-guild predation (IGP) has contributed to declines in native ladybird populations but recent field studies suggest that IGP is unlikely to drive these native population declines due to low encounter rates (Gardiner *et al.* 2011; Lucas 2005; Yasuda *et al.* 2004). Therefore, the framework I set out in chapter 2 illustrates the importance of assessing introduction and establishment biology in both field and laboratory studies. Further, field experiments comparing life history traits (i.e. dispersal ability) between native and introduced ladybirds in the context of invasibility parameters (i.e. temperature and rainfall) will improve our understanding of establishment success.

7.5 Is *Halmus chalybeus* a successfully introduced species in New Zealand?

In my thesis, ladybirds with large body sizes have shown increased ability to establish in a new environment as larger body sizes correlates with higher general activity levels and longer survival times during stressful conditions (i.e., desiccation assays). *Illeis galbula* was the largest ladybird tested and was introduced to New Zealand in 1895, earlier than the smaller *H, chalybeus* (Martin 2016). However, *I. galbula* had limited distribution across the Auckland region compared to *H. chalybeus* (Appendix D: Supplementary figure 5.2, ch 5).

Intraspecific variation may play a key role in the establishment of *H. chalybeus* in New Zealand. Intraspecific variation is increasingly being recognised as important in ecological studies (Forsman 2014; Mogi *et al.* 1996; Sweeney *et al.* 2013). In my thesis, *H. chalybeus* showed the highest level of variation in size, colour and survival times than any of the other native and introduced ladybird species (Ch's 3, 4 and 5). Size can also influence distribution via resource exploitation (e.g., prey range). *Halmus chalybeus* has a diverse dietary range, preying on both soft and armoured scales and mites (Lo and Chapman 2001). Further, *H. chalybeus* is the most widely distributed species of ladybird within the Auckland region. Therefore, this lends support to the prediction that high levels of intraspecific variation in functionally important traits such as body size, body colour and desiccation resistance can promote successful establishment and dispersal.

7.6 Conclusion

In conclusion, the study of species establishments will benefit from the examination of both successful and unsuccessful establishments. In addition, comparisons of native and introduced species biology will improve our understanding of species distributions. Combined, we can use this information in distribution modelling to accurately predict introduction success, the dispersal of introduced species and the decline of native species. My view is that research into introduction biology will benefit from a) using the framework I propose in chapter 2 for quantifying invasibility, invasiveness and stochastic events to create direct comparisons between introduced and native species, and b) studying intraspecific variation in functionally important traits such as dispersal ability and physiological hardiness to thereby improve the establishment success of introduced species in agricultural and conservation programmes.

7.7 References

Agustí, N., C. Castañé, I. Fraile, and O. Alomar. 2019. Development of a PCR-based method to monitor arthropod dispersal in agroecosystems: *Macrolophus pygmaeus* (Hemiptera: Miridae) from banker plants to tomato crops. Insect Science 27: 1125-1134.

Ameixa, O. M., J. Šipoš, M. Burda, A. M. Soares, and A. O. Soares. 2019. Factors influencing the introduction and spread of *Harmonia axyridis* in the Iberian Peninsula. Biological invasions 21: 323-331.

Austin, M. W., and A. S. Dunlap. 2019. Intraspecific variation in worker body size makes north american bumble bees (*Bombus* spp.) less susceptible to decline. The American Naturalist 194: 381-394.

Bahlai, C., J. Welsman, E. Macleod, A. Schaafsma, R. Hallett, and M. Sears. 2008. Role of visual and olfactory cues from agricultural hedgerows in the orientation behavior of multicolored Asian lady beetle (Coleoptera: Coccinellidae). Environmental Entomology 37: 973-979.

Baranovská, E., P. Chajma, and M. Knapp. 2019. Desiccation resistance in Central European carabid species: effects of body size and habitat preferences. ARPHA Conference Abstracts 2: e38513.

Blackburn, T. M., P. Pyšek, S. Bacher, J. T. Carlton, R. P. Duncan, V. Jarošík, J. R. Wilson, and D. M. Richardson. 2011. A proposed unified framework for biological invasions. Trends in Ecology & Evolution 26: 333-339.

Block, W. 1996. Cold or drought-The lesser of two evils for terrestrial arthropods? European Journal of Entomology 93: 325-340.

Bowler, D. E., and T. G. Benton. 2005. Causes and consequences of animal dispersal strategies: relating individual behaviour to spatial dynamics. Biological Reviews 80: 205-225.

Briolat, E. S., E. R. Burdfield-Steel, S. C. Paul, K. H. Rönkä, B. M. Seymoure, T. Stankowich, and A. M. M. Stuckert. 2019. Diversity in warning coloration: selective paradox or the norm? Biological Reviews 94: 388-414.

Bykova, O., and S. Blatt. 2019. Inclusion of physiological responses in insect pest risk analysis: Evaluating species potential to achieve pest status. Journal of the Acadian Entomological Society.

Cock, M. J. W., S. T. Murphy, M. T. K. Kairo, E. Thompson, R. J. Murphy, and A. W. Francis. 2016. Trends in the classical biological control of insect pests by insects: an update of the BIOCAT database. BioControl 61: 349-363.

Cummings, M. E., and L. R. Crothers. 2013. Interacting selection diversifies warning signals in a polytypic frog: an examination with the strawberry poison frog. Evolutionary Ecology 27: 693-710.

Darwin, C. 1859. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*, 1st edn. J Murray, London.

Dixon, A., and J.-L. Hemptinne. 2001. Body size distribution in predatory ladybird beetles reflects that of their prey. Ecology 82: 1847-1856.

Escalona, H. E., A. Zwick, H.-S. Li, J. Li, X. Wang, H. Pang, D. Hartley, L. S. Jermiin, O. Nedvěd, and B. Misof. 2017. Molecular phylogeny reveals food plasticity in the evolution of true ladybird beetles (Coleoptera: Coccinellidae: Coccinellini). BMC Evolutionary Biology 17: 151.

Evans, E. W., A. O. Soares, and H. Yasuda. 2011. Invasions by ladybugs, ladybirds, and other predatory beetles. BioControl 56: 597-611.

Firebaugh, A., and K. J. Haynes. 2016. Experimental tests of light-pollution impacts on nocturnal insect courtship and dispersal. Oecologia 182: 1203-1211.

Forsman, A. 2014. Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. Proceedings of the National Academy of Sciences 111: 302-307.

Gardiner, M. M., M. E. O'Neal, and D. A. Landis. 2011. Intraguild predation and native lady beetle decline. PLoS ONE 6: e23576.

Gibbs, A. G., A. K. Chippindale, and M. R. Rose. 1997. Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. Journal of Experimental Biology 200: 1821-1832.

Giorgi, J. A., N. J. Vandenberg, J. V. McHugh, J. A. Forrester, S. A. Ślipiński, K. B. Miller, L. R. Shapiro, and M. F. Whiting. 2009. The evolution of food preferences in Coccinellidae. Biological Control 51: 215-231.

González-Suárez, M., S. Bacher, and J. M. Jeschke. 2015. Intraspecific trait variation is correlated with establishment success of alien mammals. The American Naturalist 185: 737-746.

Gordon, R. D. 1985. The Coccinellidae (Coleoptera) of America north of Mexico. Journal of the New York Entomological Society 93: 912 pp.

Gutierrez, D., and R. Menendez. 1997. Patterns in the distribution, abundance and body size of carabid beetles (Coleoptera: Caraboidea) in relation to dispersal ability. Journal of Biogeography 24: 903-914.

Hulme, P. E. 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. Journal of Applied Ecology 46: 10-18.

Kawakami, Y., K. Yamazaki, and K. Ohashi. 2015. Increase in dark morphs and decrease in size during a range extension of *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae). European Journal of Entomology 112(2): 289-294.

Kertész, K., G. Piszter, Z. Bálint, and L. P. Biró. 2019a. Biogeographical patterns in the structural blue of male *Polyommatus icarus* butterflies. Scientific Reports 9: 1-9.

Kertész, K., G. Piszter, Z. Bálint, and L. P. Biró. 2019b. Biogeographical patterns in the structural blue of male *Polyommatus icarus* butterflies. Scientific Reports 9: 2338.

Knapp, M., and M. Řeřicha. 2020. Effects of the winter temperature regime on survival, body mass loss and post-winter starvation resistance in laboratory-reared and field-collected ladybirds. Scientific Reports 10: 1-10.

Llaurens, V., M. Joron, and M. Théry. 2014. Cryptic differences in colour among Müllerian mimics: how can the visual capacities of predators and prey shape the evolution of wing colours? Journal of Evolutionary Biology 27: 531-540.

Lo, P., and R. Chapman. 2001. Predation by *Halmus chalybeus* (Coleoptera: Coccinellidae) on *Ceroplastes destructor* and *C. sinensis* (Hemiptera: Coccidae: Ceroplastinae) infesting citrus in Northland, New Zealand. Biocontrol Science and Technology 11: 57-66.

Losey, J. E., J. E. Perlman, and E. R. Hoebeke. 2007. Citizen scientist rediscovers rare nine-spotted lady beetle, *Coccinella novemnotata*, in eastern North America. Journal of Insect Conservation 11: 415-417.

Lucas, É. 2005. Intraguild predation among aphidophagous predators. EJE 102: 351-364.

Maddison, P.M. 2010. Checklist of New Zealand Hexapoda. Order Coleoptera. [Chapter] NINE: Phylum Arthropoda: Subphylum Hexapoda: Protura, springtails, Diplura, and insects. *In*: Gordon, D. (Ed.), New Zealand Inventory of Biodiversity. Kingdom Animalia. Chaetognatha, Ecdysozoa, ich-nofossils Vol. 2. Canterbury University Press, Christchurch, New Zealand: 233–467.

Martin, N. A. 2016. Interesting Insects and other Invertebrates. New Zealand Arthropod Factsheet Series Number 37. Available from: http://nzacfact sheets.landcareresearch.co.nz/Index.html.

Meyerson, L. A., and H. A. Mooney. 2007. Invasive alien species in an era of globalization. Frontiers in Ecology and the Environment 5: 199-208.

Michaud, J. 2012. *Coccinellids in biological control. Ecology and Behaviour of the Ladybird Beetles (Coccinellidae)*. John Wiley & Sons, Chichester: 488-519.

Michie, L. J., F. Mallard, M. E. N. Majerus, and F. M. Jiggins. 2010. Melanic through nature or nurture: genetic polymorphism and phenotypic plasticity in *Harmonia axyridis*. Journal of Evolutionary Biology 23: 1699-1707.

Mogi, M., I. Miyagi, K. Abadi, and syafruddin. 1996. Inter- and Intraspecific Variation in Resistance to Desiccation by Adult *Aedes* (Stegomyia) spp. (Diptera: Culicidae) from Indonesia. Journal of Medical Entomology 33: 53-57.

Moser, S. E., and J. J. Obrycki. 2009. Competition and intraguild predation among three species of coccinellids (Coleoptera: Coccinellidae). Annals of the Entomological Society of America 102: 419-425.

Raak-van den Berg, C. L., P. W. de Jong, G. Gort, B. F. J. Manly, and J. C. van Lenteren. 2018. Life histories of an invasive and native ladybird under field experimental conditions in a temperate climate. Entomologia Experimentalis et Applicata 166: 151-161.

Ramani, S., P. Mohanraj, and H. Yeshwanth. 2019. *Indian Insects: Diversity and Science.* CRC Press.

Renner, S. 2004. Plant dispersal across the tropical Atlantic by wind and sea currents. International Journal of Plant Sciences 165: S23-S33.

Reznik, S. Y., M. Y. Dolgovskaya, A. N. Ovchinnikov, and N. A. Belyakova. 2015. Weak photoperiodic response facilitates the biological invasion of the harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). Journal of Applied Entomology 139: 241-249.

Reznik, S. Y., A. A. Ovchinnikova, A. N. Ovchinnikov, L. V. Barabanova, and N. A. Belyakova. 2017. Inheritance of diapause regulation in the multicoloured Asian ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae). European Journal of Entomology 114: 416–421.

Roy, H., and A. Migeon. 2010. Ladybeetles (Coccinellidae). Chapter 8.4. *In* Alien terrestrial arthropods of Europe (Eds. Roques A *et al.*). BioRisk 4: 293.

Samways, M. J., R. Osborn, H. Hastings, and V. Hattingh. 1999. Global climate change and accuracy of prediction of species' geographical ranges: establishment success of introduced ladybirds (Coccinellidae, Chilocorus spp.) worldwide. Journal of Biogeography 26: 795-812.

Seago, A. E., J. A. Giorgi, J. Li, and A. Ślipiński. 2011. Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. Molecular Phylogenetics and Evolution 60: 137-151.

Simberloff, D., I. M. Parker, and P. N. Windle. 2005. Introduced species policy, management, and future research needs. Frontiers in Ecology and the Environment 3: 12-20.

Slipinski, A., and W. Tomaszewska. 2010. Revision of the family Cavognathidae (Coleoptera: Cucujoidea). Australian Journal of Entomology 49: 256-267.

Soares, A. O., I. Borges, P. A. Borges, G. Labrie, and E. Lucas. 2008. *Harmonia axyridis*: What will stop the invader? BioControl 53: 127-145.

Soares, A. O., A. Honěk, Z. Martinkova, P. M. Brown, and I. Borges. 2018. Can native geographical range, dispersal ability and development rates predict the successful establishment of alien ladybird (Coleoptera: Coccinellidae) species in Europe? Frontiers in Ecology and Evolution 6: 57.

Soares, A. O., A. Honěk, Z. Martinkova, J. Skuhrovec, P. Cardoso, and I. Borges. 2017. *Harmonia axyridis* failed to establish in the Azores: the role of species richness, intraguild interactions and resource availability. BioControl 62: 423-434.

Strauss, S. Y., C. O. Webb, and N. Salamin. 2006. Exotic taxa less related to native species are more invasive. Proceedings of the National Academy of Sciences 103: 5841-5845.

Sweeney, K., B. Cusack, F. Armagost, T. O'Brien, C. N. Keiser, and J. N. Pruitt. 2013. Predator and prey activity levels jointly influence the outcome of long-term foraging bouts. Behavioral Ecology 24: 1205-1210.

Szawaryn, K., and R. A. Leschen. 2019. Redescription and notes on the New Zealand ladybird species *Hoangus venustus* (Pascoe, 1875)(Coleoptera: Coccinellidae). Journal of Asia-Pacific Entomology 22: 226-232.

Van Wilgen, N. J., and D. M. Richardson. 2011. Is phylogenetic relatedness to native species important for the establishment of reptiles introduced to California and Florida? Diversity and Distributions 17: 172-181.

Walther, G.-R., A. Roques, P. E. Hulme, M. T. Sykes, P. Pyšek, I. Kühn, M. Zobel, S. Bacher, Z. Botta-Dukat, and H. Bugmann. 2009. Alien species in a warmer world: risks and opportunities. Trends in Ecology & Evolution 24: 686-693.

Yasuda, H., E. W. Evans, Y. Kajita, K. Urakawa, and T. Takizawa. 2004. Asymmetric larval interactions between introduced and indigenous ladybirds in North America. Oecologia 141: 722-731.

Zenni, R. D., and M. A. Nunez. 2013. The elephant in the room: the role of failed invasions in understanding invasion biology. Oikos 122: 801-815.

8. Appendices

8.1. Appendix A: Supplements for chapter 2

We developed the below table based on studies that investigated introduced ladybirds in terms of their invasiveness, invasibility of the introduced habitat and native ranges and stochastic events. The categorization and factors/traits are as described in table 2.1 and figure 2.1 (Ch 2). Introduction indicates whether the study treats the species as biocontrol, accidental/ deliberate introduction, immigration, invasive, the introduction method is unknown or in native range. Blank lines mean that there was no indication of introduction type on the referred paper. Key effects and biological notes, indicate successful biocontrol agents, native declines/displacement, IGP, other non-target predation or instances where effects are unknown and other biological notes on ladybird species

Supplementary table 2.1 Studies on introduced ladybirds described under proposed categorization.

No.	Name	Categorisation	Factors /traits	Introduction	Key effect/ Biological notes	References
1.	*Adalia bipunctata* (Linnaeus, 1758)	Invasibility	Competitors, Intraguild predation risk – spatial distribution	Biocontrol	First record in 1993 and range expansion 2006	(Toda and Sakuratani 2006)
		Invasibility	Intraguild predation risk – IG prey	-	IGP by *Harmonia axyridis* (Pallas, 1773) limited spread of *A. bipunctata* in Japan	(Ware *et al.* 2009)
		Invasibility	Intraguild predation risk – IG prey	Biocontrol	Egg production reduced in the presence of the native *H. axyridis*	(Kajita *et al.* 2006)
2.	*Cheilomenes sexmaculata* (Fabricius, 1781)	Invasibility	Other fauna	Native region	Fecundity and hatching rate significantly reduced by ghost ants (*Tapinoma melanocephalum* (Fabricius, 1793))	(Zhou *et al.* 2014)

		Invasiveness	Development times, fecundity	Native region	Better performance than two relatively larger *Coccinella* species	(Pervez *et al.* 2005)
		Invasibility	Other fauna	Native region	Survival of lady beetle larvae was significantly decreased by fire ants (*Solenopsis invicta* Buren, 1972)	(Zhou *et al.* 2013)
		Invasibility	Other flora	Native region	Avoid mealybug infested *Eupatorium adenophorum* (invasive plant)	(Zhou *et al.* 2015)
		Invasiveness	Dispersal	Invasive	First record of *C. sexmaculata* in Colombia	(Ramírez *et al.* 2018)
		Invasiveness	Dispersal	Invasive	First record of *C. sexmaculata on* in the Caribbean island of Curaçao	(Assour and Behm 2019)
3.	*Cleobora mellyi* (Mulsant, 1850)	Invasiveness	Dietary range	Biocontrol	Biocontrol of invasive tomato-potato psyllid	(Pugh *et al.* 2015)

				(TPP), *Bactericera cockerelli* (Šulc, 1909) No difference in longevity between ladybirds supplied with TPP only or buckwheat only	
	Invasiveness	Cannibalism	Biocontrol	Increased cannibalism reduced survival in laboratory rearing on artificial diet Biocontrol of *Paropsis charybdis* Stål, 1860	(Bain *et al.* 1984)
	Invasiveness	Dispersal	Biocontrol	Inundative release of laboratory reared *C. mellyi* is possible for biocontrol of *Chrysophtharta bimaculata* (Olivier, 1807)	(Baker *et al.* 2003)

4.	*Coccinella septempunctata* Linnaeus, 1758	Invasibility	Intraguild predation	Biocontrol	Do not prey on *Aphidius colemani* Viereck, 1912	(Chacon *et al.* 2008)
		Invasiveness	Fecundity	Invasive	Adjusting reproductive output to fluctuation in prey availability	(Kajita and Evans 2009)
		Invasiveness	Dispersal	Native range		(Zadravec and Bavec 2001)
		Invasiveness, Invasibility	Resource acquisition, Intraguild predation-IG predator	Biocontrol	Could influence habitat use and dispersal of native ladybirds through changing prey abundance and IGP	(Evans 2004)
		Invasibility	Intraguild predation-IG predator	Biocontrol	Increased egg predation on native ladybird species (*Coccinella novemnotata* Herbst, 1793)	(Turnipseed *et al.* 2015)
		Invasiveness	Resource acquisition	Native range	More effective biological control of invasive species *Metcalfa pruinosa* (Say,	(Grozea *et al.* 2015)

				1830) in ornamental plants than *Propylea quatordecimpuncata* (Linnaeus, 1758), *A. bipunctata and Psyllobora vigintiduopunctata* (Linnaeus, 1758)	
	Invasibility	Intraguild predation -IG predator	Biocontrol	IGP of native ladybird species leading to declining populations	(Snyder *et al.* 2004)
	Invasiveness	Resource acquisition	Biocontrol	Has greater consumptive and non-consumptive effects on *Acyrthosiphon pisum* (Harris, 1776) than the native species (*C. novemnotata*) Could lead to decreased stability in predator and prey interactions	(Hoki *et al.* 2014)

		Invasiveness	Resource acquisition	Biocontrol	Biocontrol of *Euschistus servus* (Say, 1832) and *Nezara viridula* (Linnaeus, 1758) eggs	(Tillman 2011)
		Invasiveness	Vulnerability to change/disturbance-pesticides	Native range	Developmental time, adult weight and daily predation was affected	(Skouras *et al.* 2017)
		Invasibility	Competitors	Biocontrol	-	(Omkar *et al.* 2014)
		Invasiveness	Resource aquisition	Biocontrol	Potential biocontrol agent for *Bemisia argentifolii* (Gennadius, 1889)	(Mohamed *et al.* 2008)
5.	*Coccinella undecimpunctata* Linnaeus, 1758	Invasiveness	Resource acquisition	Biocontrol	Biocontrol of *Aphis gossypii* Glover and *Bemisia tabaci* (Gennadius, 1889)	(Zaki *et al.* 1999)
		Invasiveness, Invasibility	Intraguild predation-IG prey, Dispersal	Biocontrol and immigration	Possible decline in USA	(Wheeler and Hoebeke 2008)

	Invasiveness	Resource acquisition	Native range	Biocontrol of *Saccharicoccus sacchari* (Cockerell, 1895) in sugarcane fields in Egypt	(Tohamy *et al.* 2008)
	Invasiveness	Dispersal	Accidental	Established populations found through citizen science programs	(Smyth *et al.* 2013)
				Biocontrol of *B. tabaci*	(Simmons and Abd-Rabou 2011)
	Invasiveness	Dispersal	Biocontrol	Migration from clover fields to adjacent maize fields	(Shoeb *et al.* 2008)
	Invasiveness	Genetic variation	Biocontrol	Low genetic variability	(Sayed 2016)
	Invasiveness	Resource acquisition, fecundity	Biocontrol	Potential biocontrol agent for *B. argentifolii*	(Mohamed *et al.* 2008)

	Invasiveness	Resource acquisition	Biocontrol	Biocontrol of tomato potato psyllid (TPP), *B. cockerelli*	(MacDonald *et al.* 2016)
	Invasiveness	Resource acquisition	Native range	Biocontrol of cotton mealybug, *Phenacoccus solenopsis* Tinsley, 1898	(Hameed *et al.* 2013)
	Invasiveness	Vulnerability to change/disturbance-insecticides	Biocontrol	A synergistic effect between biocontrol and use of insecticides to control cereal aphid	(ElHag and Zaitoon 1996)
	Invasiveness	Vulnerability to change/disturbance-insecticides	Biocontrol	Voracity may be related to mobility of insecticide-treated aphids in Azores	(Cabral *et al.* 2011)
	Invasiveness	Resource acquisition, dispersal	Biocontrol	Presence of both 4th instar larvae and adults increase efficiency in biocontrol of *Myzus persicae* (Sulzer, 1776)	(Cabral *et al.* 2009)

		Invasibility	Intraguild predation-IG predator	Biocontrol	Parasitized aphids are inferior prey for larvae and may reduce intensity of IGP in the field and the negative impact IGP has on herbivore suppression	(Bilu and Coll 2007; Bilu and Coll 2009)
		Invasibility	Intraguild predation-IG predator	Biocontrol	Dominant over *Cydonia vicina nilotica* Mulsant, 1850 in shared habitats	(Bayoumy and Michaud 2015)
		Invasibility	Intraguild predation-IG prey	Biocontrol	Intraguild predation by *H. axyridis*	(Felix and Soares 2004)
6.	*Coelophora inaequalis* (Fabricius, 1775)	Invasiveness	Resource acquisition	Biocontrol	Biocontrol of yellow sugarcane aphid, *Sipha flava* (Forbes, 1884), was not effective	(Frank and McCoy 2007)
		Invasiveness	Dispersal	Introduced	-	(Maruyamai and Ohno 2011)

7.	*Cryptognatha nodiceps* Marshall, 1912	Invasiveness	Resource acquisition	Biocontrol	Biocontrol of coconut scale *Aspidiotus destuctor* Signoret, 1869	(Frank and McCoy 2007)
		Invasiveness	Resource acquisition	Biocontrol	Fed and reproduced on Diaspididae and Coccidae. Potential biocontrol agents of coconut scale *A. destructor*	(Lopez *et al.* 2004)
8.	*Curinus coeruleus* (Mulsant, 1850)	Invasiveness	Resource acquisition	Biocontrol	Biocontrol of Lepidoptera	(Sheppard *et al.* 2004)
9.	*Delphastus catalinae* (Horn, 1895)	Invasibility	Natural enemies-fungi	Introduced for Biocontrol	Biocontrol of *B. tabaci*	(Wang *et al.* 2008)
		Invasibility	Abiotic factors-temperature	Invasive/Introduced	Biocontrol of *B. tabaci*	(Simmons and Legaspi 2007)
10.	*Harmonia axyridis* (Pallas, 1773)	Invasibility	Intraguild predation-IG predator	Invasive	IGP against *A. colemani*	(Chacon *et al.* 2008)

	Invasiveness	Resource acquisition	Native range	Generalist predator that preys on exotic pests in the presence of native preferred prey.	(Zhang *et al.* 2016)
	Invasiveness	Dispersal	Deliberate	USA first release—1916 (first established population in 1988) Soviet Union first release—1927 Western Europe—1982	(Orlova-Bienkowskaja 2013)
	Invasiveness	Natural enemies	Native and invasive populations	Repeated and independent infection events by *Wolbachia pipientis* in *H. axyridis.*	(Goryacheva *et al.* 2015)
	Invasiveness	Physiology	Native range	Aggregations and invasion of human habitations as a behavioral adaptation for cold-avoidance	(Wang *et al.* 2011)
	Invasiveness	Defense mechanisms	Invasive	Microsporidia may contribute to the	(Gegner *et al.* 2015)

				unpalatability of *H. axyridis* during IGP	
	Invasiveness, Invasibility, stochastic events	Mating systems, rainfall and land use	Invasive	-	(Comont *et al.* 2014b)
	Invasibility	Intraguild predation-IG predator	Invasive/feral	IGP against *A. bipunctata* and *Oenopia conglobata* (Linnaeus, 1758) (higher predation on *O. conglobata*)	(Rondoni *et al.* 2015)
	Invasiveness	Resource acquisition	Invasive	Biocontrol and adverse impacts as a household pest, fruit production, and threat to non-target organisms	(Koch *et al.* 2006)
	Invasibility	Intraguild predation	Introduced/ invasive	Do not prey on eggs of native ladybird species in the field.	(Smith and Gardiner 2013)

	Invasiveness	Defense mechanisms	Introduced	A prominent cause of seasonal inhalant allergy	(Goetz 2009)
	Invasiveness	Resource acquisition	Biocontrol	Effective biological control of invasive species *M. pruinosa* in ornamental plants than by native ladybird species	(Grozea *et al.* 2015)
	Invasibility	Intraguild predation-IG predator	Biocontrol	Harmful IGP on both introduced and native ladybird species	(Yasuda *et al.* 2004)
	Invasibility	Intraguild predation-IG predator	Biocontrol	Biocontrol of aphid *M. persicae* and IGP on native ladybird species *E. connexa*	(Mirande *et al.* 2015a)
	Invasibility	Intraguild predation-IG predator	Biocontrol	IGP of native ladybird species leading to declining populations	(Snyder *et al.* 2004)

	Invasiveness	Dispersal	Accidental	Invasive and undergoing range expansion, first record outside Croatia	(Stankovic *et al.* 2011)
	Invasiveness, Invasibility, Stochastic events	Resource acquisition, fecundity, phenotypic variation, dispersal, natural enemies, abiotic factors, disturbance, climate change	Invasion	Review on invasiveness of *H. axyridis*, invasibility of introduced/ invaded habitats and stochastic events. Causes changes in invading/recipient coccinellid community	(Roy *et al.* 2016)
	Invasiveness	Resource acquisition	Biocontrol	Biocontrol of *E. servus* and *N. viridula* eggs	(Tillman 2011)
	Invasibility	Competitors	Biocontrol	Affect population abundance and/or could result in the competitive displacement of *C. undecimpunctata*	(Soares and Serpa 2007)

	Invasibility	Competitors	Biocontrol	May negatively affect *C. undecimpunctata* survival through resource competition	(Noia *et al.* 2008)
	Invasibility	Intraguild predation-IG predator, competitors	Invasive	IGP and resource competition with native ladybird species	(Kenis *et al.* 2017)
	Invasibility	Competitors	Invasive	Competitive displacement of native ladybird species Rapid biotic homogenization	(Roy *et al.* 2012)
	Invasiveness	Development times and fecundity	Invasive, biocontrol and native	Faster development times and high reproduction capacity and adaptability to introduced/invaded area may increase invasiveness	(Tayeh *et al.* 2015)

11.	*Harmonia conformis* (Boisduval, 1835)	Invasiveness	Resource acquisition	Biocontrol	Biocontrol of tomato potato psyllid (TPP), *B. cockerelli*	(MacDonald *et al.* 2016)
		Invasiveness	Dispersal	Native range	Biocontrol of *C. bimaculata*	(Baker *et al.* 2003)
12.	*Harmonia dimidiata* (Fabricius, 1781)	Invasiveness	Resource acquisition	Accidental/ Immigration and failed introductions	Unknown (studies on prey range needed)	(Frank and McCoy 2007)
13.	*Hippodamia variegata* (Goeze, 1777)	Invasiveness	Resource acquisition, dispersal	Unknown	Prey on aphids and psyllids on crops, weeds and ornamental plants First record in Australia	(Franzmann 2002)
		Invasibility, Invasiveness	Competition, intraguild predation, cannibalism	Biocontrol	Biocontrol of aphids May influence native species (*E. connexa*) decline	(Grez *et al.* 2012)

		Invasiveness	Dispersal	Unknown	Spread rapidly in Wisconsin in less than five years	(Williams and Young 2009)
		Invasibility	Abiotic factors-temperature	Biocontrol	Biocontrol of Russian wheat aphid	(Michels *et al.* 1997)
		Invasiveness	Dispersal	Biocontrol	Causing native ladybird population declines	(Cormier *et al.* 2000)
		Invasiveness	Genetic variability	Biocontrol	Genetic variability within and between populations of *H. variegata*	(Sayed 2016)
		Invasiveness	Resource acquisition	Native range	Biocontrol of common pistachio psylla (CPP), *A. pistaciae*	(Mehrnejad *et al.* 2011)
14.	*Mada polluta* Mulsant, 1850	Invasiveness	Resource acquisition, fecundity, developmental times	Biocontrol	Biological control agent of *Tecoma stans*	(Madire 2013)

15.	*Novius cruentatus* Mulsant, 1846	Invasiveness	Resource acquisition	Biocontrol	A major contributor to the population reduction of *Palaeococcus fuscipennis* (Burmeister, 1835)	(Mendel *et al.* 1998)
16.	*Rodolia cardinalis (Mulsant, 1850)*	Invasiveness	Resource acquisition	Biocontrol	Release does not affect finch species (*Camarhynchus parvulus* [Gould, 1837])	(Lincango *et al.* 2011)
		Invasiveness	Resource acquisition	Biocontrol	Successful biocontrol of *I. purchase*, highly unlikely to affect non-target species following establishment	(Greathead 1972; Hoddle *et al*. 2013; Frank and McCoy 2007)
		Invasibility, Invasiveness	Intraguild predation	Biocontrol	Minimum niche overlap with native predators of Homoptera and IGP should not occur	(Causton *et al*. 2004)
		Invasiveness	Resource acquisition	Biocontrol	Biocontrol of *S. sacchari* in sugarcane fields	(Tohamy *et al*. 2008)

References cited for appendix

Abram, P. K., and C. E. Moffat. 2018. Rethinking biological control programs as planned invasions. Current Opinion in Insect Science 27: 9–15.

Assour, H., and J. Behm. 2019. First occurrence of *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae) on the Caribbean island of Curaçao. Neotropical Entomology 48: 863–865.

Awad, M., P. Kalushkov, T. Nedvědová, and O. Nedvěd. 2013. Fecundity and fertility of ladybird beetle *Harmonia axyridis* after prolonged cold storage. BioControl 58: 657–666.

Awad, M., G. J. Laugier, A. Loiseau, and O. Nedvěd. 2015. Unbalanced polyandry in wild-caught ladybirds *Harmonia axyridis* (Coleoptera: Coccinellidae). Applied Entomology and Zoology 50: 427–434.

Awad, M., L. Pialek, A. Krejčí, G. Laugier, and O. Nedvěd. 2017. Paternity following multiple mating in ladybird *Harmonia axyridis*. BioControl 62: 297–307.

Bain, J., P. Singh, M. D. Ashby, and R. J. Vanboven. 1984. Laboratory rearing of the predatory coccinellid *Cleobora mellyi* Col, Coccinellidae for biological-control of *Paropsis charybdis* Col, Chrysomelidae in New Zealand. Entomophaga 29: 237–244.

Baker, S. C., J. A. Elek, R. Bashford, S. C. Paterson, J. Madden, and M. Battaglia. 2003. Inundative release of coccinellid beetles into eucalypt plantations for biological control of chrysomelid leaf beetles. Agricultural and Forest Entomology 5: 97–106.

Barahona-Segovia, R. M., A. A. Grez, and F. Bozinovic. 2015. Testing the hypothesis of greater eurythermality in invasive than in native ladybird species: from physiological performance to life-history strategies. Ecological Entomology: 182–191.

Bayoumy, M. H., and J. P. Michaud. 2015. Cannibalism in two subtropical lady beetles (Coleoptera: Coccinellidae) as a function of density, life stage, and food supply. Journal of Insect Behavior 28: 387–402.

Bayoumy, M. H., A. M. Abou-Elnaga, A. A. Ghanim, and G. A. Mashhoot. 2016. Egg cannibalism potential benefits for adult reproductive performance and offspring fitness of *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae). Egyptian Journal of Biological Pest Control 26: 35–42.

Beirne, B. 1975. Biological control attempts by introductions against pest insects in the field in Canada. The Canadian Entomologist 107: 225–236.

Benton, T. G., and D. E. Bowler. 2012. *Dispersal in invertebrates: influences on individual decisions*. Oxford University Press.

Berkvens, N., C. Landuyt, K. Deforce, D. Berkvens, L. Tirry, and P. De Clercq. 2010. Alternative foods for the multicoloured Asian lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae). European Journal of Entomology 107: 189–195.

Bilu, E., and M. Coll. 2007. The importance of intraguild interactions to the combined effect of a parasitoid and a predator on aphid population suppression. Biocontrol 52: 753–763.

Bilu, E., and M. Coll. 2009. Parasitized aphids are inferior prey for a coccinellid predator: implications for intraguild predation. Environmental Entomology 38: 153–158.

Blackburn, T. M., P. Pyšek, S. Bacher, J. T. Carlton, R. P. Duncan, V. Jarošík, J. R. Wilson, and D. M. Richardson. 2011. A proposed unified framework for biological invasions. Trends in Ecology & Evolution 26: 333–339.

Boher, F., F. M. Jaksic, S. I. Martel, M. J. Orellana, and F. Bozinovic. 2018. Does thermal physiology explain the ecological and evolutionary success of invasive species? Lessons from ladybird beetles. Evolutionary Ecology Research 19: 243–255.

Borowski, J. 2015. Beetles (Coleoptera) of the Rogow region. Part II-ladybirds (Coccinellidae). International Letters of Natural Sciences 7.

Braekman, J. C., A. Charlier, D. Daloze, S. Heilporn, J. Pasteels, V. Plasman, and S. Wang. 1999. New piperidine alkaloids from two ladybird beetles of the genus *Calvia* (Coccinellidae). European Journal of Organic Chemistry 1999: 1749–1755.

Brown, P. M., R. Frost, J. Doberski, T. Sparks, R. Harrington, and H. E. Roy. 2011. Decline in native ladybirds in response to the arrival of *Harmonia axyridis*: early evidence from England. Ecological Entomology 36: 231–240.

Cabral, S., A. O. Soares, and P. Garcia. 2009. Predation by *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) on *Myzus persicae* Sulzer (Homoptera: Aphididae): Effect of prey density. Biological Control 50: 25–29.

Cabral, S., A. O. Soares, and P. Garcia. 2011. Voracity of *Coccinella undecimpunctata*: effects of insecticides when foraging in a prey/plant system. Journal of Pest Science 84: 373–379.

Causton, C. E., Lincango, M. P., and Poulsom, T. G. A. 2004. Feeding range studies of *Rodolia cardinalis* (Mulsant), a candidate biological control agent of *Icerya purchasi* Maskell in the Galapagos islands. Biological Control 29 (3): 315-25.

Chacon, J. M., D. A. Landis, and G. E. Heimpel. 2008. Potential for biotic interference of a classical biological control agent of the soybean aphid. Biological Control 46: 216–225.

Chapin, J. B., and V. Brou. 1991. *Harmonia axyridis* (Pallas), the third species of the genus to be found in the United States (Coleoptera: Coccinellidae). Proc. Entomol. Soc. Wash 93: 630–635.

Clancy, K. M., and P. W. Price. 1987. Rapid herbivore growth enhances enemy attack: sublethal plant defenses remain a paradox. Ecology 68: 733–737.

Clark, R. E., and M. S. Singer. 2018. Differences in aggressive behaviors between two ant species determine the ecological consequences of a facultative food-for-protection mutualism. Journal of Insect Behavior 31: 510–522.

Cock, M. J. W., S. T. Murphy, M. T. K. Kairo, E. Thompson, R. J. Murphy, and A. W. Francis. 2016. Trends in the classical biological control of insect pests by insects: an update of the BIOCAT database. BioControl 61: 349–363.

Coderre, D., L. Provencher, and J.-C. Tourneur. 1987. Oviposition and niche partitioning in aphidophagous insects on maize. The Canadian Entomologist 119: 195–203.

Comont, R. F., H. E. Roy, R. Harrington, C. R. Shortall, and B. V. Purse. 2014a. Ecological correlates of local extinction and colonisation in the British ladybird beetles (Coleoptera: Coccinellidae). Biological Invasions 16: 1805–1817.

Comont, R. F., H. E. Roy, O. T. Lewis, R. Harrington, C. R. Shortall, and B. V. Purse. 2012. Using biological traits to explain ladybird distribution patterns. Journal of Biogeography 39: 1772–1781.

Comont, R. F., B. V. Purse, W. Phillips, W. E. Kunin, M. Hanson, O. T. Lewis, R. Harrington, C. R. Shortall, G. Rondoni, and H. E. Roy. 2014b. Escape from parasitism by the invasive alien ladybird, *Harmonia axyridis*. Insect Conservation and Diversity 7: 334–342.

Cormier, C. M., T. A. Forbes, T. A. Jones, R. D. Morrison, and D. B. McCorquodale. 2000. Alien invasion: The status of non-native lady beetles (Coleoptera: Coccinellidae) in industrial Cape Breton, Nova Scotia. Northeastern Naturalist 7: 241–247.

Cottrell, T. E. 2005. Predation and cannibalism of lady beetle eggs by adult lady beetles. Biological Control 34: 159–164.

Day, W., D. Prokrym, D. Ellis, and R. Chianese. 1994. The known distribution of the predator *Propylea quatuordecimpunctata* (Coleoptera: Coccinellidae) in the United States, and thoughts on the origin of this species and five other exotic lady beetles in eastern North America. Entomological News 105: 244–256.

Dick, J. T. A., C. Laverty, J. J. Lennon, D. Barrios-O'Neill, P. J. Mensink, J. Robert Britton, V. Médoc, P. Boets, M. E. Alexander, N. G. Taylor, A. M. Dunn, M. J. Hatcher, P. J. Rosewarne, S. Crookes, H. J. MacIsaac, M. Xu, A. Ricciardi, R. J. Wasserman, B. R. Ellender, O. L. F. Weyl, F. E. Lucy, P. B. Banks, J. A. Dodd, C. MacNeil, M. R. Penk, D. C. Aldridge, and J. M. Caffrey. 2017. Invader Relative Impact Potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. Journal of Applied Ecology 54: 1259–1267.

Dindo, M., S. Francati, A. Lanzoni, C. di Vitantonio, E. Marchetti, G. Burgio, and S. Maini. 2016. Interactions between the multicolored Asian lady beetle *Harmonia axyridis* and the parasitoid *Dinocampus coccinellae*. Insects 7: 67.

Dixon, A. 2007. Body size and resource partitioning in ladybirds. Population Ecology 49: 45–50.

Dixon, A., and J.-L. Hemptinne. 2001. Body size distribution in predatory ladybird beetles reflects that of their prey. Ecology 82: 1847–1856.

Dlugosch, K., and I. Parker. 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology 17: 431–449.

Drake, J. M., and D. M. Lodge. 2006. Allee effects, propagule pressure and the probability of establishment: risk analysis for biological invasions. Biological Invasions 8: 365–375.

ElHag, E. T. A., and A. A. Zaitoon. 1996. Biological parameters for four coccinellid species in Central Saudi Arabia. Biological Control 7: 316–319.

Emiljanowicz, L. M., H. A. Hager, and J. A. Newman. 2017. Traits related to biological invasion: A note on the applicability of risk assessment tools across taxa. NeoBiota 32: 31.

Éric, L. 2013. Intraguild predation among aphidophagous predators. EJE 102: 351–364.

Escalona, H. E., and A. Ślipiński. 2012. Generic revision and phylogeny of Microweiseinae (Coleoptera: Coccinellidae). Systematic Entomology 37: 125–171.

Escalona, H. E., A. Zwick, H.-S. Li, J. Li, X. Wang, H. Pang, D. Hartley, L. S. Jermiin, O. Nedvěd, and B. Misof. 2017. Molecular phylogeny reveals food plasticity in the evolution of true ladybird beetles (Coleoptera: Coccinellidae: Coccinellini). BMC Evolutionary Biology 17: 151.

Evans, E. W. 2000. Morphology of invasion: body size patterns associated with establishment of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in western North America. European Journal of Entomology 97: 469–474.

Evans, E. W. 2004. Habitat displacement of North American ladybirds by an introduced species. Ecology 85: 637–647.

Evans, E. W., and T. R. Toler. 2007. Aggregation of polyphagous predators in response to multiple prey: ladybirds (Coleoptera: Coccinellidae) foraging in alfalfa. Population Ecology 49: 29–36.

Evans, E. W., A. T. Stevenson, and D. R. Richards. 1999. Essential versus alternative foods of insect predators: benefits of a mixed diet. Oecologia 121: 107–112.

Evans, E. W., A. O. Soares, and H. Yasuda. 2011. Invasions by ladybugs, ladybirds, and other predatory beetles. BioControl 56: 597–611.

Facon, B., A. Estoup, R. A. Hufbauer, J. Foucaud, and A. Tayeh. 2017. Mating status influences cold tolerance and subsequent reproduction in the invasive ladybird *Harmonia axyridis*. Frontiers in Ecology and Evolution 5.

Facon, B., L. Crespin, A. Loiseau, E. Lombaert, A. Magro, and A. Estoup. 2011. Can things get worse when an invasive species hybridizes? The harlequin ladybird *Harmonia axyridis* in France as a case study. Evolutionary Applications 4: 71–88.

Felix, S., and A. O. Soares. 2004. Intraguild predation between the aphidophagous ladybird beetles *Harmonia axyridis* and *Coccinella undecimpunctata* (Coleoptera: Coccinellidae): the role of body weight. European Journal of Entomology 101: 237–242.

Finlayson, C., A. Alyokhin, and E. Porter. 2009. Interactions of native and non-native lady beetle species (Coleoptera: Coccinellidae) with aphid-tending ants in laboratory arenas. Environmental Entomology 38: 846–855.

Forsman, A. 2014. Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. Proceedings of the National Academy of Sciences 111: 302–307.

Frank, J. H., and E. D. McCoy. 2007. The risk of classical biological control in Florida. Biological Control 41: 151–174.

Franzmann, B. A. 2002. *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), a predacious ladybird new in Australia. Australian Journal of Entomology 41: 375–377.

Gariepy, V., G. Boivin, and J. Brodeur. 2015. Why two species of parasitoids showed promise in the laboratory but failed to control the soybean aphid under field conditions. Biological Control 80: 1–7.

Gegner, T., O. Otti, S. Tragust, and H. Feldhaar. 2015. Do microsporidia function as "biological weapon" for *Harmonia axyridis* under natural conditions? Insect Science 22: 353–359.

Ghosh, S., and B. Agarwala. 2018. Inter–specific relationship of size and walking speed in predaceous ladybirds (Insecta: Coleoptera: Coccinellidae). Current Science 115: 141.

Giorgi, J. A., N. J. Vandenberg, J. V. McHugh, J. A. Forrester, S. A. Ślipiński, K. B. Miller, L. R. Shapiro, and M. F. Whiting. 2009. The evolution of food preferences in Coccinellidae. Biological Control 51: 215–231.

Goetz, D. W. 2009. Seasonal inhalant insect allergy: *Harmonia axyridis* ladybug. Current Opinion in Allergy and Clinical Immunology 9: 329–333.

Gordon, R. D. 1985. The Coccinellidae (Coleoptera) of America north of Mexico. Journal of the New York Entomological Society 93: 912 pp.

Goryacheva, I. I., A. V. Blekhman, B. V. Andrianov, T. V. Gorelova, and I. A. Zakharov. 2015. Genotypic diversity of Wolbachia pipientis in native and invasive *Harmonia axyridis* Pall., 1773 (Coleoptera, Coccinellidae) populations. Russian Journal of Genetics 51: 731–736.

Greathead, D. J. (1972). The rediscovery in Kenya of 2 introduced coccinellids *Cryptolaemus montrouzieri* and *Rodolia cardinalis*. East African Agricultural and Forestry Journal, 38 (2): 214-14.

Grez, A. A., B. Viera, and A. O. Soares. 2012. Biotic interactions between *Eriopis connexa* and *Hippodamia variegata*, a native and an exotic coccinellid species associated with alfalfa fields in Chile. Entomologia Experimentalis Et Applicata 142: 36–44.

Grozea, I., M. Vlad, A. M. Virteiu, R. Stef, A. Carabet, L. Molnar, and V. Mazare. 2015. Biological control of invasive species *Metcalfa pruinosa* Say (Insecta: Hemiptera: Flatidae) in ornamentals plants by using Coccinelids. Journal of Biotechnology 208: S112–S112.

Hameed, A., H. M. Saleem, S. Ahmad, J. Iqbal, H. Karar, and A. Amin. 2013. Predatory Potential and Life History Characteristics of Eleven Spotted Beetle, *Coccinella undecimpunctata* L. Reared on Cotton Mealybug, *Phenacoccus solenopsis* Tinsley. Pakistan Journal of Zoology 45: 1555–1562.

Hariston, N. G., L. J. Perry, A. J. Bohonak, M. Q. Fellows, C. M. Kearns, and D. R. Engstrom. 1999. Population biology of a failed invasion: Paleolimnology of

Daphnia exilis in upstate New York. Limnology and Oceanography 44: 477–486.

Harmon, J., A. Ives, J. Losey, A. Olson, and K. Rauwald. 2000. *Coleomegilla maculata* (Coleoptera: Coccinellidae) predation on pea aphids promoted by proximity to dandelions. Oecologia 125: 543–548.

Harrison, R. G. 1980. Dispersal polymorphisms in insects. Annual Review of Ecology and Systematics 11: 95–118.

Hellmann, J. J., J. E. Byers, B. G. Bierwagen, and J. S. Dukes. 2008. Five potential consequences of climate change for invasive species. Conservation biology 22: 534–543.

Hindayana, D., R. Meyhofer, D. Scholz, and H. M. Poehling. 2001. Intraguild predation among the hoverfly *Episyrphus balteatus* de Geer (Diptera : Syrphidae) and other aphidophagous predators. Biological Control 20: 236–246.

Hodek, I., and P. Ceryngier. 2000. Sexual activity in Coccinellidae (Coleoptera): a review. European Journal of Entomology 97: 449–456.

Hodek, I., H. F. Van Emden, and A. Honěk. 2012. *Ecology and behaviour of the ladybird beetles (Coccinellidae).* Blackwell Publishing.

Hoki, E., J. Losey, and T. A. Ugine. 2014. Comparing the consumptive and non-consumptive effects of a native and introduced lady beetle on pea aphids (*Acyrthosiphon pisum*). Biological Control 70: 78–84.

Hokkanen, H. M. T., and R. I. Sailer. 1985. Success in classical biological control. Critical Reviews in Plant Sciences 3: 35–72.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship. Oikos: 483–492.

Hoddle, Mark S., Ramirez, C. and Causton, C. 2013. Post release evaluation of *Rodolia cardinalis* (Coleoptera: Coccinellidae) for control of *Icerya purchasi* (Hemiptera: Monophlebidae) in the Galapagos Islands. Biological Control 67 (2): 262-74.

Hoogendoorn, M., and G. E. Heimpel. 2002. Indirect interactions between an introduced and a native ladybird beetle species mediated by a shared parasitoid. Biological Control 25: 224–230.

Hoogendoorn, M., and G. E. Heimpel. 2004. Competitive interactions between an exotic and a native ladybeetle: a field cage study. Entomologia Experimentalis et Applicata 111: 19–28.

Hopper, K. R., and R. T. Roush. 1993. Mate finding, dispersal, number released, and the success of biological control introductions. Ecological entomology 18: 321–331.

Howarth, F. G. 1991. Environmental Impacts of Classical Biological Control. Annual Review of Entomology 36: 485–509.

Janssen, A., M. Montserrat, R. HilleRisLambers, A. M. d. Roos, A. Pallini, and M. W. Sabelis. 2006. Intraguild predation usually does not disrupt biological control. Trophic and Guild in Biological Interactions Control: 21–44.

Kajita, Y., and E. W. Evans. 2009. Ovarian dynamics and oosorption in two species of predatory lady beetles (Coleoptera: Coccinellidae). Physiological Entomology 34: 185–194.

Kajita, Y., H. Yasuda, and E. W. Evans. 2006. Effects of native ladybirds on oviposition of the exotic species, *Adalia bipunctata* (Coleoptera : Coccinellidae), in Japan. Applied Entomology and Zoology 41: 57–61.

Kajita, Y., F. Takano, H. Yasuda, and B. K. Agarwala. 2000. Effects of indigenous ladybird species (Coleoptera: Coccinellidae) on the survival of an exotic species in relation to prey abundance. Applied Entomology and Zoology 35: 473–479.

Kajita, Y., E. M. O'Neill, Y. Zheng, J. J. Obrycki, and D. W. Weisrock. 2012. A population genetic signature of human releases in an invasive ladybeetle. Molecular Ecology 21: 5473–5483.

Kajita, Y., J. J. Obrycki, J. J. Sloggett, E. W. Evans, and K. F. Haynes. 2014. Do defensive chemicals facilitate intraguild predation and influence invasion success in ladybird beetles? Journal of Chemical Ecology 40: 1212–1219.

Kalaisekar, A., V. Ramamurthy, J. Patil, A. Dhandapani, and N. Azad Thakur. 2012. Multivariate morphometrics of elytral colour polymorphism in seven-spotted ladybird beetle, *Coccinella septempunctata* L.(Coleoptera: Coccinellidae). Current Science 102: 1418–1425.

Kawakami, Y., K. Yamazaki, and K. Ohashi. 2019. Intergenerational fluctuations in colour morph frequencies may maintain elytral polymorphisms in the ladybird beetle *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae). Biological Journal of the Linnean Society 128: 725–734.

Kenis, M., T. Adriaens, P. M. J. Brown, A. Katsanis, G. San Martin, E. Branquart, D. Maes, R. Eschen, R. Zindel, J. Van Vlaenderen, D. Babendreier, H. E. Roy, L. Hautier, and R. L. Poland. 2017. Assessing the ecological risk posed by a recently established invasive alien predator: *Harmonia axyridis* as a case study. Biocontrol 62: 341–354.

Knapp, M., M. Řeřicha, S. Maršíková, F. Harabiš, T. Kadlec, O. Nedvěd, and T. Teder. 2019. Invasive host caught up with a native parasitoid: field data reveal high parasitism of *Harmonia axyridis* by *Dinocampus coccinellae* in Central Europe. Biological Invasions 21: 2795–2802.

Koch, R. L., R. C. Venette, and W. D. Hutchison. 2006. Invasions by *Harmonia axyridis* (Pallas) (Coleoptera : Coccinellidae) in the Western Hemisphere: Implications for South America. Neotropical Entomology 35: 421–434.

Krafsur, E. S., J. J. Obrycki, and J. D. Harwood. 2005. Comparative genetic studies of native and introduced Coccinellidae in North America. European Journal of Entomology 102: 469–474.

Kumschick, S., M. Gaertner, M. Vilà, F. Essl, J. M. Jeschke, P. Pyšek, A. Ricciardi, S. Bacher, T. M. Blackburn, and J. T. Dick. 2015. Ecological impacts of alien species: quantification, scope, caveats, and recommendations. BioScience 65: 55–63.

Leung, B., J. M. Drake, and D. M. Lodge. 2004. Predicting invasions: Propagule pressure and the gravity of Allee effects. Ecology 85: 1651–1660.

Lincango, M. P., Causton, C. E., Alverex, C. and Jimenez-Uzcategui, G. 2011. Evaluating the safety of *Rodolia cardinalis* to two species of Galapagos finch; *Camarhynchus parvulus* and *Geospiza fuliginosa.* Biological Control*.* 56 (2), 145-49.

Lockwood, J. L., P. Cassey, and T. Blackburn. 2005. The role of propagule pressure in explaining species invasions. Trends in Ecology & Evolution 20: 223–228.

Lombaert, E., T. Malausa, R. Devred, and A. Estoup. 2008. Phenotypic variation in invasive and biocontrol populations of the harlequin ladybird, *Harmonia axyridis*. Biocontrol 53: 89–102.

Lombaert, E., T. Guillemaud, J.-M. Cornuet, T. Malausa, B. Facon, and A. Estoup. 2010. Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. PLoS ONE 5: e9743.

Lombaert, E., A. Estoup, B. Facon, B. Joubard, J. C. Grégoire, A. Jannin, A. Blin, and T. Guillemaud. 2014. Rapid increase in dispersal during range expansion in the invasive ladybird *Harmonia axyridis*. Journal of Evolutionary Biology 27: 508–517.

Lombaert, E., T. Guillemaud, C. Thomas, L. Lawson Handley, J. Li, S. Wang, H. Pang, I. Goryacheva, I. Zakharov, and E. Jousselin. 2011. Inferring the origin of populations introduced from a genetically structured native range by approximate Bayesian computation: case study of the invasive ladybird *Harmonia axyridis*. Molecular Ecology 20: 4654–4670.

Lommen, S. T. E., C. W. Middendorp, C. A. Luijten, J. van Schelt, P. M. Brakefield, and P. W. de Jong. 2008. Natural flightless morphs of the ladybird beetle *Adalia bipunctata* improve biological control of aphids on single plants. Biological Control 47: 340–346.

Lopez, V. F., M. T. K. Kairo, and J. A. Irish. 2004. Biology and prey range of *Cryptognatha nodiceps* (Coleoptera : Coccinellidae), a potential biological control agent for the coconut scale, *Aspidiotus destructor* (Hemiptera : Diaspididae). Biocontrol Science and Technology 14: 475–485.

Losey, J. E., J. E. Perlman, and E. R. Hoebeke. 2007. Citizen scientist rediscovers rare nine-spotted lady beetle, *Coccinella novemnotata*, in eastern North America. Journal of Insect Conservation 11: 415–417.

MacDonald, F. H., P. G. Connolly, N. J. Larsen, and G. P. Walker. 2016. The voracity of five insect predators on *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) (tomato potato psyllid; TPP). New Zealand Entomologist 39: 15–22.

Madire, L. G. 2013. Biology and host range of *Mada polluta,* a potential biological control agent of *Tecoma stans* in South Africa. Biocontrol Science and Technology 23: 944–955.

Majerus, M., V. Strawson, and H. Roy. 2006. The potential impacts of the arrival of the harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera : Coccinellidae), in Britain. Ecological Entomology 31: 207–215.

Maruyamai, M., and S. Ohno. 2011. Record of a ladybird beetle *Coelophora inaequalis* (Fabricius, 1775) in Okinawa, Japan. Japanese Journal of Entomology (New Series) 14: 112–115.

McDermott, S. M., and D. C. Finnoff. 2016. Impact of repeated human introductions and the Allee effect on invasive species spread. Ecological Modelling 329: 100–111.

Meffe, G. K. 1991. Failed invasion of a southeastern blackwater stream by bluegills - implications for conservation of native communities. Transactions of the American Fisheries Society 120: 333–338.

Mehrnejad, M. R., M. A. Jalali, and R. Mirzaei. 2011. Abundance and biological parameters of psyllophagous coccinellids in pistachio orchards. Journal of Applied Entomology 135: 673–681.

Mendel, Z., F. Assael, S. Zeidan, and A. Zehavi. 1998. Classical biological control of *Palaeococcus fuscipennis* (Burmeister) (Homoptera : Margarodidae) in Israel. Biological Control 12: 151–157.

Michael, P. J., P. B. Yeoh, and J. K. Scott. 2012. Potential distribution of the australian native *Chloris truncata* based on modelling both the successful and failed global introductions. PLoS ONE 7.

Michaud, J. 2012. Coccinellids in biological control. *In* Ecology and behaviour of the ladybird beetles (Coccinellidae): 488–519.

Michaud, J., and A. Grant. 2003. Intraguild predation among ladybeetles and a green lacewing: do the larval spines of *Curinus coeruleus* (Coleoptera: Coccinellidae) serve a defensive function? Bulletin of Entomological Research 93: 499.

Michaud, J. P. 2003. A comparative study of larval cannibalism in three species of ladybird. Ecological Entomology 28: 92–101.

Michels, G. J., R. V. Flanders, and J. B. Bible. 1997. Overwintering survival of seven imported coccinellids in the Texas high plains. Southwestern Entomologist 22: 157–166.

Michie, L. J., F. Mallard, M. E. N. Majerus, and F. M. Jiggins. 2010. Melanic through nature or nurture: genetic polymorphism and phenotypic plasticity in *Harmonia axyridis*. Journal of Evolutionary Biology 23: 1699–1707.

Mirande, L., N. Desneux, M. Haramboure, and M. Ines Schneider. 2015a. Intraguild predation between an exotic and a native coccinellid in Argentina: the role of prey density. Journal of Pest Science 88: 155–162.

Mirande, L., N. Desneux, M. Haramboure, and M. I. Schneider. 2015b. Intraguild predation between an exotic and a native coccinellid in Argentina: the role of prey density. Journal of Pest Science 88: 155–162.

Mohamed, E. Nadia, and A. A. Ghanim. 2008. Biological characteristics of three predatory insects preyed on the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring under controlled conditions. Egyptian Journal of Biological Pest Control 18: 309–313.

Moser, S. E., and J. J. Obrycki. 2009. Competition and intraguild predation among three species of coccinellids (Coleoptera: Coccinellidae). Annals of the Entomological Society of America 102: 419–425.

Moulton, M. P., W. P. Cropper, and M. L. Avery. 2012. Historical records of passerine introductions to New Zealand fail to support the propagule pressure hypothesis. Biodiversity and Conservation 21: 297–307.

Musser, F. R., and A. M. Shelton. 2003. Factors altering the temporal and within-plant distribution of coccinellids in corn and their impact on potential intra-guild predation. Environmental Entomology 32: 575–583.

Nedved, O., and A. Honek. 2012. Life history and development. *In* Ecology and behaviour of the ladybird beetles: 54–109.

Nedvěd, O., M. Awad, G. Laugier, L. Piálek, A. Krejčí, F. Karabüyük, K. Pužejová, and P. Kalushkov. 2018. Paternity and sperm competition following multiple mating in Harmonia axyridis (Coleoptera: Coccinellidae). IOBC-WPRS Bulletin 137: 17–24.

Nijhout, H. 2003. The control of body size in insects. Developmental Biology 261: 1–9.

Nimmo, K. R., and P. W. Tipping. 2009. An introduced insect biological control agent preys on an introduced weed biological control agent. Florida Entomologist 92: 179–180, 172.

Noia, M., I. Borges, and A. O. Soares. 2008. Intraguild predation between the aphidophagous ladybird beetles *Harmonia axyridis* and *Coccinella undecimpunctata* (Coleoptera : Coccinellidae): The role of intra and extraguild prey densities. Biological Control 46: 140–146.

Obrycki, J. J., and T. J. Kring. 1998. Predaceous Coccinellidae in biological control. Annual Review of Entomology 43: 295–321.

Omkar, G. Mishra, B. Kumar, N. Singh, and G. Pandey. 2014. Risks associated with tandem release of large and small ladybirds (Coleoptera: Coccinellidae) in heterospecific aphidophagous guilds. Canadian Entomologist 146: 52–66.

Orlova-Bienkowskaja, M. J. 2013. Dangerous invasive harlequin ladybird *Harmonia axyridis* (Pallas, 1773) (Coleoptera, Coccinellidae) in the European Russia. Russian Journal of Biological Invasions 4: 190–193.

Paul, S. C., J. K. Pell, and J. D. Blount. 2015. Reproduction in risky environments: the role of invasive egg predators in ladybird laying strategies (dataset). PLoS ONE 10(10): e0139404.

Pervez, A., G. Mishra, S. Srivastava, S. K. Singh, and A. K. Gupta. 2005. Intrinsic advantages of *Cheilomenes sexmaculata* over two coexisting *Coccinella* species (Coleoptera: Coccinellidae). Insect Science 12: 179–184.

Polis, G. A., and R. D. Holt. 1992. Intraguild predation: the dynamics of complex trophic interactions. Trends in Ecology & Evolution 7: 151–154.

Powell, B. E., and J. Silverman. 2010. Impact of *Linepithema humile* and *Tapinoma sessile* (Hymenoptera: Formicidae) on three natural enemies of *Aphis gossypii* (Hemiptera: Aphididae). Biological Control 54: 285–291.

Pugh, A. R., D. M. O'Connell, and S. D. Wratten. 2015. Further evaluation of the southern ladybird (*Cleobora mellyi*) as a biological control agent of the invasive tomato-potato psyllid (*Bactericera cockerelli*). Biological Control 90: 157–163.

Purse, B. V., R. Comont, A. Butler, P. M. J. Brown, C. Kessel, and H. E. Roy. 2015. Landscape and climate determine patterns of spread for all colour morphs of the alien ladybird *Harmonia axyridis*. J. Biogeogr. 42: 575–588.

Raak-van den Berg, C. L., P. W. de Jong, G. Gort, B. F. J. Manly, and J. C. van Lenteren. 2018. Life histories of an invasive and native ladybird under field experimental conditions in a temperate climate. Entomologia Experimentalis et Applicata 166: 151–161.

Ramírez, J., G. González, and Y. Sánchez. 2018. First record of *Cheilomenes sexmaculata* (Fabricius, 1781)(Coleoptera, Coccinellidae) from Colombia. Check List 14: 77.

Reznik, S. Y., M. Y. Dolgovskaya, A. N. Ovchinnikov, and N. A. Belyakova. 2015. Weak photoperiodic response facilitates the biological invasion of the harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). Journal of Applied Entomology 139: 241–249.

Reznik, S. Y., A. N. Ovchinnikov, A. A. Ovchinnikova, and N. A. Belyakova. 2018. Costs and benefits of interlarval cannibalism in Harmonia axyridis. Journal of Applied Entomology 142: 223–229.

Reznik, S. Y., A. A. Ovchinnikova, A. N. Ovchinnikov, L. V. Barabanova, and N. A. Belyakova. 2017. Inheritance of diapause regulation in the multicoloured Asian ladybird Harmonia axyridis (Coleoptera: Coccinellidae). European Journal of Entomology 114.

Riddick, E. W., T. E. Cottrell, and K. A. Kidd. 2009. Natural enemies of the Coccinellidae: Parasites, pathogens, and parasitoids. Biological Control 51: 306–312.

Robertson, J. A., A. Ślipiński, M. Moulton, F. W. Shockley, A. Giorgi, N. P. Lord, D. D. Mckenna, W. Tomaszewska, J. Forrester, and K. B. Miller. 2015. Phylogeny and classification of Cucujoidea and the recognition of a new superfamily Coccinelloidea (Coleoptera: Cucujiformia). Systematic Entomology 40: 745–778.

Romanov, D. 2019. Parasitoids of the harlequin ladybeetle *Harmonia axyridis* (Pallas, 1773)(Coleotera: Coccinellidae). Russian Journal of Biological Invasions 10: 52–67.

Rondoni, G., F. Ielo, C. Ricci, and E. Conti. 2017. Behavioural and physiological responses to prey-related cues reflect higher competitiveness of invasive vs. native ladybirds. Scientific Reports 7: 9.

Rondoni, G., K. J. Athey, J. D. Harwood, E. Conti, C. Ricci, and J. J. Obrycki. 2015. Development and application of molecular gut-content analysis to detect aphid and coccinellid predation by *Harmonia axyridis* (Coleoptera: Coccinellidae) in Italy. Insect Science 22: 719–730.

Roy, H. E., T. Adriaens, N. J. Isaac, M. Kenis, T. Onkelinx, G. S. Martin, P. M. Brown, L. Hautier, R. Poland, and D. B. Roy. 2012. Invasive alien predator causes rapid declines of native European ladybirds. Diversity and Distributions 18: 717–725.

Roy, H. E., P. M. J. Brown, T. Adriaens, N. Berkvens, I. Borges, S. Clusella-Trullas, R. F. Comont, P. De Clercq, R. Eschen, A. Estoup, E. W. Evans, B. Facon, M. M. Gardiner, A. Gil, A. A. Grez, T. Guillemaud, D. Haelewaters, A. Herz, A. Honek, A. G. Howe, C. Hui, W. D. Hutchison, M. Kenis, R. L. Koch, J. Kulfan, L. L. Handley, E. Lombaert, A. Loomans, J. Losey, A. O. Lukashuk, D. Maes, A. Magro, K. M. Murray, G. San Martin, Z. Martinkova, I. A. Minnaar, O. Nedved, M. J. Orlova-Bienkowskaja, N. Osawa, W. Rabitsch, H. P. Ravn, G. Rondoni, S. L. Rorke, S. K. Ryndevich, M.-G. Saethre, J. J. Sloggett, A. O. Soares, R. Stals, M. C. Tinsley, A. Vandereycken, P. van Wielink, S. Viglasova, P. Zach, I. A. Zakharov, T. Zaviezo, and Z. Zhao. 2016. The harlequin ladybird, *Harmonia axyridis*: global perspectives on invasion history and ecology. Biological Invasions 18: 997–1044.

Rudolf, V. H. 2007. The interaction of cannibalism and omnivory: consequences for community dynamics. Ecology 88: 2697–2705.

Rudolf, V. H., and N. L. Rasmussen. 2013. Ontogenetic functional diversity: size structure of a keystone predator drives functioning of a complex ecosystem. Ecology 94: 1046–1056.

Saito, T., and S. Bjornson. 2008. Effects of a microsporidium from the convergent lady beetle, *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae), on three non-target coccinellids. Journal of Invertebrate Pathology 99: 294–301.

Sato, S., and A. F. G. Dixon. 2004. Effect of intraguild predation on the survival and development of three species of aphidophagous ladybirds: consequences for invasive species. Agricultural and Forest Entomology 6: 21–24.

Saxena, S., G. Mishra, and O. Omkar. 2019. Mating system drives the mate selection in aphidophagous ladybird, *Menochilus sexmaculatus*. Journal of Evolutionary Biology.

Sayed, S. M. 2016. Molecular diversity of the lady beetles, *Coccinella undecimpunctata* L. and *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) in Saudi Arabia. Egyptian Journal of Biological Pest Control 26: 351–355.

Schaefer, P. W., R. J. Dysart, and H. B. Specht. 1987. North-American distribution Of *Coccinella Septempunctata* (Coleoptera, Coccinellidae) and its mass appearance in coastal Delaware. Environmental Entomology 16: 368–373.

Scorsetti, A. C., S. A. Pelizza, M. N. Fogel, M. F. Vianna, and M. I. Schneider. 2017. Interactions between the entomopathogenic fungus *Beauveria bassiana* and the Neotropical predator *Eriopis connexa* (Coleoptera: Coccinellidae): Implications in Biological Control Of Pest.

Seago, A. E., J. A. Giorgi, J. Li, and A. Ślipiński. 2011. Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. Molecular Phylogenetics and Evolution 60: 137–151.

Seko, T., K.-i. Yamashita, and K. Miura. 2008. Residence period of a flightless strain of the ladybird beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in open fields. Biological Control 47: 194–198.

Seko, T., A. Sumi, A. Nakano, M. Kameshiro, T. Kaneda, and K. Miura. 2014. Suppression of aphids by augmentative release of larvae of flightless *Harmonia axyridis*. Journal of Applied Entomology 138: 326–337.

Sheppard, S. K., M. L. Henneman, J. Memmott, and W. O. C. Symondson. 2004. Infiltration by alien predators into invertebrate food webs in Hawaii: a molecular approach. Molecular Ecology 13: 2077–2088.

Shoeb, M. A., F. A. Attalla, and A. H. El-Heneidy. 2008. On the migration of common predatory species from Egyptian clover to adjacent maize fields in Egypt. Egyptian Journal of Biological Pest Control 18: 385–389.

Siljamo, P., K. Ashbrook, R. F. Comont, and C. A. Skjøth. 2020. Do atmospheric events explain the arrival of an invasive ladybird (*Harmonia axyridis*) in the UK? PLoS ONE 15: e0219335.

Simmons, A. M., and J. C. Legaspi. 2007. Ability of *Delphastus catalinae* (Coleoptera : Coccinellidae), a predator of whiteflies (Homoptera : Aleyrodidae), to survive mild winters. Journal of Entomological Science 42: 163–173.

Simmons, A. M., and S. Abd-Rabou. 2011. Inundative field releases and evaluation of three predators for *Bemisia tabaci* (Hemiptera: Aleyrodidae) management in three vegetable crops. Insect Science 18: 195–202.

Singh, S., G. Mishra, and Omkar. 2018. Plasticity in reproductive output and development in response to thermal variation in ladybird beetle, *Menochilus sexmaculatus*. Journal of Thermal Biology 71: 180–188.

Skouras, P. J., G. J. Stathas, C. C. Voudouris, A. I. Darras, J. A. Tsitsipis, and J. T. Margaritopoulos. 2017. Effect of synthetic insecticides on the larvae of

Coccinella septempunctata from Greek populations. Phytoparasitica 45: 165–173.

Ślipiński, A. 2007. *Australian ladybird beetles (Coleoptera: Coccinellidae): their biology and classification.* Csiro Publishing.

Ślipiński, S., R. Leschen, and J. Lawrence. 2011. Order Coleoptera Linnaeus, 1758. Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. Zootaxa 3148: 203–208.

Sloggett, J. J. 2005. Are we studying too few taxa? Insights from aphidophagous ladybird beetles (Coleoptera: Coccinellidae). European Journal of Entomology 102: 391.

Smith, C. A., and M. M. Gardiner. 2013. Biodiversity loss following the introduction of exotic competitors: does intraguild predation explain the decline of native lady beetles? PLoS ONE 8: e84448.

Smyth, R. R., L. L. Allee, and J. E. Losey. 2013. The status of *Coccinella undecimpunctata* (L.) (Coleoptera: Coccinellidae) in North America: An updated distribution from citizen science data. Coleopterists Bulletin 67: 532–535.

Snyder, W. E., and G. M. Clevenger. 2004. Negative dietary effects of Colorado potato beetle eggs for the larvae of native and introduced ladybird beetles. Biological Control 31: 353–361.

Snyder, W. E., G. M. Clevenger, and S. D. Eigenbrode. 2004. Intraguild predation and successful invasion by introduced ladybird beetles. Oecologia 140: 559–565.

Soares, A. O., and A. Serpa. 2007. Interference competition between ladybird beetle adults (Coleoptera: Coccinellidae): effects on growth and reproductive capacity. Population Ecology 49: 37–43.

Soares, A. O., I. Borges, P. A. Borges, G. Labrie, and E. Lucas. 2008. *Harmonia axyridis*: What will stop the invader? BioControl 53: 127–145.

Soares, A. O., A. Honěk, Z. Martinkova, J. Skuhrovec, P. Cardoso, and I. Borges. 2017. *Harmonia axyridis* failed to establish in the Azores: the role of species richness, intraguild interactions and resource availability. BioControl 62: 423–434.

Stankovic, V. M., T. Koren, and I. Stankovic. 2011. The harlequin ladybird continues to invade southeastern Europe. Biological Invasions 13: 1711–1716.

Steele, T., and S. Bjørnson. 2019. Effects of microsporidiosis and food availability on the two-spotted lady beetle, *Adalia bipunctata* L., and convergent lady beetle, *Hippodamia convergens* Guérin-Méneville. Journal of Invertebrate Pathology 161: 7–13.

Szawaryn, K., L. Bocak, A. Ślipiński, H. E. Escalona, and W. Tomaszewska. 2015. Phylogeny and evolution of phytophagous ladybird beetles (Coleoptera: Coccinellidae: Epilachnini), with recognition of new genera. Systematic Entomology 40: 547–569.

Takizawa, T., and H. Yasuda. 2006. The effects of attacks by the mutualistic ant, *Lasius japonicus* Santschi (Hymenoptera: Formicidae) on the foraging behavior of the two aphidophagous ladybirds, *Coccinella septempunctata* brucki Mulsant (Coleoptera: Coccinellidae) and *Propylea japonica* (Thunberg)(Coleoptera: Coccinellidae). Applied Entomology And Zoology 41: 161–169.

Tayeh, A., R. A. Hufbauer, A. Estoup, V. Ravigné, L. Frachon, and B. Facon. 2015. Biological invasion and biological control select for different life histories. Nature Communications 6: 1–5.

Tayeh, A., A. Estoup, G. Laugier, A. Loiseau, J. Turgeon, S. Toepfer, and B. Facon. 2012. Evolution in biocontrol strains: insight from the harlequin ladybird *Harmonia axyridis*. Evolutionary Applications 5: 481–488.

Tillman, P. G. 2011. Natural Biological Control of Stink Bug (Heteroptera: Pentatomidae) Eggs in Corn, Peanut, and Cotton Farmscapes in Georgia. Environmental Entomology 40: 303–314.

Toda, Y., and Y. Sakuratani. 2006. Expansion of the geographical distribution of an exotic ladybird beetle, *Adalia bipunctata* (Coleoptera : Coccinellidae), and its interspecific relationships with native ladybird beetles in Japan. Ecological Research 21: 292–300.

Togni, P. H., M. Venzon, C. A. Muniz, E. F. Martins, A. Pallini, and E. R. Sujii. 2016. Mechanisms underlying the innate attraction of an aphidophagous coccinellid to coriander plants: Implications for conservation biological control. Biological Control 92: 77–84.

Tohamy, T. H., A. A. A. El-Raheem, and A. M. El-Rawy. 2008. Role of the cultural practices and natural enemies for suppressing infestation of the pink sugarcane mealybug, *Saccharicoccus sacchari* (Cockerell) (Hemiptera: Pseudococcidae) in sugarcane fields at Minia Governorate, Middle Egypt. Egyptian Journal of Biological Pest Control 18: 177–188.

Tscharntke, T., I. Steffan-Dewenter, A. Kruess, and C. Thies. 2002. Characteristics of insect populations on habitat fragments: A mini review. Ecological Research 17: 229–239.

Tumminello, G., T. A. Ugine, and J. E. Losey. 2015. Intraguild interactions of native and introduced coccinellids: the decline of a flagship species. Environmenatal Entomology 44: 64–72.

Turgeon, J., A. Tayeh, B. Facon, E. Lombaert, P. De Clercq, N. Berkvens, J. G. Lundgren, and A. Estoup. 2011. Experimental evidence for the phenotypic impact of admixture between wild and biocontrol Asian ladybird (*Harmonia axyridis*) involved in the European invasion. Journal of Evolutionary Biology 24: 1044–1052.

Turnipseed, R., T. A. Ugine, and J. E. Losey. 2015. Egg predation by the introduced lady beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae), lowers mortality but raises relative risk for the native lady beetle, *Coccinella novemnotata*. PLoS ONE 10.

Ueno, H. 1996. Estimate of multiple insemination in a natural population of *Harmonia axyridis* (Coleoptera: Coccinellidae). Applied Entomology And Zoology 31: 621–623.

Veran, S., S. Piry, V. Ternois, C. N. Meynard, B. Facon, and A. Estoup. 2016. Modeling spatial expansion of invasive alien species: relative contributions of environmental and anthropogenic factors to the spreading of the harlequin ladybird in France. Ecography 39: 665–675.

Viglášová, S., O. Nedvěd, P. Zach, J. Kulfan, M. Parák, A. Honěk, Z. Martinková, and H. E. Roy. 2017. Species assemblages of ladybirds including the harlequin ladybird *Harmonia axyridis*: a comparison at large spatial scale in urban habitats. BioControl 62: 409–421.

Wallach, A. D., A. H. Dekker, M. Lurgi, J. M. Montoya, D. A. Fordham, and E. G. Ritchie. 2017. Trophic cascades in 3D: network analysis reveals how apex predators structure ecosystems. Methods in Ecology and Evolution: 135–142.

Wang, L., J. Huang, and H. Luo. 2008. Response by a Whitefly predator, *Delphastus catalinae* to toxins from *Verticillium lecanii* and mass rearing techniques for the ladybeetle. Journal of Insect Science 8: 51–51.

Wang, S., X. L. Tan, X. J. Guo, and F. Zhang. 2013. Effect of temperature and photoperiod on the development, reproduction, and predation of the predatory ladybird *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae). Journal of Economical Entomology 106: 2621–2629.

Wang, S., J. P. Michaud, X. L. Tan, F. Zhang, and X. J. Guo. 2011. The aggregation behavior of *Harmonia axyridis* in its native range in Northeast China. BioControl 56: 193–206.

Ware, R., B. Yguel, and M. Majerus. 2009. Effects of competition, cannibalism and intra-guild predation on larval development of the European coccinellid *Adalia bipunctata* and the invasive species *Harmonia axyridis*. Ecological Entomology 34: 12–19.

Wells, P. M., J. Baverstock, S. J. Clark, F. M. Jiggins, H. E. Roy, and J. K. Pell. 2017. Determining the effects of life stage, shared prey density and host plant on intraguild predation of a native lacewing (*Chrysoperla carnea*) by an invasive coccinellid (*Harmonia axyridis*). BioControl 62: 373–384.

Werf, V. W., E. W. Evans, and J. Powell. 2000. Measuring and modelling the dispersal of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in alfalfa fields. European Journal of Entomology 97: 487–493.

Wheeler, A. G., and E. R. Hoebeke. 2008. Rise and fall of an immigrant lady beetle: Is *Coccinella undecimpunctata* L. (Coleoptera : Coccinellidae) still present in North America? Proceedings of the Entomological Society of Washington 110: 817–823.

Williams, A. H., and D. K. Young. 2009. The alien *Hippodamia variegata* (Coleoptera: Coccinellidae) quickly establishes itself throughout wisconsin. Great Lakes Entomologist 42: 100–100.

Yasuda, H., E. W. Evans, Y. Kajita, K. Urakawa, and T. Takizawa. 2004. Asymmetric larval interactions between introduced and indigenous ladybirds in North America. Oecologia 141: 722–731.

Zadravec, D., and M. Bavec. 2001. Natural enemies against vegetable pests in the Northeast of Slovenia. 5th Slovenian Conference on Plant Protection.

Zaki, F. N., M. F. El-Shaarawy, and N. A. Farag. 1999. Release of two predators and two parasitoids to control aphids and whiteflies. Anzeiger Fur Schadlingskunde-Journal of Pest Science 72: 19–20.

Zenni, R. D., and M. A. Nunez. 2013. The elephant in the room: the role of failed invasions in understanding invasion biology. Oikos 122: 801–815.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annual Review of Entomology 42: 207–230.

Zerebecki, R. A., and C. J. Sorte. 2011. Temperature tolerance and stress proteins as mechanisms of invasive species success. PLoS ONE 6: e14806.

Zhang, G. F., G. L. Lovei, X. Wu, and F. H. Wan. 2016. Presence of native prey does not divert predation on exotic pests by *Harmonia axyridis* in its indigenous range. PLoS ONE 11.

Zhou, A., J. Bi, J. Xu, and M.-Q. Wang. 2015. Effects of the invasive plant *Eupatorium adenophorum* on the exotic mealybug *Phenacoccus solenopsis* (Hemiptera:

Pseudococcidae) and their natural enemies. Agricultural and Forest Entomology 17: 389–399.

Zhou, A., Y. Lu, L. Zeng, Y. Xu, and G. Liang. 2013. *Solenopsis invicta* (Hymenoptera: Formicidae), defend *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) against its natural enemies. Environmental Entomology 42: 247–252.

Zhou, A. M., G. W. Liang, L. Zeng, Y. Y. Lu, and Y. J. Xu. 2014. Interactions between ghost ants and invasive mealybugs: The case of *Tapinoma melanocephalum* (Hymenoptera: Formicidae) and *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae). Florida Entomologist 97: 1474–1480.

Zhou, X., A. Honek, W. Powell, and N. Carter. 1995. Variations in body length, weight, fat content and survival in *Coccinella septempunctata* at different hibernation sites. Entomologia Experimentalis et Applicata 75: 99–107.

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INSIGHTS INTO THE ESTABLISHMENT OF INTRODUCED SPECIES USING COCCINELLINES (COLEOPTERA: COCCINELLIDAE) AS A MODEL SYSTEM

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ABSTRACT

Species introductions occur around the world both inadvertently and deliberately (typically for conservation, agriculture or fisheries). However, not all introduced species become established. Understanding the factors that affect the establishment success of introduced species will help us improve species introductions for biocontrol and conservation purposes. Here we argue that important generalist arthropod predators, the Coccinellini ladybirds (Coleoptera: Coccinellidae), are an ideal taxon for investigating the establishment process of introduced species. Coccinellini are introduced accidently via plant exports, as well as deliberately as biocontrol agents to reduce agricultural pests, and a few have become invasive species. Here, using work from invasive biology and biocontrol systems, we categorize the factors affecting the successful establishment of introduced species. These factors are 1) invasiveness of the species, 2) invasibility of the recipient ecosystem and 3) stochastic events that occur after the introduction. We review how factors such as diet and competition, dispersal ability, propagule population, disturbances and climate change can be studied within these three categories to better predict the establishment success of introduced ladybirds. Our review highlights that our current understanding of the differences between successful and unsuccessful species establishments is limited. To address this, we need direct comparisons of dispersal ability and both interspecific and intraspecific variation in ladybirds. We conclude that studies of ladybirds will help to develop theories that better characterize and predict establishment success and invasive potential.

Keywords: categorization, dispersal ability, intraspecific variation, habitat invasibility, intraguild predation

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INTRODUCTION

Around the world, species are introduced into new locations both inadvertently and deliberately for biological control, conservation and sport. However, not all introduced species become established. A recent update on biological control agents suggests that only a maximum of 32.6% of introduced biocontrol agents become established after introduction (Cock *et al.* 2016). These low success rates have always been a problem in biological control programs. Therefore, it is important to understand the factors that affect the successful establishment of introduced species. Although introduced biological control agents are studied extensively in the screening process, we are still unable to predict their establishment success. To develop an overall model of establishment success of introduced species, Abram and Moffat (2018) suggested looking at the introduction of biological control agents as planned invasions to understand the factors enabling species to establish and disperse in a new environment. For instance, biological invasions are a four-part process comprising transport, introduction, establishment and spread (Blackburn *et al.* 2011). Current literature has mostly considered the factors that enable an introduced population to go from establishment to the spreading stage with much less attention to a) factors that drive earlier stages of the invasion process (Blackburn *et al.* 2011) and b) factors that affected failed introductions (Cock *et al.* 2016; Gariepy *et al.* 2015; Hariston *et al.* 1999; Meffe 1991; Zenni and Nunez 2013). The stage when most introductions fail is establishment—when the introduced population begins to reproduce and maintain (or grow) its population in its new habitat (Blackburn *et al.* 2011). Identifying the factors that drive the establishment of introduced species remains challenging and controversial (*e.g.*, see Dick *et al.* [2017] and associated commentary). Therefore, we propose studying factors that promote and inhibit the successful establishment of introduced species.

To determine the establishment success of introduced species, we need to evaluate factors under three categories (*sensu* Abram and Moffat 2018): a) invasibility of the habitat, b) invasiveness of the introduced species and c) stochastic events (Fig. 1). We use the terms invasibility and invasiveness as defined in invasive biology, as we are considering species introductions as planned invasions to better understand the factors affecting successful establishment as an adaptation of the framework proposed by Abram and Moffat (2018) for biocontrol.

Our current approach assesses the characteristics of the introduced species to identify its effectiveness for

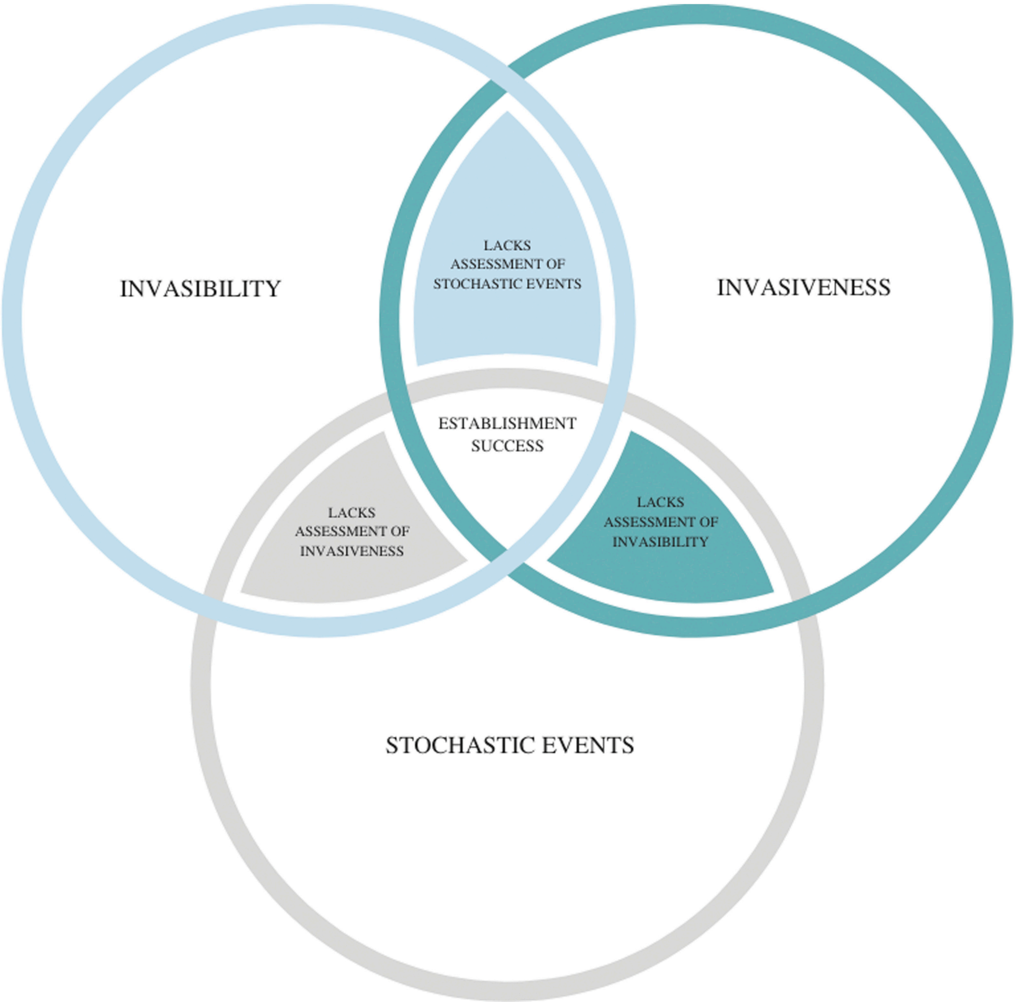

Fig. 1. Categorization of factors to assess establishment success of introduced species. Invasibility is defined as the characteristics of a habitat that makes it vulnerable to be invaded by introduced species. Invasiveness is defined as those characteristics or traits of introduced species that enable them to establish in a new area. Stochastic events are events that may or may not change invasibility and invasiveness affecting the establishment process. We predict the rate of successful establishment will increase at the mid-point where all three categories overlap. However, in the absence of stochastic events that would influence the establishment process, we may predict the success rate of establishment by assessing only the species' match in invasiveness with invasibility of the recipient ecosystem.

the purpose with which it is going to be introduced (*i.e.*, biocontrol) and assess whether the habitat parameters of the introduced area match the current known distribution or native habitats of the introduced species. This creates a limit on our understanding of how other factors in both species (*i.e.*, variation and intraguild predation vulnerability, see Table 1) and in the introduced area (*i.e.*, other species present or absent, see Table 1) may affect establishment of the introduced species after its introduction. Potential interactions between the introduced species and recipient ecosystem are not studied enough. Using the proposed three categories to guide research effort would allow us to gain an overall understanding of species traits (invasiveness) and habitat parameters (invasibility) of the introduced area, how they interact with each other and any stochastic events occurring during the establishment process. For an introduced species to establish successfully in a new habitat, invasiveness of the species (species-specific characteristics) should match with habitat invasibility and, finally, should overcome any stochastic events (such as sudden weather changes) that may occur. For example, ladybirds generally acclimatize well in fluctuating temperatures, and this would not affect the establishment of introduced ladybirds in habitats with fluctuating temperatures (Singh *et al.* 2018). However, some species (*i.e.*, *Harmonia axyridis* [Pallas, 1773]) require lower temperatures to induce and complete their overwintering diapause (Soares *et al.* 2008). Therefore, understanding variation in temperature tolerance of the introduced species as well as the temperature profile of the introduced area will help us to predict how establishment of the introduced species would be affected by temperature fluctuations. Here we review studies of coccinelline ladybirds (Coleoptera: Coccinellidae) within the three-category framework to identify gaps in our understanding and suggest what we can do to improve the establishment success of ladybirds in biocontrol studies.

Coccinellines as a Model System. Ladybirds, also called ladybird beetles, lady beetles, ladybugs and coccinellids (Hodek *et al.* 2012), are mostly predators that play a complex role in community dynamics (Rudolf 2007; Rudolf and Rasmussen 2013; Wallach *et al.* 2017). There are approximately 6,000 described species of ladybird (Ślipiński *et al.* 2011). They are currently the most species-rich of the 15 families in the superfamily Coccinelloidea (Robertson *et al.* 2015). The coccinellids have been the focus of several recent molecular phylogenetic studies (Escalona *et al.* 2017; Giorgi *et al.* 2009; Seago *et al.* 2011; Szawaryn *et al.* 2015), and based on these studies the classification has changed significantly, although it still needs further revision. The family is currently divided into two subfamilies, the less diverse Microweiseinae (Escalona and Ślipiński 2012), with only a handful of often cryptic genera, and the larger Coccinellinae (Ślipiński 2007), containing up to 20 tribes whose relationships are poorly known and which includes all of the taxa discussed in this review and familiar to most biologists. Ladybirds are generally thought of as specialized predators of stenorrhynchan Hemiptera, but their prey includes many other insects (including other ladybirds) and arthropods (particularly mites), and they may also feed opportunistically on honeydew and pollen (Giorgi *et al.* 2009; Seago *et al.* 2011). Some, like the Epilachnini, are plant feeding, while some Coccinellini are specialists on fungi (Escalona *et al.* 2017; Giorgi *et al.* 2009).

While ladybirds have a wide range of dietary specificities, coccinellines are important generalist arthropod predators (preying on soft-bodied arthropods) that have been, and continue to be, introduced around the world for biological control of agricultural pests (Rudolf 2007; Rudolf and Rasmussen 2013; Wallach *et al.* 2017). Broad dietary range in generalist predators may increase their ability to establish in a wide range of habitats and increase their risk of becoming invasive (Emiljanowicz *et al.* 2017). Moreover, several Coccinellini species used in biocontrol programs are known to be invasive (Lombaert *et al.* 2014; Snyder *et al.* 2004). Ladybirds can have strong effects on the recipient ecosystem, and some introduced ladybird species have played a role in the displacement and decline of intraguild competitors, including other native ladybird species (Brown *et al.* 2011; Paul *et al.* 2015; Tumminello *et al.* 2015). Therefore, coccinellines serve as an ideal model system to evaluate the factors driving the establishment of introduced species. In this following section, we review the distribution of introduced Coccinellini and what is currently understood about the factors that influence their successful establishment. This review provides the first systematic compilation of our knowledge of factors affecting the successful establishment of introduced Coccinellini considering traits that influence both biological control and invasive potential.

FACTORS AFFECTING ESTABLISHMENT OF INTRODUCED SPECIES

Methods of Introduction. Ladybirds are introduced into new habitats in two main ways: first, they are intentionally introduced as a biological control agent in agricultural crops (Gordon 1985; Snyder and Clevenger 2004), or, second, they are accidently introduced via exports of plants and other cargo (Schaefer *et al.* 1987). Current pest control and management programs include the introduction of ladybirds for: a) classical biocontrol, which involves the establishment of an introduced population for permanent suppression of pests, and b) lab rearing of adults to augment wild populations for immediate

Table 1. Factors affecting the successful establishment of introduced ladybirds. Examples are illustrative rather than exhaustive.

Category	Factor/Trait	Class	References
Invasibility	Abiotic factors	Temperature (*e.g.*, differences in performance during temperature extremes)	Singh *et al.* 2018; Soares *et al.* 2008
		Rainfall (*e.g.*, high rainfall decreases probability of colonization)	Comont *et al.* 2014b
	Resource availability	Prey density	Evans and Toler 2007; Moser and Obrycki 2009
	Natural enemies	Parasites	Dindo *et al.* 2016
		Fungi	Scorsetti *et al.* 2017
		Pathogens	Steele and Bjørnson 2019; Tayeh *et al.* 2012
	Competitors		Smith and Gardiner 2013; Soares *et al.* 2017
	Intraguild predation risk	IG predator	Kajita *et al.* 2000
		IG prey	Moser and Obrycki 2009
		Colonization times	Éric 2013
		Spatial and temporal distribution	Musser and Shelton 2003
	Other flora and fauna	Innate responses to particular plant(s)	Togni *et al.* 2016
Invasiveness	Resource acquisition	Dietary range	Berkvens *et al.* 2010; Emiljanowicz *et al.* 2017; Franzmann 2002; Majerus *et al.* 2006
	Female fecundity		Awad *et al.* 2013; Tayeh *et al.* 2012; Turgeon *et al.* 2011
	Male reproductive success	Selection of traits that increase fitness in males in captive conditions	Facon *et al.* 2011
	Vulnerability to natural enemies		Riddick *et al.* 2009; Romanov 2019
	Defense mechanisms	Behavioral and physical adaptations	Sato and Dixon 2004; Smith and Gardiner 2013; Snyder *et al.* 2004
		Chemical defenses	Kajita *et al.* 2014
	Mating systems	Melanism	Nedvěd *et al.* 2018
		Voltinism	Comont *et al.* 2014b
	Developmental times	Faster developmental times and higher investment in reproduction	Raak-van den Berg *et al.* 2018; Tayeh *et al.* 2015
	Dispersal ability	Chemical cues	Werf *et al.* 2000
		Flight speed	Lombaert *et al.* 2014
	Body size		Dixon 2007; Éric 2013; Nóia *et al.* 2008
	Phenotypic variation	Phenotypic plasticity	Boher *et al.* 2018; Lombaert *et al.* 2008
		Seasonal fluctuations – morph frequencies	Kawakami *et al.* 2019
		Acclimatization	Singh *et al.* 2018
		Intraspecific variation	Evans 2000
	Genetic variation	Genetic diversity	Krafsur *et al.* 2005
		Bridgehead effect, Multiple mating	Awad *et al.* 2015; Hodek and Ceryngier 2000; Lombaert *et al.* 2011
	Cannibalism		Tumminello *et al.* 2015
	Interspecific interaction	Aggressiveness of introduced species	Kajita *et al.* 2014
	Diapause		Soares *et al.* 2008
	Physiology	Eurythermicity	Barahona-Segovia *et al.* 2015
	Vulnerability to change/ disturbance	Rapid responses	Kajita and Evans 2009

Continued on next page

Table 1. Continued.

Category	Factor/Trait	Class	References
Stochastic events	Propagule pressure		Kajita *et al.* 2012
	Climate change	Global warming	Barahona-Segovia *et al.* 2015; Boher *et al.* 2018
	Habitat degradation/management/land use/disturbance	Increases in urban land cover	Comont *et al.* 2014b

Note: Effects of these traits on invasibility, invasiveness and stochastic events vary depending on the species and its interaction with the introduced habitat.

reduction of pest populations, such as is done for the non-coccinellines *Cryptolaemus montrouzieri* Mulsant, 1853 and *Delphastus pusillus* (LeConte, 1852) (Michaud 2012; Obrycki and Kring 1998). The supplementary table provided in Appendix 1 summarizes studies on introduced ladybirds described under the proposed categorization.

Establishment and Distribution. Successful establishment occurs when a species that has settled into a location that is outside its natural geographical range can maintain a self-sustaining population with natural dispersal (Hokkanen and Sailer 1985; Howarth 1991). Introduced populations face several barriers to dispersal and the maintenance of a self-sustaining population, such as: geography, captivity/cultivation, survival, reproduction and environment (Blackburn *et al.* 2011). Successful establishment occurs only once, and repeated introductions may not necessarily improve the probability of an introduced ladybird establishing (Day *et al.* 1994). Establishment success varies in ladybirds, with instances where only one introduction was sufficient for establishment (Kajita *et al.* 2000), while in other instances several introductions did not result in population establishment (Kajita *et al.* 2000).

There are many factors hypothesized to influence the successful establishment and dispersal of introduced ladybird species in novel environments. Table 1 summarizes the factors that have been investigated to predict successful establishment. Note that this includes studies carried out on invasive species as well as considering species introductions for biological control and conservation.

Habitat Invasibility. Many factors influence the invasibility of a habitat and hence the probability of establishment, including abiotic resistance, resource availability, the presence of natural enemies, mutualists, intraguild predators and other native flora and fauna (discussed in the following sections). Our rationale is to compile literature on ladybirds to provide hypotheses about habitat characteristics that may influence invasibility of an introduced area. To date, less attention has been given to investigating invasibility in the recipient habitat than to the characteristics of the species to be introduced. Therefore, we gain insights into invasibility by contrasting traits between the native and introduced species, which also in turn provides data on invasiveness of the species. The exception to this is the effect of temperature on life history traits which has been studied extensively in order to understand the habitat match between native and introduced habitats (Facon *et al.* 2017; Singh *et al.* 2018). Temperature affects ladybird physiology. For example, winter diapause may affect reproductive success in *H. axyridis*, where one of the reasons for failed introductions in the Azores is thought to be the unavailability of preferred winter temperatures (Soares *et al.* 2008). However, studies that compare photoperiodic regulation of diapause and its effect on reproduction in invasive and native populations of *H. axyridis* shows that there is a weak effect of photoperiod on diapause induction. Therefore, the invasibility of the habitat should be investigated within the framework of the invasiveness of the species with respect to photoperiod, food quality and temperature (Reznik *et al.* 2015, 2017). For instance, if the introduced species needs low rainfall, preferred prey with availability of alternative food resources and winter temperatures lower than 12 °C to diapause then the habitat should be assessed within those parameters. Further, we need to understand how habitat invasibility would change following a stochastic event and how it would affect the invasiveness of the introduced species. For example, we need to identify the prey preferences of the introduced species (invasiveness) in addition to how prey densities and their fluctuations (invasibility) in the introduced habitat and changes in prey populations due to diseases or human-mediated activities would interact during the establishment period. Therefore, below we discuss factors affecting the invasibility of the habitat, including interactions with species' invasiveness and potential stochastic changes, to better understand how these three categories affect successful establishment.

Abiotic Factors. Rainfall and temperature factors directly affect the invasiveness and therefore establishment success of ladybirds. For example, in ladybirds, increased rainfall decreases the probability of colonization locally (Comont *et al.* 2014b). As mentioned earlier, few studies compare the thermal capacities (see table of thermal constants of 25 ladybird species in Nedvěd and Honěk [2012]) of native and introduced species including ladybirds (Majerus *et al.* 2006; Singh *et al.* 2018; Zerebecki and Sorte 2011). In contrast to introduced ladybirds, most native ladybirds appear to be less able to cope with climatic changes, such as those induced by global warming (Majerus *et al.* 2006). Since most endemic ladybirds are habitat and niche specific (Majerus *et al.* 2006), they lack the ability to establish in novel habitats. On the other hand, introduced ladybirds often easily adapt/acclimatize to novel habitats as well as climatic changes (Singh *et al.* 2018). This gives them a competitive advantage over native ladybirds (Majerus *et al.* 2006). Comparative studies on the physiological performance of native and introduced species along a range of temperatures show that introduced species have broader temperature tolerance ranges than native species (Zerebecki and Sorte 2011). Higher temperature tolerances were related to broader geographic temperature ranges and higher maximum habitat temperatures but were unrelated to minimum habitat temperatures (Zerebecki and Sorte 2011). Therefore, it is more likely that introduced species that successfully establish in new environments have higher growth and survival rates at high temperatures and eurythermality (having a wider range of thermal comfort) than species that do not successfully establish (Hellmann *et al.* 2008; Zerebecki and Sorte 2011).

Some laboratory studies indicate that, contrary to field studies and predictions, both introduced and native ladybird species show eurythermality, although they show differences in performance (fecundity, locomotor performance, development time) in temperature extremes (Barahona-Segovia *et al.* 2015; Zerebecki and Sorte 2011). For example, neither a native species (*Eriopis chilensis* Hofmann, 1970) nor an introduced species (*H. axyridis*) performed well at high temperatures, but the introduced species showed comparatively better performance at lower temperatures compared to *E. chilensis* (Barahona-Segovia *et al.* 2015). This suggests that there is variation in the degree that invasive/introduced ladybirds can respond to climate change (Barahona-Segovia *et al.* 2015). However, invasive species that perform better at lower temperatures, such as *H. axyridis*, may be challenged in terms of distribution following global warming due to a lack of thermal plasticity compared to native species (Boher *et al.* 2018). However, fluctuating temperatures can increase the reproductive capacity and development of ladybirds such as *Menochilus sexmaculatus* (Fabricius, 1781) (Singh *et al.* 2018). Investigating further the physiological performance of native and introduced species will help us better predict establishment success and reduce the number of failed introductions. For instance, assessing how different temperatures, temperature fluctuations (invasibility of the habitat) and changes in lowest and highest temperatures (stochastic events) due to climate change interact with preferred temperature ranges (invasiveness) of introduced ladybirds may provide insights into their establishment success.

In some species, variation in larval and adult coloration can be influenced by environmental factors such as temperature rather than through genetic inheritance (Majerus *et al.* 2006). Color patterns can therefore be an indication of climate during development. For example, *H. axyridis* has a wide range of color plasticity in pupae and adults which is linked to temperature (Michie *et al.* 2010). Final-instar larvae that experience low temperatures produce dark pupae which are adapted to absorb more heat than light-colored pupae, facilitating faster development and earlier eclosion in cool conditions (Majerus *et al.* 2006). Further, fluctuations in morph frequencies as a result of seasonal changes or temporality (temporal environmental heterogeneity) play an important role in controlling for the selection pressures faced by ladybirds due to changes in climatic conditions (Kawakami *et al.* 2019). We need comparisons of the level of color variation between native and introduced ladybird populations to identify their differences in invasiveness and tests of how color variation interacts with different climates or invasibility of different habitats.

Disturbance. Anthropogenic disturbance is a major factor influencing species establishment and distribution. A spatial distribution model of *H. axyridis* in France suggests that its establishment is predicted better by anthropogenic factors (such as urbanization, presence/absence of highways [disturbance], agriculture and vineyards) than environmental factors (temperature, wind speed and rainfall) (Veran *et al.* 2016). The establishment and rapid expansion of *H. axyridis* in urban areas of the UK is explained by its ability to use a wide range of habitats (such as having overwintering sites inside buildings) and prey species (Purse *et al.* 2015). Rapid responses to changes in the external environment provide a competitive advantage for introduced species (Rondoni *et al.* 2017), enabling successful establishment. Introduced species (*Coccinella septempunctata* Linnaeus, 1758) show more rapid responses to changes in prey density than the native species (*Coccinella tranversoguttata richardsoni* Brown, 1962), adopting oosorption as a strategy to conserve resources at low prey densities (Kajita and Evans 2009). Such rapid responses to sudden changes in prey densities may account for the greater reproductive success and abundance of introduced ladybirds over native ladybirds (Kajita and Evans 2009). Further, introduction of natural predators in the habitat can also affect the abundance of introduced species. For instance, populations of *Boreioglycaspis melaleucae* Moore, 1964 (Hemiptera: Psyllidae; an introduced weed biocontrol agent) were affected by the introduction of *H. axyridis* in Florida (Nimmo and Tipping 2009). Therefore, adaptability of reproductive behavior in response to anthropogenic disturbances provides a competitive advantage in the establishment of introduced species. Disturbances that are caused by habitat degradation and land use or urbanization reduces colonization success in ladybirds (Comont *et al.* 2014b). Therefore, assessing how the invasibility of the habitat in terms of the level of disturbance in the recipient ecosystem (sudden changes in prey and predator densities) and sudden changes to the environment such as deforestation (stochastic events) interact with invasiveness of ladybirds by monitoring changes their development, fecundity and dispersal will provide a better understanding of establishment processes.

Spatial and Temporal Distribution of Intraguild Competitors. The spatial and temporal distribution of ladybirds affects intraguild interactions between introduced species and native congeners (Musser and Shelton 2003). Changes in the spatial distribution of populations can allow individuals to reduce negative interactions with other species. The spatial and temporal distribution of native and introduced ladybirds and competitors (such as lacewings, braconids and other aphidophagous predators) are important regulators of the intensity of interactions between native and introduced populations. For example, the invasive species *H. axyridis* avoided interactions with native species, *Coleomegilla maculata* (DeGeer, 1775) by changing its spatial distribution on host plants, where *H. axyridis* adults moved further away from *C. maculata* adults and larvae of *H. axyridis* moved towards larvae of *C. maculata* (Musser and Shelton 2003). The different spatial distribution within the plant has been possible for *C. maculata* mainly because its distribution is not associated with that of aphid populations; therefore, it is less vulnerable to predation by introduced species which have a spatial distribution associated with aphid density (Coderre *et al.* 1987; Harmon *et al.* 2000). However, a large-scale study showed that the abundance of native and other ladybirds was not influenced by the abundance of *H. axyridis* but was influenced by the abundance of aphids (Viglášová *et al.* 2017). Temporal changes in the distribution of ladybird species feeding on aphids can be caused by geometric and physiological constraints associated with size (Dixon 2007). Smaller aphidophagous species have an advantage over larger species, as they can exploit aphids when they are relatively uncommon. Hence, small aphidophagous species vary less in abundance and in size than larger species each year (Dixon 2007). If we approach species introductions as planned invasions, understanding the presence of competitors and their spatial and temporal distribution (invasibility) may provide insights on how it will affect invasiveness of the introduced species, increasing predictability of establishment success. This will further help us identify suitable times and places to introduce species and manage introduction programs to increase establishment success.

Other Flora and Fauna in Recipient Ecosystem. Despite ladybirds having been studied for years as biocontrol agents, few studies have examined interactions between introduced ladybird species and native flora and fauna other than native ladybirds. The dispersal and colonization of ladybirds are affected by innate responses to plants, *e.g.*, the presence of coriander increased longevity and dispersal in *Cycloneda sanguinea* (Linnaeus, 1763) (Togni *et al.* 2016). However, there are few studies that have investigated these interactions to understand their effect on establishment success.

Ladybirds that feed on aphids can be negatively affected by ants protecting the honeydew-excreting hemipterans from their natural enemies (Powell and Silverman 2010). A laboratory study conducted to assess the interactions of native and introduced ladybirds with *Myrmica rubra* (Linnaeus, 1758) found no evidence that the introduced species have a competitive advantage over native ladybirds against aphid-tending ants (Finlayson *et al.* 2009). Further, the intensity of attacks from ant populations are influenced by resource availability (Clark and Singer 2018; Takizawa and Yasuda 2006); therefore, fluctuations in resources following introductions of ladybirds may change the intensity of attacks from mutualists such as ants and should be monitored closely to ensure successful establishment.

Further, ladybirds themselves have a variety of natural enemies including bacteria, nematodes and fungi to wasps and flies, and when these natural enemies are absent, ladybird establishment success may be increased (enemy release hypothesis; see review by Riddick *et al.* 2009). Studies show that parasites and entomopathogenic fungi native to recipient ecosystems can reduce fitness in terms of delayed development, reduced female fecundity and higher mortality of introduced species than native species (Dindo *et al.* 2016; Scorsetti *et al.* 2017; Steele and Bjørnson 2019).

Introduced ladybird species can influence parasitism rates on native ladybirds. Parasitism rates of native ladybirds (*C. maculata*) decrease in the presence of introduced ladybirds (*H. axyridis*) as the introduced species are relatively non-suitable hosts and serve as a sink (ecological trap), decreasing parasitoid egg recruitment and native host death (Hoogendoorn and Heimpel 2002). However, the prevalence of natural enemies such as parasites is lower for introduced species than for native species during the early stages of establishment (Comont *et al.* 2014a). Saito and Bjørnson (2008) studied the effects of a microsporidium transmitted horizontally from *Hippodamia convergens* Guérin-Méneville, 1842 to three other non-target ladybird species. Results suggest that native species (*Adalia bipunctata* [Linnaeus, 1758]) are a more suitable host for the microsporidium compared to the introduced species (*C. septempunctata* and *H. axyridis*) (Saito and Bjørnson 2008). Similarly, *H. axyridis* is better able to cope with bacteria in the genus *Wolbachia*, which kill males early in development, compared to *A. bipunctata* (Goryacheva *et al.* 2015). However, *Dinocampus coccinellae* (Schrank, 1802) negatively affected the fitness of *H. axyridis* more than that of *A. bipunctata* (Dindo *et al.* 2016). Further, vulnerability to natural enemies such as parasites and pathogens may vary between species (Riddick *et al.* 2009; Romanov 2019), may change over time (*i.e.*, increase sensitivity; Knapp *et al.* 2019) and may depend on invasibility factors such as resource availability and temperature (Steele and Bjørnson 2019). Comparative studies on the interactions and effects of these parasites on native and successfully established ladybirds with ladybirds that failed to establish will provide insights on how native natural enemies may influence establishment success.

Invasiveness of Ladybirds. Factors affecting invasiveness are resource acquisition, female fecundity, mating system, developmental time, size, diapause, physiology, dispersal ability, inter- or intraspecific variation (phenotypic variation, genetic variation), social interaction, cannibalism, vulnerability to natural enemies, defense mechanisms and vulnerability to change/disturbance. We note that the invasiveness of species with regard to resource acquisition, cannibalism, competition and intraguild predation are closely associated with each other and with the invasibility of the habitat. For example, the effect of intraguild members present in the habitat (invasibility) depends on how vulnerable the introduced species is to these interactions or encounters (invasiveness). Therefore, below we discuss how the invasiveness of a species interacts with invasibility (and stochastic events) to better understand how invasiveness affects the establishment success of introduced ladybirds.

Diet and Competition. Prey identity and density has a significant impact on the fitness of the ladybirds, and therefore dietary range is a key factor influencing invasiveness in ladybirds and the establishment success of introduced populations. Predatory ladybirds have a wide dietary range, including Sternorrhyncha (*e.g.*, aphids and whiteflies), other Hemiptera, Thysanoptera, young instars of holometabolan insects, phytophagous mites, fungi (mildew) and pollen (Berkvens *et al.* 2010; Hodek *et al.* 2012). Dietary range correlates with the range of habitats occupied by ladybirds. Ladybirds with wider dietary ranges are likely to occupy more habitats and disperse farther than ladybirds with narrow dietary ranges (Comont *et al.* 2012; Giorgi *et al.* 2009).

The voracity and reproductive capacity of ladybirds depends on prey density (Soares and Serpa 2007). However, the effect of prey density on larval survival is species specific. For example, in successfully-established introduced species such as *C. septempunctata* and *H. axyridis*, survival increased with prey density, but native *C. maculata* population sizes remained relatively low in the presence of heterospecific larvae regardless of prey density (Moser and Obrycki 2009). Therefore, in the presence of heterospecific ladybird larvae (native and introduced species), high prey densities may increase the establishment success of the larvae of introduced species.

A wider dietary range increases invasiveness (Berkvens *et al.* 2010), which increases the ability to establish in a habitat with low densities of preferred prey by allowing ladybirds to feed on alternate prey (invasibility of the habitat). For example, *H. axyridis*, a biocontrol agent that has now become invasive, has a wide dietary range beyond target aphid and scale insects (Berkvens *et al.* 2010; Majerus *et al.* 2006). *Harmonia axyridis* consumes exotic pests in its native range, even in the presence of preferred native prey (Zhang *et al.* 2016). Berkvens *et al.* (2010) studied the development, reproduction and survival of *H. axyridis* on diets of fruits (apple, pear and raspberry), fungi (*Botrytis cinerea* Persoon, *Sclerotinia sclerotiorum* [Lib.] de Bary and *Rhizoctonia solani* Kühn) and pollen in the laboratory by offering one of the food sources to one individual. *Harmonia axyridis* larval and adult survival was prolonged when fruits were offered compared with water as a control, but *H. axyridis* maintained on a diet of fruits failed to complete development or reproduce (Berkvens *et al.* 2010). However, females that were fed solely on pollen in the laboratory successfully completed development and reproduced (Berkvens *et al.* 2010). Pollinivory probably provided complementary and sufficient nutrients for *H. axyridis* development and is an alternative food source in the absence of aphids and scale insects (Berkvens *et al.* 2010). Further, Tayeh *et al.* (2015) showed that even though biocontrol populations have developed faster life histories than native and invasive populations during their laboratory rearing, invasive populations allocate more resources to reproduction than both biocontrol and native populations of *H. axyridis*. However, successful establishments are not simply associated with faster life histories and reproductive investments but with the ability to adapt and evolve life history strategies to match the introduced area (Tayeh *et al.* 2015).

As opposed to invasive *H. axyridis*, there are only a few studies that have investigated the effects of mixed diet on the growth and colonization of native coccinellid species (Evans *et al.* 1999). This limits our understanding of how having a mixed diet may increase establishment success by comparing differences between successfully-established and native ladybirds. Egg production in both native and introduced aphidophagous ladybirds is higher when a mixed diet is provided as opposed to the essential food only (Evans *et al.* 1999). Most ladybirds that are introduced around the world are aphidophagous or polyphagous. However, the availability of aphid populations changes rapidly over the year, which relates to changes in the intensity of cannibalism and intraguild predation (IGP) in ladybirds (Yasuda *et al.* 2004). IGP is the killing and consumption of competitors or individuals of the same guild regardless of taxonomic relationship or mode of resource acquisition (see section “Intraguild Predation”) (Yasuda *et al.* 2004). Cannibalism and IGP are two major factors that determine population dynamics and the community structure of coccinellid assemblages.

Cannibalism. Reduced cannibalism in introduced species facilitates species establishment and provides a competitive advantage over native species. Cannibalism is observed in both native and introduced ladybirds, and rates of cannibalism differ between native and introduced species (Michaud 2003). Some studies show that the differences in the rate of cannibalism between native and introduced ladybirds did not change in absence or reduced prey densities (Michaud 2003), but contrastingly some native ladybird species showed higher levels of cannibalism compared to introduced ladybird species in the absence of prey (*e.g.*, the native species *Coccinella novemnotata* Herbst, 1793 and the introduced species *C. septempunctata* [Cottrell 2005; Turnipseed *et al.* 2015]). However, a recent study comparing cannibalism between *H. axyridis* larvae kept in isolation and in groups shows that the survival time of the last larva in a sample was increased in the larvae kept in groups in the presence of cannibalism (Reznik *et al.* 2018). This may suggest that in the absence of prey, having a higher cannibalism rate may influence survival of a population by increasing maximum survival time (Reznik *et al.* 2018). Cannibalism is a bigger threat for the eggs of introduced species like *H. axyridis* rather than IGP, because native ladybirds tend not to prey on *H. axyridis* (Cottrell 2005; Snyder *et al.* 2004). Rates of egg cannibalism in ladybirds change with gender, life stage, reproductive status and the presence of heterospecifics, regardless of prey availability (Bayoumy *et al.* 2016). A laboratory experiment that provided options to cannibalize conspecifics and intraguild predation revealed that the presence of heterospecifics reduced cannibalism in introduced ladybird species (such as *C. septempunctata*), promoting its dominance over native species (Tumminello *et al.* 2015). Therefore, the overall decline of native ladybird species like *C. novemnotata* could be a result of increased interactions between native and introduced ladybirds leading to asymmetric egg predation by introduced species (IGP) and cannibalism (Turnipseed *et al.* 2015).

Intraguild Predation. Intraguild predation (IGP) can have a dramatic effect on the establishment success of introduced species (Éric 2013; Felix and Soares 2004; Nóia *et al.* 2008). An intraguild predation relationship occurs when one guild member (intraguild predator—IG predator) attacks another guild member (intraguild prey—IG prey) despite the presence of common prey (extraguild prey) (Janssen *et al.* 2006; Polis and Holt 1992). The direction, intensity and symmetry of IGP is affected by many factors including life stage, extraguild prey densities, intraguild prey densities and body size/mass (Éric 2013; Felix and Soares 2004; Nóia *et al.* 2008). The ability of an introduced species to become an IG predator (aggression, invasiveness) interacts with vulnerability of native species (invasibility of habitat) (Hindayana *et al.* 2001; Kajita *et al.* 2006). Similarly, the vulnerability of introduced species to become an IG prey (invasiveness) interacts with aggressiveness of native species (invasibility) in the introduced habitat (Hindayana *et al.* 2001; Kajita *et al.* 2000). This results in changes in community structure, niche shifts (mostly in intraguild prey), changes in foraging behavior and indirect effects on prey. For example, a laboratory study that investigated the role of native ladybirds (*H. axyridis* and *C. septempunctata*) in preventing the establishment of *A. bipunctata* in Japan revealed that despite high availability of prey, *H. axyridis* prolonged the development and changed the foraging behavior of *A. bipunctata* through IGP (Kajita *et al.* 2000).

The vulnerability of IG prey depends on the density, mobility, distastefulness, strength of the integument (processes on the body of ladybird larvae, such as the non-coccinelline species *Curinus coeruleus* [Mulsant, 1850]; Michaud and Grant [2003]) and size of the prey (Nóia *et al.* 2008). An increase in extraguild prey density results in significantly lower rates of IGP (Kajita *et al.* 2000; Tumminello *et al.* 2015). For example, *C.*

septempunctata shows no signs of IGP against *A. bipunctata* when prey are abundant, yet in scarcity of aphids, adults and larvae of *C. septempunctata* prey on *A. bipunctata* (Kajita *et al.* 2006). Among many IGP types such as protective, competitive, nutritional (when the nutritive value of the IG prey exceeds the value of the alternative food sources available) and opportunistic (when prey selection is based on size selection rather than the guild), only nutritional and opportunistic IGP has been studied under laboratory conditions (Éric 2013).

The level of competition (invasibility) in the introduced habitat and vulnerability of the species to becoming intraguild prey (invasiveness) is a major factor influencing establishment success. The body size and weight of individuals in a population can indicate levels of competition in the environment (Moser and Obrycki 2009; Snyder *et al.* 2004) and hence the invasibility of the habitat. The body weight of native ladybirds (*C. maculata*; Moser and Obrycki 2009*, Eriopis connexa* [Germar, 1824]; Mirande *et al.* 2015) did not increase with prey density and presence of introduced ladybirds (*H. axyridis* and *C. septempunctata*) but the body weight of introduced ladybirds increased when they were reared in the presence of native ladybirds and native IG predators (Wells *et al.* 2017). The weight gains of introduced ladybirds are thought to be a competitive advantage because they could use native ladybirds as an additional food source (Moser and Obrycki 2009; Snyder *et al.* 2004). Similar results were found in a field cage study in which *H. axyridis* gained more weight when they were reared with native ladybirds (*C. maculata*) than with conspecifics (Hoogendoorn and Heimpel 2004). The body weight and mobility of individuals also influences the intensity of IGP (Felix and Soares 2004). Rates of IGP increase with larval instar when *H. axyridis* is the IG predator, but IGP does not change when *Coccinella undecimpunctata* Linnaeus, 1758 is the IG predator (Felix and Soares 2004). IGP almost always results when the IG predator and prey are very different in size (*e.g.*, larvae vs. eggs, 4th-instar larvae vs 1st-instar larvae) and larvae in the same instar rarely prey on each other (Snyder *et al.* 2004). Further, ladybird species whose target prey are large or active have larger body sizes and lay larger eggs than ladybirds that target small or less active prey (Dixon and Hemptinne 2001). Although size influences IGP, mass does not seem to matter, as the rate of IGP does not change when the body weight is different between native and introduced ladybirds (Nóia *et al.* 2008). Therefore, when introducing ladybirds to a new habitat, understanding the differences in body sizes between native members of the guild in the introduced area and introduced species would help predict the level of competition and how that would influence the establishment success of the introduced species.

Developmental stage and the characteristics of other species in the ecosystem play a major role in deciding the intensity and direction of IGP. Small and slow larvae are the most vulnerable IG prey (Éric 2013; Nóia *et al.* 2008). However, foraging speed is influenced by body size and adults of larger ladybirds have been shown to have slower walking speeds compared to smaller ladybirds (Ghosh and Agarwala 2018). According to the slow growth high mortality hypothesis, faster growth influences less mortality (Clancy and Price 1987). Therefore, larvae of smaller predator species with faster developmental times could be larger than co-occurring larvae of a larger predator species with slow developmental times, changing the direction of IGP (Evans 2004). Therefore, differences in colonization time (time when eggs start hatching during the season) could change the direction of IGP and affect the survival of offspring during IGP interactions (Éric 2013). Adverse effects of IGP on native species are strongest when interactions occur between older introduced and younger native larvae (Nóia *et al.* 2008; Yasuda *et al.* 2004). However, reproduction and development time of coccinellids may be affected by temperature and photoperiod (Singh *et al.* 2018; Wang *et al.* 2013). Therefore, it is important to investigate how changes in temperature and photoperiod may affect intensity and direction of intraguild predation.

Relative body size influences the outcome of interspecific interactions by favoring larger individuals, which are more likely to become IG predators. For example, larvae of *H. convergens*, the smallest species, is preyed on by *H. axyridis*, *C. septempunctata* and *C. tranversoguttata* (Yasuda *et al.* 2004) when reared together. However, the intensity of the interactions was weaker when aphids were in abundance (Yasuda *et al.* 2004). Further, Snyder *et al.* (2004) argued that the likelihood of IGP is not related to size differences between predator and prey but is dependent on the ability to capture IG prey and the ability to avoid attack. Accordingly, small lacewing larvae are able to kill large ladybird larvae (Nedvěd *et al.* 2013). The survival of introduced aggressive ladybirds such as *C. septempunctata* and *H. axyridis* is higher when they share habitat with native species because natives become relatively easy IG prey, resulting in an increased probability of establishment (Moser and Obrycki 2009).

Cannibalism and egg predation by IG predators is common despite all ladybirds having alkaloid chemical defenses in all life stages (Braekman *et al.* 1999; Sloggett 2005). These alkaloids appear to have no harmful effects on the development or survival of first instars (Kajita *et al.* 2014) when digested by other ladybird species in IGP. Only *Calvia quatuordecimguttata* (Linnaeus, 1758) eggs were found to be well protected against *H. axyridis* (Katsanis *et al.* 2017). While introduced ladybirds may not have specific chemical defenses (nor do native species) to protect against IGP, introduced ladybirds are often more aggressive than native ladybirds, which results in asymmetric IGP (Kajita *et al.* 2014). However, invasive ladybirds have adaptations to protect eggs and larvae, which are advantageous for establishment in new habitats. For example, *H. axyridis* has a species-specific chemical defense system (alkaloids) to protect its eggs from predation (Sato and Dixon 2004; Smith and Gardiner 2013). In addition to chemical defenses, *H. axyridis* larvae also have strongly adhesive tarsi that facilitated more successful attacks and escapes against predation (Snyder *et al.* 2004).

Even though there is no evidence of egg predation by introduced ladybirds, being less vulnerable to predation by other generalist predators may increase the population growth of introduced species (Smith and Gardner 2013). Therefore, native and introduced species may have complex interactions, with introduced ladybirds having more effective chemical defenses and aggressive foraging behavior than native ladybirds (Hoogendoorn and Heimpel 2002) that indirectly result in exploitative and competitive relationships with native ladybirds (Smith and Gardiner 2013).

IGP directly favors IG predators by providing an additional nutritional source and indirectly favors IG predators by reducing exploitative competition (Yasuda *et al.* 2004). Therefore, when the IG predator is an introduced species, IGP directly and indirectly favors establishment of the introduced species (Yasuda *et al.* 2004). However, a recent study which investigated the unsuccessful introduction of *H. axyridis* suggests that both a) the availability of resources sufficient to sustain large populations of large species, and b) the presence of less aggressive large coccinellid species which would not outcompete *H. axyridis*, are important to achieve successful establishment in a novel environment (Soares *et al.* 2017). Therefore, when introducing a species to a new habitat as a planned invasion, we should investigate the invasibility of the habitat based on body size and vulnerability to become IG prey of local members of the food guild, and potential interactions between these factors to better predict establishment success and plan introduction processes (Soares *et al.* 2017).

Dispersal Ability. Dispersal ability is a critical factor that facilitates the establishment of introduced species (Harrison 1980; Tscharntke *et al.* 2002) and varies between individuals. Dispersal is movement creating gene flow and can be driven by many different behaviors such as foraging, finding mates, laying eggs, avoiding predators or even moving or drifting in wind (Benton and Bowler 2012). Understanding how invasibility and the dispersal ability of introduced species interact will help improve our ability to predict successful establishments. Ladybirds have specific machinery, such as wings, and specific developmental stages (adults) associated with dispersal. Therefore, wing polymorphism and variation in wing shape, size of the wings and flight muscles in monomorphic insects suggest that physiological mechanisms and ecological factors determine the dispersal ability of individuals (Zera and Denno 1997). However, flightless morphs of biocontrol agents such as *A. bipunctata* (Lommen *et al.* 2008) and *H. axyridis* (Seko *et al.* 2008, 2014) may be more effective in biocontrol due to an increased residence time in the target field compared to flying ladybirds. Migration behavior may also influence dispersal ability (Zhou *et al.* 1995). For example, adult *C. septempunctata* choose hibernation sites away from breeding and foraging sites and their dispersal ability and accumulation of nutrients and energy before hibernation play a major role in their survival during and after hibernation (Zhou *et al.* 1995).

Dispersal ability can vary within a population. The ability of invasive populations of *H. axyridis* ladybirds to disperse is higher in individuals at the edge of the population than that at the core of the population, with an increase in flight speed at the edge of the population (Lombaert *et al.* 2014). Dispersal is also influenced by environmental factors. For example, a field experiment on *C. septempunctata* showed that dispersal and navigation of ladybirds towards foraging and mating habitats may be influenced by chemical cues (Werf *et al.* 2000). Further, species may disperse via human transportation and disperse into synanthropic areas such as walls of residential buildings (Borowski 2015). However, very little research has compared the dispersal abilities of native and introduced ladybird populations. This hinders our understanding of the importance of natural dispersal ability on establishment success.

Stochastic Events Affecting Establishment Success. Stochastic factors that may affect establishment success of introduced species are propagule pressure, climate change and human mediated changes in the habitat (*e.g.*, habitat degradation, management, land use and disturbance). For instance, a recent study suggests that atmospheric events such as changes in wind speed and direction may affect flying and migratory ladybirds such as *H. axyridis* and its invasion in the UK (Siljamo *et al.* 2020). We discuss how stochastic events such as climate change and disturbance interact with the invasiveness of species and invasibility of habitats in earlier sections. We review studies that assess propagule pressure to understand how it interacts with invasiveness, invasibility and the establishment success of introduced populations.

Propagule Population and Variation. Propagule pressure or characteristics of the propagule (*i.e.*, founding) population, including the number of individuals in the propagule population (propagule size) and number of times the species is introduced to the recipient environment (propagule number), may strongly influence establishment success (Lockwood *et al.* 2005). While propagule pressure is hypothesized to influence establishment in diverse taxa, there are relatively few observational (Beirne 1975; Hopper and Roush 1993) or experimental studies that have investigated the effect of propagule pressure on the establishment success of introduced species (Moulton *et al.* 2012), including ladybirds. A population genetic study on *C. septempunctata* suggests higher propagule pressure has supported the establishment of this species in the USA (Kajita *et al.* 2012). Further, *H. axyridis* was first introduced in the USA in 1916 but was not established until 1988, which included several failed introductions including a single release of 32 specimens in 1979 and eight releases of 3,781 specimens in 1980 (Chapin and Brou 1991). Further, investigating other stochastic events such as unusual weather patterns that occurred following introductions may help in understanding factors affecting the successful establishment of the propagule population (Moulton *et al.* 2012). Further, strong Allee effects may influence the establishment success of introduced species. Allee effects may define establishment success only above a certain number of individuals (propagule size) independent of their variation (Drake and Lodge 2006) and may be influenced by repeated introductions or immigration events (Drake and Lodge 2006) and time between repeated introductions (McDermott and Finnoff 2016). Therefore, further investigation of strong and weak Allee effects in natural populations (Leung *et al.* 2004) may increase the predictability of establishment success of introduced ladybird species and the invasive potential of ladybirds such as *H. axyridis*. Further, few studies suggest that failed establishment may have been influenced by other factors such as IGP (*e.g.*, *A. bipunctata*; Kajita et al. 2000). Species such as *Propylea quatuordecimpunctata* (Linnaeus, 1758) and *C. septempunctata* may have successfully established near port sites but lack of documentation may have implied failed introductions (Day *et al.* 1994). Additionally, the effect of propagule pressure is also influenced by genetic variability in the propagule, species-level traits and the location (such as level of disturbance, effects of other non-native species and the intensity of competition or predation) (Lockwood *et al.* 2005).

Genetic Variation. Introduced populations should be less genetically diverse in introduced areas than in their native areas because they are founded by a small proportion of a larger metapopulation (Lombaert *et al.* 2011). Inbreeding depression can follow if the population remains small for several generations (Laugier *et al.* 2016). However, multiple introductions can increase the genetic variation of introduced populations to similar levels of source populations (Dlugosch and Parker 2008). Studies that quantitatively measure genetic loss due to introduction are limited, and there are few studies that compare the genetic variation of the source population to genetic variation of the introduced population in ladybirds (see study of *H. axyridis* by Krafsur *et al.* [2005] and Tayeh *et al.* [2012]).

A comparison of allele diversity and heterozygosities between native and introduced ladybird species in North America suggests that there is no relationship between genetic diversity and establishment success (Krafsur *et al.* 2005). However, the genetic variability of the population is related to its dietary range (Krafsur *et al.* 2005), with the most variation observed in populations of ladybirds that depend on ephemeral and discontinuously distributed prey (Krafsur *et al.* 2005).

Multiple mating, well documented in many ladybirds (Hodek and Ceryngier 2000), increases the genetic variability of the population. The mean number of males contributing to the fertilization of an *H. axyridis* egg cluster was 3.8 (Ueno 1996). The progeny of a single multiply-mated female can establish a new genetically variable population after an accidental dispersal to a new distant area (Awad *et al.* 2015, 2017). Studying genetic variation in invasive populations may also provide insights into how variability in gene flow may affect establishment success and how we can use genetic variability to increase establishment success in introductions carried out as planned invasions. For example, despite several unsuccessful attempts to actively introduce *H. axyridis* in North America for biocontrol purposes, successful establishment was not detected until after 1988 (Chapin and Brou 1991). However, *H. axyridis* has become invasive in later decades, suggesting that these invasive populations could have originated or established from successful invasive populations that were not previously detected rather than native ranges (Lombaert *et al.* 2010). This is an example of the invasive bridgehead effect in which an invasive population serves as the source for population expansions and successful subsequent invasions into new environments (Lombaert *et al.* 2010). Lombaert *et al.* (2011) examined the bridgehead population of *H. axyridis* to identify its source population and revealed an admixture of genetic markers from both eastern and western parts of its native area, increasing its natural genetic diversity, which facilitated it to further spread to other locations. Further, recent studies indicate that genetic bottlenecks are not as frequent in successfully established invasive populations as was once assumed (Lombaert *et al.* 2014) and that having an admixture may facilitate the establishment of introduced populations in novel environments or even help them become bridgehead populations (Lombaert *et al.* 2011).

Phenotypic Variation. Phenotypic variation within and among populations can aid the establishment of a population in different environments because it generates variation in individual fitness (Kalaisekar *et al.* 2012). Similarly, individuals with the ability to adjust their phenotype in response to the environment are expected to have an increased probability of establishing compared to individuals with little phenotypic plasticity.

For a given genotype, environmental conditions may have a profound effect on the phenotype, including traits such as body size, color and/or size of the wings (Nijhout 2003). Adult body size represents the cumulative effect of genetic composition and diverse environmental conditions faced by the developing individual (Evans 2000). The body size of insects can correlate with fitness-related traits such as fecundity, growth rate and survival (Honěk 1993). A comparative study between *C. septempunctata* (invasive in North America) and native North American ladybird species (*C. tranversoguttata*, *H. convergens*, *Hippodamia quinquesignata* [Kirby, 1837], *Hippodamia sinuata* Mulsant, 1850 and *Hippodamia tredecimpunctata* [Linnaeus, 1758]), showed that *C. septempunctata* has greater variation in adult body size than native species (Evans 2000). A comparison of phenotypic variation between invasive and biocontrol populations of *H. axyridis* provided evidence of increased adaptive phenotypic plasticity of European invasive populations of *H. axyridis* compared to biocontrol populations (Lombaert *et al.* 2008). Nevertheless, we still have few comparisons of the level of phenotypic variation between sympatric native and introduced ladybird populations. Understanding how phenotypic variation in propagule populations increases the invasiveness of invasive populations and comparing phenotypic variation between successful and failed introductions may help us manage species introductions to increase the rate of successful establishment of introduced species.

SUMMARY

Factors that affect the establishment of introduced populations can be categorized broadly into invasiveness of species, invasibility of recipient ecosystem and stochastic events. Fig. 2 summarizes how factors affecting successful establishment interact within the proposed framework of invasibility, invasiveness and stochastic events and how these factors may increase the predictability of establishment success. This provides a useful guide for researchers wanting to study establishment success and can be used as a checklist of assessments to be carried out when introducing a new species for biocontrol and conservation. For example, abiotic factors such as temperature may increase the invasibility of a habitat by interacting with the life history traits of introduced species such as female fecundity, body size and developmental time. However, the outcomes of these interactions are affected by stochastic events such as climate change induced by global warming, fluctuations in prey populations, anthropogenic disturbances and biological homogenization. Therefore, to predict establishment success accurately we need to have insights into the interactions occurring between these factors.

The presence and density of biotic factors such as preferred prey, alternate prey, natural enemies and native ladybirds affects the invasibility of a habitat and interacts with the invasiveness of the species by influencing its competitiveness and dietary range. The effect of both biotic and abiotic factors in the habitat interact with inter- and intraspecific variation and the dispersal ability of the introduced species, which may in turn depend on the characteristics of the propagule population.

Greater genotypic variation and phenotypic plasticity in functionally important traits has recently been hypothesized to increase establishment success, which implies that there are higher levels of variation in successfully established ladybirds than less successful populations (Forsman 2014). However, more studies comparing the genetic and phenotypic variation between introduced populations and native populations in the recipient ecosystems are needed (Kumschick *et al.* 2015). Investigating the level of correlation between trait variation and establishment success of invasive ladybirds will provide insights to improve the success of species introductions for biocontrol and conservation purposes. The characteristics of the propagule population and propagule pressure play a key role in the successful establishment of ladybirds (Kajita *et al.* 2012; Lockwood *et al.* 2005). The effect of propagule pressure depends on the biodiversity of the recipient population, climate and competition (Kajita *et al.* 2012; Lockwood *et al.* 2005). However, higher propagule pressure facilitates the establishment and range expansion of introduced populations by increasing the genetic variability of the introduced population (Lockwood *et al.* 2005).

Mating systems also influence the establishment success of introduced species by influencing the

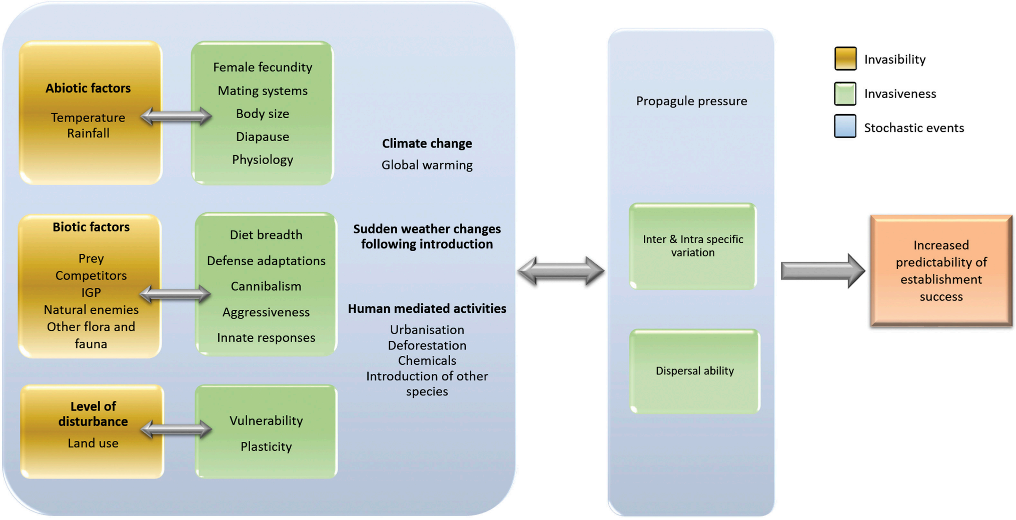

Fig. 2. Summary of factors to assess within the proposed categorization to increase predictability of establishment success.

genetic variability and survival of the offspring. However, we need to assess how fecundity and egg viability is influenced by different mating systems to better understand how to improve the establishment of introduced species. Further, assessing rearing conditions in the laboratory before release, to ensure that we preserve genetic diversity in the population (Gariepy *et al.* 2015) will increase the establishment success of introduced species in biocontrol and conservation programs.

Conclusions and Future Directions

Ladybirds in the tribe Coccinellini are generalist predators introduced around the world for biological control purposes. The success of introduction is variable, and some introductions have inadvertently resulted in invasive populations. Research on invasive ladybirds, ladybird introductions and distributions can provide key insights into the factors that influence the successful establishment of introduced species. Currently, species introductions have very low establishment rates. We propose three categories based on invasion biology (*sensu* Abrams and Moffat 2018) that will aid planning research effort to increase the establishment success of introduced ladybirds: investigating factors affecting invasibility, invasiveness and stochastic events (Table 1), and the interactions between these categories (Fig. 2).

Most research on ladybirds has concentrated on a few widely distributed species and has focused on their establishment and effect on native ladybirds. Among the 49 ladybird species studied around the world for their use as biocontrol agents (Appendix 1), research assessing the success of these introductions is limited to only six species: *H. axyridis*, *C. septempunctata*, *A. bipunctata*, *C. undecimpunctata*, *H. chalybeus*, and the non-coccinelline *C. montrouzieri.* Future efforts may be helped via citizen science programs (Losey *et al.* 2007). Given the important role that ladybirds play as predators in ecosystems, there is an urgent need to diversify research (Sloggett 2005) into ladybird ecology to better understand ways to improve the establishment success of introduced ladybirds. Further, given the recent contradictory evidence on interactions between introduced and native ladybird populations (*e.g.*, the relative influence of IGP on native ladybird population declines), we need more studies that assess these complex interactions in order to accurately project the impacts of introduced ladybird species establishments and assess their invasive potential.

Studies that have looked at unsuccessful establishments of introduced species—including ladybirds—is limited (Cock *et al.* 2016; Zenni and Nunez 2013). However, there are a few studies that have investigated failed invasions in *H. axyridis* (Evans *et al.* 2011; Soares *et al.* 2008, 2017; Zenni and Nunez 2013) which makes ladybirds an ideal model system to begin a comprehensive understanding of establishment success. Studying failed establishments will help us in two ways: 1) to understand the factors that limit establishment (Abram and Moffat 2018) and 2) predict the dispersal and distribution of introduced or invasive species accurately (Michael *et al.* 2012). Therefore, it is important to monitor and record species introductions, successful and unsuccessful establishments and use this information in distribution modelling to accurately predict the introduction success and dispersal of ladybirds.

Future work on ladybirds should concentrate on investigating differences in phenotypic and genetic variation between invasive/widely distributed ladybirds and less distributed/unsuccessful introductions of ladybirds. We also suggest investigation of the role of climatic parameters on species introductions and distributions, the role of climate change in range expansions and the role of IGP in native species declines. Further, understanding differences and effects of dispersal ability on species establishment will improve the predictability of establishment success of introduced species. Field experiments comparing life history traits between native and introduced or invasive ladybirds considering invasibility parameters of the introduced area (Raak-van den Berg *et al.* 2018) will provide insights into how abiotic and biotic factors interact with invasiveness of introduced species, improving our understanding of establishment success.

We believe the three categories (invasibility, invasiveness and stochastic events) will aid direct comparisons between successful and unsuccessful establishments for a cohesive theory of introduction biology. By targeting our research effort on the vulnerable establishment stage of introductions, we will be better placed to design and implement environmental management programs.

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References Cited

Abram, P. K., and C. E. Moffat. 2018. Rethinking biological control programs as planned invasions. Current Opinion in Insect Science 27: 9–15.

Assour, H., and J. Behm. 2019. First occurrence of *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae) on the Caribbean island of Curaçao. Neotropical Entomology 48: 863–865.

Awad, M., P. Kalushkov, T. Nedvědová, and O. Nedvěd. 2013. Fecundity and fertility of ladybird beetle *Harmonia axyridis* after prolonged cold storage. BioControl 58: 657–666.

Awad, M., G. J. Laugier, A. Loiseau, and O. Nedvěd. 2015. Unbalanced polyandry in wild-caught ladybirds *Harmonia axyridis* (Coleoptera: Coccinellidae). Applied Entomology and Zoology 50: 427–434.

Awad, M., L. Pialek, A. Krejčí, G. Laugier, and O. Nedvěd. 2017. Paternity following multiple mating in ladybird *Harmonia axyridis.* BioControl 62: 297–307.

Bain, J., P. Singh, M. D. Ashby, and R. J. Vanboven. 1984. Laboratory rearing of the predatory coccinellid *Cleobora mellyi* [Col.: Coccinellidae] for biological control of *Paropsis charybdis* [Col.: Chrysomelidae] in New Zealand. Entomophaga 29: 237–244.

Baker, S. C., J. A. Elek, R. Bashford, S. C. Paterson, J. Madden, and M. Battaglia. 2003. Inundative release of coccinellid beetles into eucalypt plantations for biological control of chrysomelid leaf beetles. Agricultural and Forest Entomology 5: 97–106.

Barahona-Segovia, R. M., A. A. Grez, and F. Bozinovic. 2015. Testing the hypothesis of greater eurythermality in invasive than in native ladybird species: From physiological performance to life-history strategies. Ecological Entomology 41: 182–191.

Bayoumy, M. H., A. M. Abou-Elnaga, A. A. Ghanim, and G. A. Mashhoot. 2016. Egg cannibalism potential benefits for adult reproductive performance and offspring fitness of *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae). Egyptian Journal of Biological Pest Control 26: 35–42.

Bayoumy, M. H., and J. P. Michaud. 2015. Cannibalism in two subtropical lady beetles (Coleoptera: Coccinellidae) as a function of density, life stage, and food supply. Journal of Insect Behavior 28: 387–402.

Beirne, B. 1975. Biological control attempts by introductions against pest insects in the field in Canada. The Canadian Entomologist 107: 225–236.

Benton, T. G., and D. E. Bowler. 2012. Dispersal in invertebrates: Influences on individual decisions [pp. 41–49]. *In*: Dispersal Ecology and Evolution (J. Clobert, M. Baguette, T. G. Benton, and J. M. Bullock, editors). Oxford University Press, Oxford, 462 pp.

Berkvens, N., C. Landuyt, K. Deforce, D. Berkvens, L. Tirry, and P. De Clercq. 2010. Alternative foods for the multicoloured Asian lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae). European Journal of Entomology 107: 189–195.

Bilu, E., and M. Coll. 2007. The importance of intraguild interactions to the combined effect of a parasitoid and a predator on aphid population suppression. Biocontrol 52: 753–763.

Bilu, E., and M. Coll. 2009. Parasitized aphids are inferior prey for a coccinellid predator: Implications for intraguild predation. Environmental Entomology 38: 153–158.

Blackburn, T. M., P. Pyšek, S. Bacher, J. T. Carlton, R. P. Duncan, V. Jarošík, J. R. Wilson, and D. M. Richardson. 2011. A proposed unified framework for biological invasions. Trends in Ecology and Evolution 26: 333–339.

Boher, F., F. M. Jaksic, S. I. Martel, M. J. Orellana, and F. Bozinovic. 2018. Does thermal physiology explain the ecological and evolutionary success of invasive species? Lessons from ladybird beetles. Evolutionary Ecology Research 19: 243–255.

Borowski, J. 2015. Beetles (Coleoptera) of the Rogow region. Part II - ladybirds (Coccinellidae). International Letters of Natural Sciences 34: 90–101.

Braekman, J. C., A. Charlier, D. Daloze, S. Heilporn, J. Pasteels, V. Plasman, and S. Wang. 1999. New piperidine alkaloids from two ladybird beetles of the genus *Calvia* (Coccinellidae). European Journal of Organic Chemistry 1999: 1749–1755.

Brown, P. M., R. Frost, J. Doberski, T. Sparks, R. Harrington, and H. E. Roy. 2011. Decline in native ladybirds in response to the arrival of *Harmonia axyridis*: Early evidence from England. Ecological Entomology 36: 231–240.

Cabral, S., A. O. Soares, and P. Garcia. 2009. Predation by *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) on *Myzus persicae* Sulzer (Homoptera: Aphididae): Effect of prey density. Biological Control 50: 25–29.

Cabral, S., A. O. Soares, and P. Garcia. 2011. Voracity of *Coccinella undecimpunctata*: Effects of insecticides when foraging in a prey/plant system. Journal of Pest Science 84: 373–379.

Chacon, J. M., D. A. Landis, and G. E. Heimpel. 2008. Potential for biotic interference of a classical biological control agent of the soybean aphid. Biological Control 46: 216–225.

Chapin, J. B., and V. Brou. 1991. *Harmonia axyridis* (Pallas), the third species of the genus to be found in the United States (Coleoptera: Coccinellidae). Proceedings of the Entomological Society of Washington 93: 630–635.

Clancy, K. M., and P. W. Price. 1987. Rapid herbivore growth enhances enemy attack: Sublethal plant defenses remain a paradox. Ecology 68: 733–737.

Clark, R. E., and M. S. Singer. 2018. Differences in aggressive behaviors between two ant species determine the ecological consequences of a facultative food-for-protection mutualism. Journal of Insect Behavior 31: 510–522.

Cock, M. J. W., S. T. Murphy, M. T. K. Kairo, E. Thompson, R. J. Murphy, and A. W. Francis. 2016. Trends in the classical biological control of insect pests by insects: An update of the BIOCAT database. BioControl 61: 349–363.

Coderre, D., L. Provencher, and J.-C. Tourneur. 1987. Oviposition and niche partitioning in aphidophagous insects on maize. The Canadian Entomologist 119: 195–203.

Comont, R. F., B. V. Purse, W. Phillips, W. E. Kunin, M. Hanson, O. T. Lewis, R. Harrington, C. R. Shortall, G. Rondoni, and H. E. Roy. 2014a. Escape from parasitism by the invasive alien ladybird, *Harmonia axyridis.* Insect Conservation and Diversity 7: 334–342.

Comont, R. F., H. E. Roy, R. Harrington, C. R. Shortall, and B. V. Purse. 2014b. Ecological correlates of local extinction and colonisation in the British ladybird beetles (Coleoptera: Coccinellidae). Biological Invasions 16: 1805–1817.

Comont, R. F., H. E. Roy, O. T. Lewis, R. Harrington, C. R. Shortall, and B. V. Purse. 2012. Using biological traits to explain ladybird distribution patterns. Journal of Biogeography 39: 1772–1781.

Cormier, C. M., T. A. Forbes, T. A. Jones, R. D. Morrison, and D. B. McCorquodale. 2000. Alien invasion: The status of non-native lady beetles (Coleoptera: Coccinellidae) in industrial Cape Breton, Nova Scotia. Northeastern Naturalist 7: 241–247.

Cottrell, T. E. 2005. Predation and cannibalism of lady beetle eggs by adult lady beetles. Biological Control 34: 159–164.

Day, W., D. Prokrym, D. Ellis, and R. Chianese. 1994. The known distribution of the predator *Propylea quatuordecimpunctata* (Coleoptera: Coccinellidae) in the United States, and thoughts on the origin of this species and five other exotic lady beetles in eastern North America. Entomological News 105: 244–256.

Dick, J. T. A., C. Laverty, J. J. Lennon, D. Barrios-O'Neill, P. J. Mensink, J. Robert Britton, V. Médoc, P. Boets, M. E. Alexander, N. G. Taylor, A. M. Dunn, M. J. Hatcher, P. J. Rosewarne, S. Crookes, H. J. MacIsaac, M. Xu, A. Ricciardi, R. J. Wasserman, B. R. Ellender, O. L. F. Weyl, F. E. Lucy, P. B. Banks, J. A. Dodd, C. MacNeil, M. R. Penk, D. C. Aldridge, and J. M. Caffrey. 2017. Invader Relative Impact Potential: A new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species. Journal of Applied Ecology 54: 1259–1267.

Dindo, M., S. Francati, A. Lanzoni, C. di Vitantonio, E. Marchetti, G. Burgio, and S. Maini. 2016. Interactions between the multicolored Asian lady beetle *Harmonia axyridis* and the parasitoid *Dinocampus coccinellae.* Insects 7: 67.

Dixon, A. 2007. Body size and resource partitioning in ladybirds. Population Ecology 49: 45–50.

Dixon, A., and J.-L. Hemptinne. 2001. Body size distribution in predatory ladybird beetles reflects that of their prey. Ecology 82: 1847–1856.

Dlugosch, K., and I. Parker. 2008. Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology 17: 431–449.

Drake, J. M., and D. M. Lodge. 2006. Allee effects, propagule pressure and the probability of establishment: Risk analysis for biological invasions. Biological Invasions 8: 365–375.

ElHag, E. T. A., and A. A. Zaitoon. 1996. Biological parameters for four coccinellid species in central Saudi Arabia. Biological Control 7: 316–319.

Emiljanowicz, L. M., H. A. Hager, and J. A. Newman. 2017. Traits related to biological invasion: A note on the applicability of risk assessment tools across taxa. NeoBiota 32: 31.

Éric, L. 2013. Intraguild predation among aphidophagous predators. European Journal of Entomology 102: 351–364.

Escalona, H. E., and A. Ślipiński. 2012. Generic revision and phylogeny of Microweiseinae (Coleoptera: Coccinellidae). Systematic Entomology 37: 125–171.

Escalona, H. E., A. Zwick, H.-S. Li, J. Li, X. Wang, H. Pang, D. Hartley, L. S. Jermiin, O. Nedvěd, and

B. Misof. 2017. Molecular phylogeny reveals food plasticity in the evolution of true ladybird beetles (Coleoptera: Coccinellidae: Coccinellini). BMC Evolutionary Biology 17: 151.

Evans, E. W. 2000. Morphology of invasion: Body size patterns associated with establishment of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in western North America. European Journal of Entomology 97: 469–474.

Evans, E. W. 2004. Habitat displacement of North American ladybirds by an introduced species. Ecology 85: 637–647.

Evans, E. W., A. O. Soares, and H. Yasuda. 2011. Invasions by ladybugs, ladybirds, and other predatory beetles. BioControl 56: 597–611.

Evans, E. W., A. T. Stevenson, and D. R. Richards. 1999. Essential versus alternative foods of insect predators: Benefits of a mixed diet. Oecologia 121: 107–112.

Evans, E. W., and T. R. Toler. 2007. Aggregation of polyphagous predators in response to multiple prey: Ladybirds (Coleoptera: Coccinellidae) foraging in alfalfa. Population Ecology 49: 29–36.

Facon, B., L. Crespin, A. Loiseau, E. Lombaert, A. Magro, and A. Estoup. 2011. Can things get worse when an invasive species hybridizes? The harlequin ladybird *Harmonia axyridis* in France as a case study. Evolutionary Applications 4: 71–88.

Facon, B., A. Estoup, R. A. Hufbauer, J. Foucaud, and A. Tayeh. 2017. Mating status influences cold tolerance and subsequent reproduction in the invasive ladybird *Harmonia axyridis.* Frontiers in Ecology and Evolution 5: 108.

Felix, S., and A. O. Soares. 2004. Intraguild predation between the aphidophagous ladybird beetles *Harmonia axyridis* and *Coccinella undecimpunctata* (Coleoptera: Coccinellidae): The role of body weight. European Journal of Entomology 101: 237–242.

Finlayson, C., A. Alyokhin, and E. Porter. 2009. Interactions of native and non-native lady beetle species (Coleoptera: Coccinellidae) with aphid-tending ants in laboratory arenas. Environmental Entomology 38: 846–855.

Forsman, A. 2014. Effects of genotypic and phenotypic variation on establishment are important for conservation, invasion, and infection biology. Proceedings of the National Academy of Sciences 111: 302–307.

Frank, J. H., and E. D. McCoy. 2007. The risk of classical biological control in Florida. Biological Control 41: 151–174.

Franzmann, B. A. 2002. *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae), a predacious ladybird new in Australia. Australian Journal of Entomology 41: 375–377.

Gariepy, V., G. Boivin, and J. Brodeur. 2015. Why two species of parasitoids showed promise in the laboratory but failed to control the soybean aphid under field conditions. Biological Control 80: 1–7.

Gegner, T., O. Otti, S. Tragust, and H. Feldhaar. 2015. Do microsporidia function as "biological weapon" for *Harmonia axyridis* under natural conditions? Insect Science 22: 353–359.

Ghosh, S., and B. Agarwala. 2018. Inter–specific relationship of size and walking speed in predaceous ladybirds (Insecta: Coleoptera: Coccinellidae). Current Science 115: 141–146.

Giorgi, J. A., N. J. Vandenberg, J. V. McHugh, J. A. Forrester, S. A. Ślipiński, K. B. Miller, L. R. Shapiro, and M. F. Whiting. 2009. The evolution of food preferences in Coccinellidae. Biological Control 51: 215–231.

Goetz, D. W. 2009. Seasonal inhalant insect allergy: *Harmonia axyridis* ladybug. Current Opinion in Allergy and Clinical Immunology 9: 329–333.

Gordon, R. D. 1985. The Coccinellidae (Coleoptera) of America north of Mexico. Journal of the New York Entomological Society 93: 1–912.

Goryacheva, I. I., A. V. Blekhman, B. V. Andrianov, T. V. Gorelova, and I. A. Zakharov. 2015. Genotypic diversity of *Wolbachia pipientis* in native and invasive *Harmonia axyridis* Pall., 1773 (Coleoptera, Coccinellidae) populations. Russian Journal of Genetics 51: 731–736.

Grez, A. A., B. Viera, and A. O. Soares. 2012. Biotic interactions between *Eriopis connexa* and *Hippodamia variegata*, a native and an exotic coccinellid species associated with alfalfa fields in Chile. Entomologia Experimentalis Et Applicata 142: 36–44.

Grozea, I., M. Vlad, A. M. Virteiu, R. Stef, A. Carabet, L. Molnar, and V. Mazare. 2015. Biological control of invasive species *Metcalfa pruinosa* Say (Insecta: Hemiptera: Flatidae) in ornamentals plants by using coccinelids [*sic*]. Journal of Biotechnology 208: S112.

Hameed, A., H. M. Saleem, S. Ahmad, J. Iqbal, H. Karar, and A. Amin. 2013. Predatory Potential and life history characteristics of eleven spotted beetle, *Coccinella undecimpunctata* L. reared on cotton mealybug, *Phenacoccus solenopsis* Tinsley. Pakistan Journal of Zoology 45: 1555–1562.

Hariston, N. G., L. J. Perry, A. J. Bohonak, M. Q. Fellows, C. M. Kearns, and D. R. Engstrom. 1999. Population biology of a failed invasion: Paleolimnology of *Daphnia exilis* in upstate New York. Limnology and Oceanography 44: 477–486.

Harmon, J., A. Ives, J. Losey, A. Olson, and K. Rauwald. 2000. *Coleomegilla maculata* (Coleoptera: Coccinellidae) predation on pea aphids promoted by proximity to dandelions. Oecologia 125: 543–548.

Harrison, R. G. 1980. Dispersal polymorphisms in insects. Annual Review of Ecology and Systematics 11: 95–118.

Hellmann, J. J., J. E. Byers, B. G. Bierwagen, and J. S. Dukes. 2008. Five potential consequences of climate change for invasive species. Conservation Biology 22: 534–543.

Hindayana, D., R. Meyhofer, D. Scholz, and H. M. Poehling. 2001. Intraguild predation among the hoverfly *Episyrphus balteatus* de Geer (Diptera: Syrphidae) and other aphidophagous predators. Biological Control 20: 236–246.

Hodek, I., and P. Ceryngier. 2000. Sexual activity in Coccinellidae (Coleoptera): A review. European Journal of Entomology 97: 449–456.

Hodek, I., H. F. Van Emden, and A. Honěk. 2012. Ecology and Behaviour of the Ladybird Beetles (Coccinellidae). Blackwell Publishing Ltd., Chichester, West Sussex, UK, 561 pp.

Hoki, E., J. Losey, and T. A. Ugine. 2014. Comparing the consumptive and non-consumptive effects of a native and introduced lady beetle on pea aphids (*Acyrthosiphon pisum*). Biological Control 70: 78–84.

Hokkanen, H. M. T., and R. I. Sailer. 1985. Success in classical biological control. Critical Reviews in Plant Sciences 3: 35–72.

Honěk, A. 1993. Intraspecific variation in body size and fecundity in insects: A general relationship. Oikos 66: 483–492.

Hoogendoorn, M., and G. E. Heimpel. 2002. Indirect interactions between an introduced and a native ladybird beetle species mediated by a shared parasitoid. Biological Control 25: 224–230.

Hoogendoorn, M., and G. E. Heimpel. 2004. Competitive interactions between an exotic and a native ladybeetle: A field cage study. Entomologia Experimentalis et Applicata 111: 19–28.

Hopper, K. R., and R. T. Roush. 1993. Mate finding, dispersal, number released, and the success of biological control introductions. Ecological Entomology 18: 321–331.

Howarth, F. G. 1991. Environmental impacts of classical biological control. Annual Review of Entomology 36: 485–509.

Janssen, A., M. Montserrat, R. HilleRisLambers, A. M. de Roos, A. Pallini, and M. W. Sabelis. 2006. Intraguild predation usually does not disrupt biological control [pp. 21–44]. *In*: Trophic and Guild Interactions in Biological Control (J. Brodeur and G. Boivin, editors). Springer, Dordrecht, 250 pp.

Kajita, Y., and E. W. Evans. 2009. Ovarian dynamics and oosorption in two species of predatory lady beetles (Coleoptera: Coccinellidae). Physiological Entomology 34: 185–194.

Kajita, Y., J. J. Obrycki, J. J. Sloggett, E. W. Evans, and K. F. Haynes. 2014. Do defensive chemicals facilitate intraguild predation and influence invasion success in ladybird beetles? Journal of Chemical Ecology 40: 1212–1219.

Kajita, Y., E. M. O'Neill, Y. Zheng, J. J. Obrycki, and D. W. Weisrock. 2012. A population genetic signature of human releases in an invasive ladybeetle. Molecular Ecology 21: 5473–5483.

Kajita, Y., F. Takano, H. Yasuda, and B. K. Agarwala. 2000. Effects of indigenous ladybird species (Coleoptera: Coccinellidae) on the survival of an exotic species in relation to prey abundance. Applied Entomology and Zoology 35: 473–479.

Kajita, Y., H. Yasuda, and E. W. Evans. 2006. Effects of native ladybirds on oviposition of the exotic species, *Adalia bipunctata* (Coleoptera: Coccinellidae), in Japan. Applied Entomology and Zoology 41: 57–61.

Kalaisekar, A., V. Ramamurthy, J. Patil, A. Dhandapani, and N. Azad Thakur. 2012. Multivariate morphometrics of elytral colour polymorphism in seven-spotted ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). Current Science 102: 1418–1425.

Katsanis, A., A. Magro, F. Ramon-Portugal, M. Kenis, and D. Babendreir. 2017. Chemical defences of native European coccinellid eggs against intraguild predation by the invasive Asian coccinellid, *Harmonia axyridis* (Pallas). BioControl 62: 385–396.

Kawakami, Y., K. Yamazaki, and K. Ohashi. 2019. Intergenerational fluctuations in colour morph frequencies may maintain elytral polymorphisms in the ladybird beetle *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae). Biological Journal of the Linnean Society 128: 725–734.

Kenis, M., T. Adriaens, P. M. J. Brown, A. Katsanis, G. San Martin, E. Branquart, D. Maes, R. Eschen, R. Zindel, J. Van Vlaenderen, D. Babendreier, H. E. Roy, L. Hautier, and R. L. Poland. 2017. Assessing the ecological risk posed by a recently established invasive alien predator: *Harmonia axyridis* as a case study. Biocontrol 62: 341–354.

Knapp, M., M. Řeřicha, S. Maršíková, F. Harabiš, T. Kadlec, O. Nedvěd, and T. Teder. 2019. Invasive host caught up with a native parasitoid: Field data reveal high parasitism of *Harmonia axyridis* by *Dinocampus coccinellae* in Central Europe. Biological Invasions 21: 2795–2802.

Koch, R. L., R. C. Venette, and W. D. Hutchison. 2006. Invasions by *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in the Western Hemisphere: Implications for South America. Neotropical Entomology 35: 421–434.

Krafsur, E. S., J. J. Obrycki, and J. D. Harwood. 2005. Comparative genetic studies of native and introduced Coccinellidae in North America. European Journal of Entomology 102: 469–474.

Kumschick, S., M. Gaertner, M. Vilà, F. Essl, J. M. Jeschke, P. Pyšek, A. Ricciardi, S. Bacher, T. M. Blackburn, and J. T. Dick. 2015. Ecological impacts of alien species: Quantification, scope, caveats, and recommendations. BioScience 65: 55–63.

Laugier, G. J. M., G. Le Moguédec, W. Su, A. Tayeh, L. Soldati, B. Serrate, A. Estoup, and B. Facon. 2016. Reduced population size can induce quick evolution of inbreeding depression in the invasive ladybird *Harmonia axyridis.* Biological Invasions 18: 2871–2881.

Leung, B., J. M. Drake, and D. M. Lodge. 2004. Predicting invasions: Propagule pressure and the gravity of Allee effects. Ecology 85: 1651–1660.

Lockwood, J. L., P. Cassey, and T. Blackburn. 2005. The role of propagule pressure in explaining species invasions. Trends in Ecology and Evolution 20: 223–228.

Lombaert, E., A. Estoup, B. Facon, B. Joubard, J. C. Grégoire, A. Jannin, A. Blin, and T. Guillemaud. 2014. Rapid increase in dispersal during range expansion in the invasive ladybird *Harmonia axyridis.* Journal of Evolutionary Biology 27: 508–517.

Lombaert, E., T. Guillemaud, J.-M. Cornuet, T. Malausa, B. Facon, and A. Estoup. 2010. Bridgehead effect in the worldwide invasion of the biocontrol harlequin ladybird. PLoS ONE 5(3): e9743.

Lombaert, E., T. Guillemaud, C. Thomas, L. Lawson Handley, J. Li, S. Wang, H. Pang, I. Goryacheva, I. Zakharov, and E. Jousselin. 2011. Inferring the origin of populations introduced from a genetically structured native range by approximate Bayesian computation: Case study of the invasive ladybird *Harmonia axyridis.* Molecular Ecology 20: 4654–4670.

Lombaert, E., T. Malausa, R. Devred, and A. Estoup. 2008. Phenotypic variation in invasive and biocontrol populations of the harlequin ladybird, *Harmonia axyridis.* Biocontrol 53: 89–102.

Lommen, S. T. E., C. W. Middendorp, C. A. Luijten, J. van Schelt, P. M. Brakefield, and P. W. de Jong. 2008. Natural flightless morphs of the ladybird beetle *Adalia bipunctata* improve biological control of aphids on single plants. Biological Control 47: 340–346.

Lopez, V. F., M. T. K. Kairo, and J. A. Irish. 2004. Biology and prey range of *Cryptognatha nodiceps* (Coleoptera: Coccinellidae), a potential biological control agent for the coconut scale, *Aspidiotus destructor* (Hemiptera: Diaspididae). Biocontrol Science and Technology 14: 475–485.

Losey, J. E., J. E. Perlman, and E. R. Hoebeke. 2007. Citizen scientist rediscovers rare nine-spotted lady beetle, *Coccinella novemnotata*, in eastern North America. Journal of Insect Conservation 11: 415–417.

MacDonald, F. H., P. G. Connolly, N. J. Larsen, and G. P. Walker. 2016. The voracity of five insect predators on *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae) (tomato potato psyllid; TPP). New Zealand Entomologist 39: 15–22.

Madire, L. G. 2013. Biology and host range of *Mada polluta*, a potential biological control agent of *Tecoma stans* in South Africa. Biocontrol Science and Technology 23: 944–955.

Maruyamai, M., and S. Ohno. 2011. Record of a ladybird beetle *Coelophora inaequalis* (Fabricius, 1775) in Okinawa, Japan. Japanese Journal of Entomology (New Series) 14: 112–115.

Majerus, M., V. Strawson, and H. Roy. 2006. The potential impacts of the arrival of the harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), in Britain. Ecological Entomology 31: 207–215.

McDermott, S. M., and D. C. Finnoff. 2016. Impact of repeated human introductions and the Allee effect on invasive species spread. Ecological Modelling 329: 100–111.

Meffe, G. K. 1991. Failed invasions of a southeastern blackwater stream by bluegills-implications for conservation of native communities. Transactions of the American Fisheries Society 120: 333–338.

Mehrnejad, M. R., M. A. Jalali, and R. Mirzaei. 2011. Abundance and biological parameters of psyllophagous coccinellids in pistachio orchards. Journal of Applied Entomology 135: 673–681.

Mendel, Z., F. Assael, S. Zeidan, and A. Zehavi. 1998. Classical biological control of *Palaeococcus fuscipennis* (Burmeister) (Homoptera: Margarodidae) in Israel. Biological Control 12: 151–157.

Michael, P. J., P. B. Yeoh, and J. K. Scott. 2012. Potential distribution of the Australian native *Chloris truncata* based on modelling both the successful and failed global introductions. PLoS ONE 7(7): e42140.

Michaud, J. 2012. Coccinellids in biological control [pp. 488–519]. *In*: Ecology and Behaviour of the Ladybird Beetles (Coccinellidae) (I. Hodek, H. F. Van Emden, and A. Honěk, editors). Blackwell Publishing Ltd., Chichester, West Sussex, UK, 561 pp.

Michaud, J., and A. Grant. 2003. Intraguild predation among ladybeetles and a green lacewing: Do the larval spines of *Curinus coeruleus* (Coleoptera: Coccinellidae) serve a defensive function? Bulletin of Entomological Research 93: 499–505.

Michaud, J. P. 2003. A comparative study of larval cannibalism in three species of ladybird. Ecological Entomology 28: 92–101.

Michels, G. J., R. V. Flanders, and J. B. Bible. 1997. Overwintering survival of seven imported coccinellids in the Texas High Plains. Southwestern Entomologist 22: 157–166.

Michie, L. J., F. Mallard, M. E. N. Majerus, and F. M. Jiggins. 2010. Melanic through nature or nurture: Genetic polymorphism and phenotypic plasticity in *Harmonia axyridis.* Journal of Evolutionary Biology 23: 1699–1707.

Mirande, L., N. Desneux, M. Haramboure, and M. I. Schneider. 2015. Intraguild predation between an exotic and a native coccinellid in Argentina: The role of prey density. Journal of Pest Science 88: 155–162.

Mohamed, E. N., and A. A. Ghanim. 2008. Biological characteristics of three predatory insects preyed on the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring under controlled conditions. Egyptian Journal of Biological Pest Control 18: 309–313.

Moser, S. E., and J. J. Obrycki. 2009. Competition and intraguild predation among three species of coccinellids (Coleoptera: Coccinellidae). Annals of the Entomological Society of America 102: 419–425.

Moulton, M. P., W. P. Cropper, and M. L. Avery. 2012. Historical records of passerine introductions to New Zealand fail to support the propagule pressure hypothesis. Biodiversity and Conservation 21: 297–307.

Musser, F. R., and A. M. Shelton. 2003. Factors altering the temporal and within-plant distribution of coccinellids in corn and their impact on potential intra-guild predation. Environmental Entomology 32: 575–583.

Nedvěd, O., M. Awad, G. Laugier, L. Piálek, A. Krejčí, F. Karabüyük, K. Pužejová, and P. Kalushkov. 2018. Paternity and sperm competition following multiple mating in *Harmonia axyridis* (Coleoptera: Coccinellidae). IOBC-WPRS Bulletin 137: 17–24.

Nedvěd, O., X. Fois, D. Ungerová, and P. Kalushkov. 2013. Alien vs. Predator – the native lacewing *Chrysoperla carneais* the superior intraguild predator in trials against the invasive ladybird *Harmonia axyridis.* Bulletin of Insectology 66(1): 73–78.

Nedvěd, O., and A. Honěk. 2012. Life history and development [pp. 54–109]. *In*: Ecology and Behaviour of the Ladybird Beetles (Coccinellidae) (I. Hodek, H. F. Van Emden, and A. Honěk, editors). Blackwell Publishing Ltd., Chichester, West Sussex, UK, 561 pp.

Nijhout, H. 2003. The control of body size in insects. Developmental Biology 261: 1–9.

Nimmo, K. R., and P. W. Tipping. 2009. An introduced insect biological control agent preys on an introduced weed biological control agent. Florida Entomologist 92: 179–180.

Nóia, M., I. Borges, and A. O. Soares. 2008. Intraguild predation between the aphidophagous ladybird beetles *Harmonia axyridis* and *Coccinella undecimpunctata* (Coleoptera: Coccinellidae): The role of intra and extraguild prey densities. Biological Control 46: 140–146.

Obrycki, J. J., and T. J. Kring. 1998. Predaceous Coccinellidae in biological control. Annual Review of Entomology 43: 295–321.

Omkar, G. Mishra, B. Kumar, N. Singh, and G. Pandey. 2014. Risks associated with tandem release of large and small ladybirds (Coleoptera: Coccinellidae) in heterospecific aphidophagous guilds. The Canadian Entomologist 146: 52–66.

Orlova-Bienkowskaja, M. J. 2013. Dangerous invasive harlequin ladybird *Harmonia axyridis* (Pallas, 1773) (Coleoptera, Coccinellidae) in the European Russia. Russian Journal of Biological Invasions 4: 190–193.

Paul, S. C., J. K. Pell, and J. D. Blount. 2015. Reproduction in risky environments: The role of invasive egg predators in ladybird laying strategies (dataset). PLoS ONE 10(10): e0139404.

Pervez, A., G. Mishra, S. Srivastava, S. K. Singh, and A. K. Gupta. 2005. Intrinsic advantages of *Cheilomenes sexmaculata* over two coexisting *Coccinella* species (Coleoptera: Coccinellidae). Insect Science 12: 179–184.

Polis, G. A., and R. D. Holt. 1992. Intraguild predation: The dynamics of complex trophic interactions. Trends in Ecology and Evolution 7: 151–154.

Powell, B. E., and J. Silverman. 2010. Impact of *Linepithema humile* and *Tapinoma sessile* (Hymenoptera: Formicidae) on three natural enemies of *Aphis gossypii* (Hemiptera: Aphididae). Biological Control 54: 285–291.

Pugh, A. R., D. M. O'Connell, and S. D. Wratten. 2015. Further evaluation of the southern ladybird (*Cleobora mellyi*) as a biological control agent of the invasive tomato-potato psyllid (*Bactericera cockerelli*). Biological Control 90: 157–163.

Purse, B. V., R. Comont, A. Butler, P. M. J. Brown, C. Kessel, and H. E. Roy. 2015. Landscape and climate determine patterns of spread for all colour morphs of the alien ladybird *Harmonia axyridis.* Journal of Biogeography 42: 575–588.

Raak-van den Berg, C. L., P. W. de Jong, G. Gort, B. F. J. Manly, and J. C. van Lenteren. 2018. Life histories of an invasive and native ladybird under field experimental conditions in a temperate climate. Entomologia Experimentalis et Applicata 166: 151–161.

Ramírez, J., G. González, and Y. Sánchez. 2018. First record of *Cheilomenes sexmaculata* (Fabricius, 1781) (Coleoptera, Coccinellidae) from Colombia. CheckList 14: 77–80.

Reznik, S. Y., M. Y. Dolgovskaya, A. N. Ovchinnikov, and N. A. Belyakova. 2015. Weak photoperiodic response facilitates the biological invasion of the harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). Journal of Applied Entomology 139: 241–249.

Reznik, S. Y., A. N. Ovchinnikov, A. A. Ovchinnikova, and N. A. Belyakova. 2018. Costs and benefits of interlarval cannibalism in *Harmonia axyridis.* Journal of Applied Entomology 142: 223–229.

Reznik, S. Y., A. A. Ovchinnikova, A. N. Ovchinnikov, L. V. Barabanova, and N. A. Belyakova. 2017. Inheritance of diapause regulation in the multicoloured Asian ladybird *Harmonia axyridis* (Coleoptera: Coccinellidae). European Journal of Entomology 114: 416–421.

Riddick, E. W., T. E. Cottrell, and K. A. Kidd. 2009. Natural enemies of the Coccinellidae: Parasites, pathogens, and parasitoids. Biological Control 51: 306–312.

Robertson, J. A., A. Ślipiński, M. Moulton, F. W. Shockley, A. Giorgi, N. P. Lord, D. D. McKenna, W. Tomaszewska, J. Forrester, and K. B. Miller. 2015. Phylogeny and classification of Cucujoidea and the recognition of a new superfamily Coccinelloidea (Coleoptera: Cucujiformia). Systematic Entomology 40: 745–778.

Romanov, D. 2019. Parasitoids of the harlequin ladybeetle *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae). Russian Journal of Biological Invasions 10: 52–67.

Rondoni, G., K. J. Athey, J. D. Harwood, E. Conti, C. Ricci, and J. J. Obrycki. 2015. Development and application of molecular gut-content analysis to detect aphid and coccinellid predation by *Harmonia axyridis* (Coleoptera: Coccinellidae) in Italy. Insect Science 22: 719–730.

Rondoni, G., F. Ielo, C. Ricci, and E. Conti. 2017. Behavioural and physiological responses to prey-related cues reflect higher competitiveness of invasive vs. native ladybirds. Scientific Reports 7: 3716.

Roy, H. E., T. Adriaens, N. J. Isaac, M. Kenis, T. Onkelinx, G. S. Martin, P. M. Brown, L. Hautier, R. Poland, and D. B. Roy. 2012. Invasive alien predator causes rapid declines of native European ladybirds. Diversity and Distributions 18: 717–725.

**Roy, H. E., P. M. J. Brown, T. Adriaens, N. Berkvens, I. Borges, S. Clusella-Trullas, R. F. Comont, P. De Clercq, R. Eschen, A. Estoup, E. W. Evans, B. Facon, M. M. Gardiner, A. Gil, A. A. Grez, T. Guillemaud, D. Haelewaters, A. Herz, A. Honek, A. G. Howe, C. Hui, W. D. Hutchison, M. Kenis, R. L. Koch, J. Kulfan, L. L. Handley, E. Lombaert, A. Loomans, J. Losey, A. O. Lukashuk, D. Maes, A. Magro, K. M. Murray, G. San Martin, Z. Martinkova, I. A. Minnaar, O. Nedved, M. J. Orlova-Bienkowskaja, N. Osawa, W. Rabitsch, H. P. Ravn, G. Rondoni, S.

L. Rorke, S. K. Ryndevich, M.-G. Saethre, J. J. Sloggett, A. O. Soares, R. Stals, M. C. Tinsley, A. Vandereycken, P. van Wielink, S. Viglasova, P. Zach, I. A. Zakharov, T. Zaviezo, and Z. Zhao. 2016. The harlequin ladybird, *Harmonia axyridis*: Global perspectives on invasion history and ecology. Biological Invasions 18: 997–1044.

Rudolf, V. H. 2007. The interaction of cannibalism and omnivory: Consequences for community dynamics. Ecology 88: 2697–2705.

Rudolf, V. H., and N. L. Rasmussen. 2013. Ontogenetic functional diversity: Size structure of a keystone predator drives functioning of a complex ecosystem. Ecology 94: 1046–1056.

Saito, T., and S. Bjørnson. 2008. Effects of a microsporidium from the convergent lady beetle, *Hippodamia convergens* Guerin-Meneville (Coleoptera: Coccinellidae), on three non-target coccinellids. Journal of Invertebrate Pathology 99: 294–301.

Sato, S., and A. F. G. Dixon. 2004. Effect of intraguild predation on the survival and development of three species of aphidophagous ladybirds: Consequences for invasive species. Agricultural and Forest Entomology 6: 21–24.

Sayed, S. M. 2016. Molecular diversity of the lady beetles, *Coccinella undecimpunctata* L. and *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae) in Saudi Arabia. Egyptian Journal of Biological Pest Control 26: 351–355.

Schaefer, P. W., R. J. Dysart, and H. B. Specht. 1987. North American distribution of *Coccinella septempunctata* (Coleoptera: Coccinellidae) and its mass appearance in coastal Delaware. Environmental Entomology 16: 368–373.

Scorsetti, A. C., S. A. Pelizza, M. N. Fogel, M. F. Vianna, and M. I. Schneider. 2017. Interactions between the entomopathogenic fungus *Beauveria bassiana* and the Neotropical predator *Eriopis connexa* (Coleoptera: Coccinellidae): Implications in biological control of pest. Journal of Plant Protection Research 57(4): 389–395.

Seago, A. E., J. A. Giorgi, J. Li, and A. Ślipiński. 2011. Phylogeny, classification and evolution of ladybird beetles (Coleoptera: Coccinellidae) based on simultaneous analysis of molecular and morphological data. Molecular Phylogenetics and Evolution 60: 137–151.

Seko, T., A. Sumi, A. Nakano, M. Kameshiro, T. Kaneda, and K. Miura. 2014. Suppression of aphids by augmentative release of larvae of flightless *Harmonia axyridis.* Journal of Applied Entomology 138: 326–337.

Seko, T., K.-i. Yamashita, and K. Miura. 2008. Residence period of a flightless strain of the ladybird beetle *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in open fields. Biological Control 47: 194–198.

Sheppard, S. K., M. L. Henneman, J. Memmott, and W. O. C. Symondson. 2004. Infiltration by alien predators into invertebrate food webs in Hawaii: A molecular approach. Molecular Ecology 13: 2077–2088.

Shoeb, M. A., F. A. Attalla, and A. H. El-Heneidy. 2008. On the migration of common predatory species from Egyptian clover to adjacent maize fields in Egypt. Egyptian Journal of Biological Pest Control 18: 385–389.

Siljamo, P., K. Ashbrook, R. F. Comont, and C. A. Skjøth. 2020. Do atmospheric events explain the arrival of an invasive ladybird (*Harmonia axyridis*) in the UK? PLoS ONE 15: e0219335.

Simmons, A. M., and S. Abd-Rabou. 2011. Inundative field releases and evaluation of three predators for *Bemisia tabaci* (Hemiptera: Aleyrodidae) management in three vegetable crops. Insect Science 18: 195–202.

Simmons, A. M., and J. C. Legaspi. 2007. Ability of *Delphastus catalinae* (Coleoptera: Coccinellidae), a predator of whiteflies (Homoptera: Aleyrodidae), to survive mild winters. Journal of Entomological Science 42: 163–173.

Singh, S., G. Mishra, and Omkar. 2018. Plasticity in reproductive output and development in response to thermal variation in ladybird beetle, *Menochilus sexmaculatus.* Journal of Thermal Biology 71: 180–188.

Skouras, P. J., G. J. Stathas, C. C. Voudouris, A. I. Darras, J. A. Tsitsipis, and J. T. Margaritopoulos. 2017. Effect of synthetic insecticides on the larvae of *Coccinella septempunctata* from Greek populations. Phytoparasitica 45: 165–173.

Ślipiński, A. 2007. Australian Ladybird Beetles (Coleoptera: Coccinellidae): Their Biology and Classification. CSIRO Publishing, Canberra, 286 pp.

Ślipiński, S., R. Leschen, and J. Lawrence. 2011. Order Coleoptera Linnaeus, 1758. Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. Zootaxa 3148: 203–208.

Sloggett, J. J. 2005. Are we studying too few taxa? Insights from aphidophagous ladybird beetles (Coleoptera: Coccinellidae). European Journal of Entomology 102: 391–398.

Smith, C. A., and M. M. Gardiner. 2013. Biodiversity loss following the introduction of exotic competitors: Does intraguild predation explain the decline of native lady beetles? PLoS ONE 8: e84448.

Smyth, R. R., L. L. Allee, and J. E. Losey. 2013. The status of *Coccinella undecimpunctata* (L.) (Coleoptera: Coccinellidae) in North America: An updated distribution from citizen science data. The Coleopterists Bulletin 67: 532–535.

Snyder, W. E., and G. M. Clevenger. 2004. Negative dietary effects of Colorado potato beetle eggs for the larvae of native and introduced ladybird beetles. Biological Control 31: 353–361.

Snyder, W. E., G. M. Clevenger, and S. D. Eigenbrode. 2004. Intraguild predation and successful invasion by introduced ladybird beetles. Oecologia 140: 559–565.

Soares, A. O., I. Borges, P. A. Borges, G. Labrie, and E. Lucas. 2008. *Harmonia axyridis*: What will stop the invader? BioControl 53: 127–145.

Soares, A. O., A. Honěk, Z. Martinkova, J. Skuhrovec, P. Cardoso, and I. Borges. 2017. *Harmonia axyridis* failed to establish in the Azores: The role of species richness, intraguild interactions and resource availability. BioControl 62: 423–434.

Soares, A. O., and A. Serpa. 2007. Interference competition between ladybird beetle adults (Coleoptera: Coccinellidae): Effects on growth and reproductive capacity. Population Ecology 49: 37–43.

Stankovic, V. M., T. Koren, and I. Stankovic. 2011. The harlequin ladybird continues to invade southeastern Europe. Biological Invasions 13: 1711–1716.

Steele, T., and S. Bjørnson. 2019. Effects of microsporidiosis and food availability on the two-spotted lady beetle, *Adalia bipunctata* L., and convergent lady beetle, *Hippodamia convergens* Guérin-Méneville. Journal of Invertebrate Pathology 161: 7–13.

Szawaryn, K., L. Bocak, A. Ślipiński, H. E. Escalona, and W. Tomaszewska. 2015. Phylogeny and evolution of phytophagous ladybird beetles (Coleoptera: Coccinellidae: Epilachnini), with recognition of new genera. Systematic Entomology 40: 547–569.

Takizawa, T., and H. Yasuda. 2006. The effects of attacks by the mutualistic ant, *Lasius japonicus* Santschi (Hymenoptera: Formicidae) on the foraging behavior of the two aphidophagous ladybirds, *Coccinella septempunctata brucki* Mulsant (Coleoptera: Coccinellidae) and *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae). Applied Entomology and Zoology 41: 161–169.

Tayeh, A., R. A. Hufbauer, A. Estoup, V. Ravigné, L. Frachon, and B. Facon. 2015. Biological invasion and biological control select for different life histories. Nature Communications 6: 1–5.

Tayeh, A., A. Estoup, G. Laugier, A. Loiseau, J. Turgeon, S. Toepfer, and B. Facon. 2012. Evolution in biocontrol strains: Insight from the harlequin ladybird *Harmonia axyridis.* Evolutionary Applications 5: 481–488.

Tillman, P. G. 2011. Natural biological control of stink bug (Heteroptera: Pentatomidae) eggs in corn, peanut, and cotton farmscapes in Georgia. Environmental Entomology 40: 303–314.

Toda, Y., and Y. Sakuratani. 2006. Expansion of the geographical distribution of an exotic ladybird beetle, *Adalia bipunctata* (Coleoptera: Coccinellidae), and its interspecific relationships with native ladybird beetles in Japan. Ecological Research 21: 292–300.

Togni, P. H., M. Venzon, C. A. Muniz, E. F. Martins, A. Pallini, and E. R. Sujii. 2016. Mechanisms underlying the innate attraction of an aphidophagous coccinellid to coriander plants: Implications for conservation biological control. Biological Control 92: 77–84.

Tohamy, T. H., A. A. A. El-Raheem, and A. M. El-Rawy. 2008. Role of the cultural practices and natural enemies for suppressing infestation of the pink sugarcane mealybug, *Saccharicoccus sacchari* (Cockerell) (Hemiptera: Pseudococcidae) in sugarcane fields at Minia Governorate, Middle Egypt. Egyptian Journal of Biological Pest Control 18: 177–188.

Tscharntke, T., I. Steffan-Dewenter, A. Kruess, and C. Thies. 2002. Characteristics of insect populations on habitat fragments: A mini review. Ecological Research 17: 229–239.

Tumminello, G., T. A. Ugine, and J. E. Losey. 2015. Intraguild interactions of native and introduced coccinellids: The decline of a flagship species. Environmental Entomology 44: 64–72.

Turgeon, J., A. Tayeh, B. Facon, E. Lombaert, P. De Clercq, N. Berkvens, J. G. Lundgren, and A. Estoup. 2011. Experimental evidence for the phenotypic impact of admixture between wild and biocontrol Asian ladybird (*Harmonia axyridis*) involved in the European invasion. Journal of Evolutionary Biology 24: 1044–1052.

Turnipseed, R., T. A. Ugine, and J. E. Losey. 2015. Egg predation by the introduced lady beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae), lowers mortality but raises relative risk for the native lady beetle, *Coccinella novemnotata.* PLoS ONE 10(6): e0118493.

Ueno, H. 1996. Estimate of multiple insemination in a natural population of *Harmonia axyridis* (Coleoptera: Coccinellidae). Applied Entomology and Zoology 31: 621–623.

Veran, S., S. Piry, V. Ternois, C. N. Meynard, B. Facon, and A. Estoup. 2016. Modeling spatial expansion of invasive alien species: Relative contributions of environmental and anthropogenic factors to the spreading of the harlequin ladybird in France. Ecography 39: 665–675.

Viglášová, S., O. Nedvěd, P. Zach, J. Kulfan, M. Parák, A. Honěk, Z. Martinková, and H. E. Roy. 2017. Species assemblages of ladybirds including the harlequin ladybird *Harmonia axyridis*: A comparison at large spatial scale in urban habitats. BioControl 62: 409–421.

Wallach, A. D., A. H. Dekker, M. Lurgi, J. M. Montoya, D. A. Fordham, and E. G. Ritchie. 2017. Trophic cascades in 3D: Network analysis reveals how apex predators structure ecosystems. Methods in Ecology and Evolution 8(1): 135–142.

Wang, L., J. Huang, and H. Luo. 2008. Response by a whitefly predator, *Delphastus catalinae* to toxins from *Verticillium lecanii* and mass rearing techniques for the ladybeetle. Journal of Insect Science 8: 51.

Wang, S., J. P. Michaud, X. L. Tan, F. Zhang, and X. J. Guo. 2011. The aggregation behavior of *Harmonia axyridis* in its native range in northeast China. BioControl 56: 193–206.

Wang, S., X. L. Tan, X. J. Guo, and F. Zhang. 2013. Effect of temperature and photoperiod on the development, reproduction, and predation of the predatory ladybird *Cheilomenes sexmaculata* (Coleoptera: Coccinellidae). Journal of Economic Entomology 106: 2621–2629.

Ware, R., B. Yguel, and M. Majerus. 2009. Effects of competition, cannibalism and intra-guild predation on larval development of the European coccinellid *Adalia bipunctata* and the invasive species *Harmonia axyridis.* Ecological Entomology 34: 12–19.

Wells, P. M., J. Baverstock, S. J. Clark, F. M. Jiggins, H. E. Roy, and J. K. Pell. 2017. Determining the effects of life stage, shared prey density and host plant on intraguild predation of a native lacewing (*Chrysoperla carnea*) by an invasive coccinellid (*Harmonia axyridis*). BioControl 62: 373–384.

Werf, V. W., E. W. Evans, and J. Powell. 2000. Measuring and modelling the dispersal of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in alfalfa fields. European Journal of Entomology 97: 487–493.

Wheeler, A. G., and E. R. Hoebeke. 2008. Rise and fall of an immigrant lady beetle: Is *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) still present in North America? Proceedings of the Entomological Society of Washington 110: 817–823.

Williams, A. H., and D. K. Young. 2009. The alien *Hippodamia variegata* (Coleoptera: Coccinellidae) quickly establishes itself throughout Wisconsin. Great Lakes Entomologist 42: 100.

Yasuda, H., E. W. Evans, Y. Kajita, K. Urakawa, and T. Takizawa. 2004. Asymmetric larval interactions between introduced and indigenous ladybirds in North America. Oecologia 141: 722–731.

Zadravec, D., and M. Bavec. 2001. Natural enemies against vegetable pests in the northeast of Slovenia [pp. 477–480]. Proceedings of the 5th Slovenian Conference on Plant Protection, Catez ob Savi, Slovenia, 6–8 March 2001.

Zaki, F. N., M. F. El-Shaarawy, and N. A. Farag. 1999. Release of two predators and two parasitoids to control aphids and whiteflies. Anzeiger Fur Schadlingskunde-Journal of Pest Science 72: 19–20.

Zenni, R. D., and M. A. Nunez. 2013. The elephant in the room: The role of failed invasions in understanding invasion biology. Oikos 122: 801–815.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annual Review of Entomology 42: 207–230.

Zerebecki, R. A., and C. J. Sorte. 2011. Temperature tolerance and stress proteins as mechanisms of invasive species success. PLoS ONE 6: e14806.

Zhang, G. F., G. L. Lovei, X. Wu, and F. H. Wan. 2016. Presence of native prey does not divert predation on exotic pests by *Harmonia axyridis* in its indigenous range. PLoS ONE 11(7): e0159048.

Zhou, A., J. Bi, J. Xu, and M.-Q. Wang. 2015. Effects of the invasive plant *Eupatorium adenophorum* on the exotic mealybug *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) and their natural enemies. Agricultural and Forest Entomology 17: 389–399.

Zhou, A., Y. Lu, L. Zeng, Y. Xu, and G. Liang. 2013. *Solenopsis invicta* (Hymenoptera: Formicidae), defend *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) against its natural enemies. Environmental Entomology 42: 247–252.

Zhou, A. M., G. W. Liang, L. Zeng, Y. Y. Lu, and Y. J. Xu. 2014. Interactions between ghost ants and invasive mealybugs: The case of *Tapinoma melanocephalum* (Hymenoptera: Formicidae) and *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae). Florida Entomologist 97: 1474–1480.

Zhou, X., A. Honek, W. Powell, and N. Carter. 1995. Variations in body length, weight, fat content and survival in *Coccinella septempunctata* at different hibernation sites. Entomologia Experimentalis et Applicata 75: 99–107.

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APPENDIX 1

STUDIES ON INTRODUCED COCCINELLINES DESCRIBED UNDER PROPOSED CATEGORIZATION

We developed the below table based on studies that investigated introduced ladybirds in terms of their invasiveness, invasibility of the introduced habitat and native ranges and stochastic events. "Categorization" and "Factors/Traits" are as described in Table 1 and Fig. 1. "Introduction" indicates whether the study treats the species as biocontrol, accidental/deliberate introduction, immigration, invasive, or the introduction method is unknown or in native range. Blank lines mean that there was no indication of introduction type on the referred paper. "Key effects/Biological notes" indicates successful biocontrol agents, native declines/displacement, IGP, other non-target predation or instances where effects are unknown, and other biological notes on ladybird species.

No.	Coccinelline name	Categorization	Factors/Traits	Introduction	Key effect/Biological notes	References
1.	*Adalia bipunctata* (Linnaeus, 1758)	Invasibility	Competitors, Intraguild predation risk – spatial distribution	Biocontrol	First record in 1993 and range expansion 2006	Toda and Sakuratani 2006
		Invasibility	Intraguild predation risk – IG prey	-	IGP by *Harmonia axyridis* limited spread of *A. bipunctata* in Japan	Ware *et al.* 2009
		Invasibility	Intraguild predation risk – IG prey	Biocontrol	Egg production reduced in the presence of the native *H. axyridis*	Kajita *et al.* 2006
2.	*Cheilomenes sexmaculata* (Fabricius, 1781)	Invasibility	Other fauna	Native region	Fecundity and hatching rate significantly reduced by ghost ants [*Tapinoma melanocephalum* (Fabricius, 1793); Hymenoptera: Formicidae]	Zhou *et al.* 2014
		Invasiveness	Development times, Fecundity	Native region	Better performance than two relatively larger *Coccinella* species	Pervez *et al.* 2005
		Invasibility	Other fauna	Native region	Survival of lady beetle larvae was significantly decreased by fire ants (*Solenopsis invicta* Buren, 1972)	Zhou *et al.* 2013
		Invasibility	Other flora	Native region	Avoid mealybug infested *Eupatorium adenophorum* Spreng. (Asteraceae) (invasive plant)	Zhou *et al.* 2015
		Invasiveness	Dispersal	Invasive	First record of *C. sexmaculata* in Colombia	Ramírez *et al.* 2018
		Invasiveness	Dispersal	Invasive	First record of *C. sexmaculata on* on the Caribbean island of Curaçao	Assour and Behm 2019
3.	*Cleobora mellyi* (Mulsant, 1850)	Invasiveness	Dietary range	Biocontrol	Biocontrol of invasive tomato-potato psyllid (TPP), *Bactericera cockerelli* (Šulc, 1909) (Hemiptera: Triozidae); no difference in longevity between ladybirds supplied with TPP only or buckwheat only	Pugh *et al.* 2015
		Invasiveness	Cannibalism	Biocontrol	Increased cannibalism reduced survival in laboratory rearing on artificial diet; biocontrol of *Paropsis charybdis* Stål, 1860 (Coleoptera: Chrysomelidae)	Bain *et al.* 1984
		Invasiveness	Dispersal	Biocontrol	Inundative release of laboratory reared *C. mellyi* is possible for biocontrol of *Paropsisterna bimaculata* (Olivier, 1807) (Coleoptera: Chrysomelidae)	Baker *et al.* 2003

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Appendix 1. Continued.

No.	Coccinelline name	Categorization	Factors/Traits	Introduction	Key effect/Biological notes	References
4.	*Coccinella septempunctata* Linnaeus, 1758	Invasibility	Intraguild predation	Biocontrol	Do not prey on *Aphidius colemani* Viereck, 1912 (Hymenoptera: Braconidae)	Chacon *et al.* 2008
		Invasiveness	Fecundity	Invasive	Adjusting reproductive output to fluctuation in prey availability	Kajita and Evans 2009
		Invasiveness	Dispersal	Native range	-	Zadravec and Bavec 2001
		Invasiveness, Invasibility	Resource acquisition, Intraguild predation – IG predator	Biocontrol	Could influence habitat use and dispersal of native ladybirds through changing prey abundance and IGP	Evans 2004
		Invasibility	Intraguild predation – IG predator	Biocontrol	Increased egg predation on native ladybird species (*Coccinella novemnotata*)	Turnipseed *et al.* 2015
		Invasiveness	Resource acquisition	Native range	More effective biological control of invasive species *Metcalfa pruinosa* (Say, 1830) (Hemiptera: Flatidae) in ornamental plants than *Propylea quatordecimpuncata*, *A. bipunctata* and *Psyllobora vigintiduopunctata* (Linnaeus, 1758)	Grozea *et al.* 2015
		Invasibility	Intraguild predation – IG predator	Biocontrol	IGP of native ladybird species leading to declining populations	Snyder *et al.* 2004
		Invasiveness	Resource acquisition	Biocontrol	Has greater consumptive and non-consumptive effects on *Acyrthosiphon pisum* (Harris, 1776) (Hemiptera: Aphididae) than the native species (*C. novemnotata*); could lead to decreased stability in predator and prey interactions	Hoki *et al.* 2014
		Invasiveness	Resource acquisition	Biocontrol	Biocontrol of *Euschistus servus* (Say, 1832) and *Nezara viridula* (Linnaeus, 1758) (Hemiptera: Pentatomidae) eggs	Tillman 2011
		Invasiveness	Vulnerability to change/ disturbance – pesticides	Native range	Developmental time, adult weight and daily predation was affected	Skouras *et al.* 2017
		Invasibility	Competitors	Biocontrol	-	Omkar *et al.* 2014
		Invasiveness	Resource aquisition	Biocontrol	Potential biocontrol agent for *Bemisia argentifolii* (Gennadius, 1889) (Hemiptera: Aleyrodidae)	Mohamed *et al.* 2008

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Appendix 1. Continued.

No.	Coccinelline name	Categorization	Factors/Traits	Introduction	Key effect/Biological notes	References
5.	*Coccinella undecimpunctata* Linnaeus, 1758	Invasiveness	Resource acquisition	Biocontrol	Biocontrol of *Aphis gossypii* Glover (Hemiptera: Aphididae) and *Bemisia tabaci* (Gennadius, 1889)	Zaki *et al.* 1999
		Invasiveness, Invasibility	Intraguild predation – IG prey, Dispersal	Biocontrol and immigration	Possible decline in USA	Wheeler and Hoebeke 2008
		Invasiveness	Resource acquisition	Native range	Biocontrol of *Saccharicoccus sacchari* (Cockerell, 1895) (Hemiptera: Pseudococcidae) in sugarcane fields in Egypt	Tohamy *et al.* 2008
		Invasiveness	Dispersal	Accidental	Established populations found through citizen science programs	Smyth *et al.* 2013
					Biocontrol of *B. tabaci*	Simmons and Abd-Rabou 2011
		Invasiveness	Dispersal	Biocontrol	Migration from clover fields to adjacent maize fields	Shoeb *et al.* 2008
		Invasiveness	Genetic variation	Biocontrol	Low genetic variability	Sayed 2016
		Invasiveness	Resource acquisition, Fecundity	Biocontrol	Potential biocontrol agent for *B. argentifolii*	Mohamed *et al.* 2008
		Invasiveness	Resource acquisition	Biocontrol	Biocontrol of tomato potato psyllid (TPP), *B. cockerelli*	MacDonald *et al.* 2016
		Invasiveness	Resource acquisition	Native range	Biocontrol of cotton mealybug, *Phenacoccus solenopsis* Tinsley, 1898 (Pseudococcidae)	Hameed *et al.* 2013
		Invasiveness	Vulnerability to change/ disturbance – insecticides	Biocontrol	A synergistic effect between biocontrol and use of insecticides to control cereal aphid	ElHag and Zaitoon 1996
		Invasiveness	Vulnerability to change/ disturbance – insecticides	Biocontrol	Voracity may be related to mobility of insecticide-treated aphids in Azores	Cabral *et al.* 2011
		Invasiveness	Resource acquisition, Dispersal	Biocontrol	Presence of both 4th instar larvae and adults increase efficiency in biocontrol of *Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae)	Cabral *et al.* 2009
		Invasibility	Intraguild predation – IG predator	Biocontrol	Parasitized aphids are inferior prey for larvae and may reduce intensity of IGP in the field and the negative impact IGP has on herbivore suppression	Bilu and Coll 2007, 2009
		Invasibility	Intraguild predation – IG predator	Biocontrol	Dominant over *Cydonia vicina nilotica* Mulsant, 1850 in shared habitats	Bayoumy and Michaud 2015
		Invasibility	Intraguild predation – IG prey	Biocontrol	Intraguild predation by *H. axyridis*	Felix and Soares 2004

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Appendix 1. Continued.

No.	Coccinelline name	Categorization	Factors/Traits	Introduction	Key effect/Biological notes	References
6.	*Coelophora inaequalis* (Fabricius, 1775)	Invasiveness	Resource acquisition	Biocontrol	Biocontrol of yellow sugarcane aphid, *Sipha flava* (Forbes, 1884) (Hemiptera: Aphididae), was not effective	Frank and McCoy 2007
		Invasiveness	Dispersal	Introduced	-	Maruyamai and Ohno 2011
7.	*Cryptognatha nodiceps* Marshall, 1912	Invasiveness	Resource acquisition	Biocontrol	Biocontrol of coconut scale, *Aspidiotus destructor* Signoret, 1869 (Hemiptera: Diaspididae)	Frank and McCoy 2007
		Invasiveness	Resource acquisition	Biocontrol	Fed and reproduced on Diaspididae and Coccidae; potential biocontrol agents of coconut scale *A. destructor*	Lopez *et al.* 2004
8.	*Curinus coeruleus* (Mulsant, 1850)	Invasiveness	Resource acquisition	Biocontrol	Biocontrol of Lepidoptera	Sheppard *et al.* 2004
9.	*Delphastus catalinae* (Horn, 1895)	Invasibility	Natural enemies – fungi	Introduced for Biocontrol	Biocontrol of *B. tabaci*	Wang *et al.* 2008
		Invasibility	Abiotic factors – temperature	Invasive/ Introduced	Biocontrol of *B. tabaci*	Simmons and Legaspi 2007
10.	*Harmonia axyridis* (Pallas, 1773)	Invasibility	Intraguild predation – IG predator	Invasive	IGP against *A. colemani*	Chacon *et al.* 2008
		Invasiveness	Resource acquisition	Native range	Generalist predator that preys on exotic pests in the presence of native preferred prey	Zhang *et al.* 2016
		Invasiveness	Dispersal	Deliberate	USA first release—1916 (first established population in 1988); Soviet Union first release—1927; Western Europe—1982	Orlova-Bienkowskaja 2013
		Invasiveness	Natural enemies	Native and invasive populations	Repeated and independent infection events by *Wolbachia pipientis* in *H. axyridis.*	Goryacheva *et al.* 2015
		Invasiveness	Physiology	Native range	Aggregations and invasion of human habitations as a behavioral adaptation for cold-avoidance	Wang *et al.* 2011
		Invasiveness	Defense mechanisms	Invasive	Microsporidia may contribute to the unpalatability of *H. axyridis* during IGP	Gegner *et al.* 2015
		Invasiveness, Invasibility, Stochastic events	Mating systems, rainfall and land use	Invasive	-	Comont *et al.* 2014a
		Invasibility	Intraguild predation – IG predator	Invasive/Feral	IGP against *A. bipunctata* and *Oenopia conglobata* (Linnaeus, 1758) (higher predation on *O. conglobata*)	Rondoni *et al.* 2015
		Invasiveness	Resource acquisition	Invasive	Biocontrol and adverse impacts as a household pest, fruit production, and threat to non-target organisms	Koch *et al.* 2006
		Invasibility	Intraguild predation	Introduced/ Invasive	Do not prey on eggs of native ladybird species in the field	Smith and Gardiner 2013

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Appendix 1. Continued.

No.	Coccinelline name	Categorization	Factors/Traits	Introduction	Key effect/Biological notes	References
		Invasiveness	Defense mechanisms	Introduced	A prominent cause of seasonal inhalant allergy	Goetz 2009
		Invasiveness	Resource acquisition	Biocontrol	Effective biological control of invasive species *M. pruinosa* in ornamental plants than by native ladybird species	Grozea *et al.* 2015
		Invasibility	Intraguild predation – IG predator	Biocontrol	Harmful IGP on both introduced and native ladybird species	Yasuda *et al.* 2004
		Invasibility	Intraguild predation – IG predator	Biocontrol	Biocontrol of aphid *M. persicae* and IGP on native ladybird species *E. connexa*	Mirande *et al.* 2015
		Invasibility	Intraguild predation – IG predator	Biocontrol	IGP of native ladybird species leading to declining populations	Snyder *et al.* 2004
		Invasiveness	Dispersal	Accidental	Invasive and undergoing range expansion, first record outside Croatia	Stankovic *et al.* 2011
		Invasiveness, Invasibility, Stochastic events	Resource acquisition, Fecundity, Phenotypic variation, Dispersal, Natural enemies, Abiotic factors, Disturbance, Climate change	Invasion	Review on invasiveness of *H. axyridis*, invasibility of introduced/invaded habitats and stochastic events; causes changes in invading/recipient coccinellid community	Roy *et al.* 2016
		Invasiveness	Resource acquisition	Biocontrol	Biocontrol of *E. servus* and *N. viridula* eggs	Tillman 2011
		Invasibility	Competitors	Biocontrol	Affect population abundance and/or could result in the competitive displacement of *C. undecimpunctata*	Soares and Serpa 2007
		Invasibility	Competitors	Biocontrol	May negatively affect *C. undecimpunctata* survival through resource competition	Nóia *et al.* 2008
		Invasibility	Intraguild predation – IG predator, Competitors	Invasive	IGP and resource competition with native ladybird species	Kenis *et al.* 2017
		Invasibility	Competitors	Invasive	Competitive displacement of native ladybird species; rapid biotic homogenization	Roy *et al.* 2012
		Invasiveness	Development times and fecundity	Invasive, Biocontrol and native	Faster development times and high reproduction capacity and adaptability to introduced/invaded area may increase invasiveness	Tayeh *et al.* 2015
11.	*Harmonia conformis* (Boisduval, 1835)	Invasiveness	Resource acquisition	Biocontrol	Biocontrol of tomato potato psyllid (TPP), *B. cockerelli*	MacDonald *et al.* 2016
		Invasiveness	Dispersal	Native range	Biocontrol of *C. bimaculata*	Baker *et al.* 2003
12.	*Harmonia dimidiata* (Fabricius, 1781)	Invasiveness	Resource acquisition	Accidental/ Immigration and failed introductions	Unknown (studies on prey range needed)	Frank and McCoy 2007

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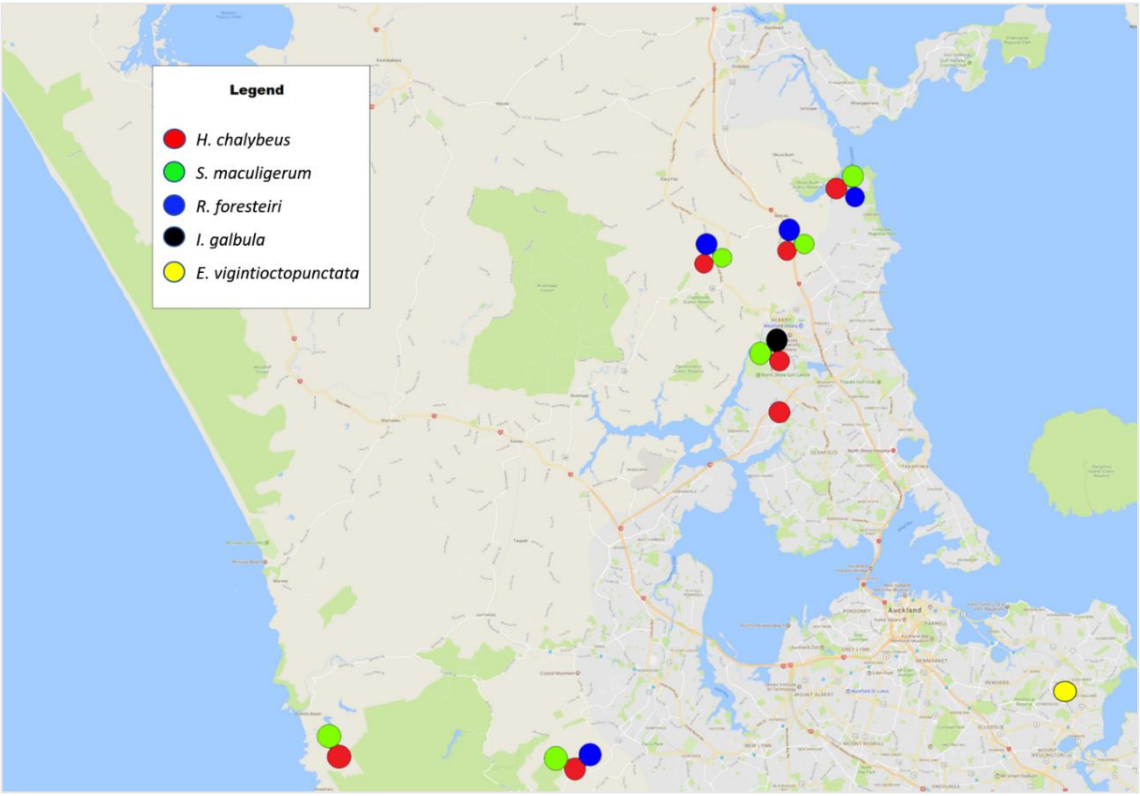
Appendix 1. Continued.

No.	Coccinelline name	Categorization	Factors/Traits	Introduction	Key effect/Biological notes	References
13.	*Hippodamia variegata* (Goeze, 1777)	Invasiveness	Resource acquisition, Dispersal	Unknown	Prey on aphids and psyllids on crops, weeds and ornamental plants; first record in Australia	Franzmann 2002
		Invasibility, Invasiveness	Competition, Intraguild predation, Cannibalism	Biocontrol	Biocontrol of aphids; may influence native species (*E. connexa*) decline	Grez *et al.* 2012
		Invasiveness	Dispersal	Unknown	Spread rapidly in Wisconsin in less than five years	Williams and Young 2009
		Invasibility	Abiotic factors – temperature	Biocontrol	Biocontrol of Russian wheat aphid	Michels *et al.* 1997
		Invasiveness	Dispersal	Biocontrol	Causing native ladybird population declines	Cormier *et al.* 2000
		Invasiveness	Genetic variability	Biocontrol	Genetic variability within and between populations of *H. variegata*	Sayed 2016
		Invasiveness	Resource acquisition	Native range	Biocontrol of common pistachio psylla (CPP), *Agonoscena pistaciae* Burckhardt and Lauterer, 1989 (Hemiptera: Aphalaridae)	Mehrnejad *et al.* 2011
14.	*Mada polluta* Mulsant, 1850	Invasiveness	Resource acquisition, Fecundity, Developmental times	Biocontrol	Biological control agent of *Tecoma stans* (L.) Juss. ex Kunth (Bignoniaceae)	Madire 2013
15.	*Novius cruentatus* Mulsant, 1846	Invasiveness	Resource acquisition	Biocontrol	A major contributor to the population reduction of *Palaeococcus fuscipennis* (Burmeister, 1835) (Hemiptera: Monophlebidae)	Mendel *et al.* 1998

Appendix B: Supplements for chapter 3



Supplementary figure 3.1 Geographic distribution of the five ladybird species along the field sites

Supplementary table 3.1 Details of sampling sites (habitat type and GPS coordinates)

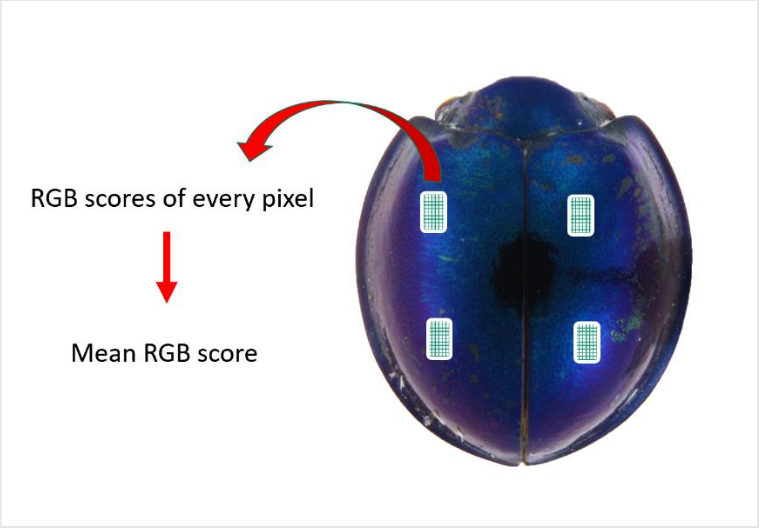
Site name	Habitat type*	Latitude	Longitude
Foley Quarry Road	Exotic	36.70455S	174.67023E
Glendhu Scenic Reserve	Native	36.76974S	174.71075E
Anawhata	Native	36.92103S	174.47015E
Ruru Reserve, West Coast Road	Native	36.92849S	174.59953E
Long Bay	Scrub	36.67330S	174.74518E
Lonely Track Road	Scrub	36.69912S	174.71628E
Rosedale Park North	Native	36.74757S	174.71001E
Landcare research premises	Exotic	36.883364S	174.848759E

*Definitions of habitat types as described by LINZ data services (URL: https://data.linz.govt.nz/license/attribution-3-0-new-zealand).

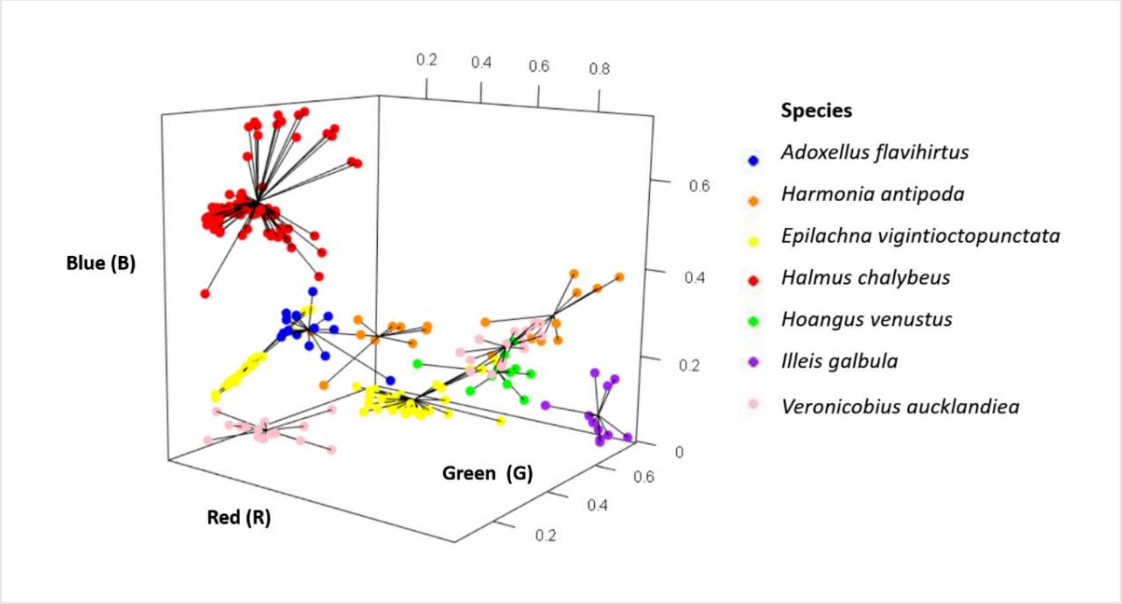
Scrub is a tract of land covered by vegetation less than 3m high

Native is a tract of land covered by trees native to New Zealand

Exotic is a tract of land covered by trees not native to New Zealand



Supplementary figure 3.2 Illustration of four colour patches selected on the elytra of a ladybird (*H. chalybeus*)



Supplementary figure 3.3 Distribution of RGB scores of colour patches of ladybird species.

Points in the graph indicate the RGB score for a colour of each individual. Black colour centroids are the patch specific centroid for each colour of each species. Lines connecting patch specific centroid and each point are distances that together indicate the variability of each colour in each species from the respective centroid in 3D space.

8.2. Appendix C: Supplements for chapter 4

PCA analysis

Supplementary table 4.1 Principal component loading scores and eigenvalues from a PCA of ladybird size variables

Size parameter*	PC1	PC2
Width of the pronotum	0.45	0.38
Width of the head	0.45	0.49
Distance between two points in the elytra	0.45	-0.01
Width of the body	0.44	-0.78
Average length of the elytra	0.45	-0.05
Eigen value	4.89	0.06
Variance explained	98.72%	1.27%

Extraction method: Principal Component Analysis using the NIPALS algorithm to accommodate missing values.
*Only **factor loading values >0.40** are considered to influence the principal components.

General activity

Mean velocity

Supplementary table 4.2 Results of asymptotic test to compare coefficients of variation of mean velocity between species

	Halmus chalybeus D_AD (p values)	*Illeis galbula* D_AD (p values)	*Rhyzobius foresteri* D_AD (p values)
Illeis galbula	0.04 (0.83)		
Rhyzobius foresteri	2.60 (0.11)	2.80 (0.09)	
Serangium maculigerum	3.03 (0.08)	3.32 (0.07)	0.02 (0.88)

Immediate response analysis

Supplementary table 4.3 Gamma hurdle model GLM for the change in behaviour in response to the simulated predator attack using *H. chalybeus* as the base species

Predictor	**Estimate**	**Std. Error**	**Z value**	**P**
Intercept	-0.51	1.11	-0.46	0.64
PC1	36.13	20.40	1.77	0.08
WPC1	-0.59	0.62	-0.95	0.34
Species *Illeis galbula*	-0.18	1.07	-0.17	0.87
Species *Rhyzobius foresteri*	-0.67	1.45	-0.46	0.64
Species *Serangium maculigerum*	3.19	3.96	0.81	0.42

Supplementary table 4.4 Results of asymptotic test to compare coefficients of variation of duration of behavioural changes to the simulated predator attack between species

	Halmus chalybeus D_AD (p value)	*Illeis galbula* D_AD (p value)	*Rhyzobius foresteri* D_AD (p value)
Illeis galbula	0.14 (0.71)		
Rhyzobius foresteri	0.01 (0.93)	0.07 (0.79)	
Serangium maculigerum	0.48 (0.49)	0.02 (0.89)	0.24 (0.63)

8.3. Appendix D: Supplements for chapter 5

Supplementary table 5.1 Details of sampling sites (habitat type and GPS coordinates)

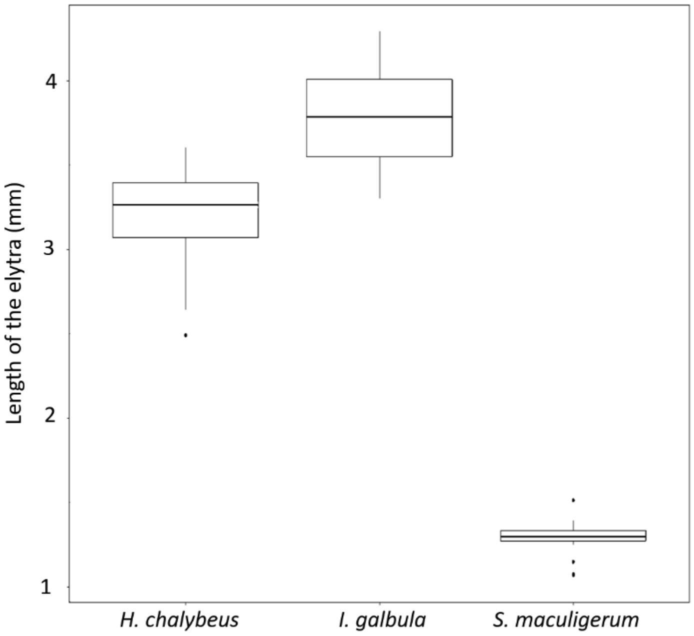
Site	**Habitat type***	**Latitude**	**Longitude**
Long Bay	Scrub	36.67330S	174.74518E
Rosedale Park North	Native	36.74757S	174.71628E
Massey University Albany campus	Native	36.73488S	174.69568E
Auckland Botanical Gardens	Scrub	37.00790S	174.90615E

*Definitions of habitat types as described by LINZ data services (URL: https://data.linz.govt.nz/license/attribution-3-0-new-zealand).

Scrub is a tract of land covered by vegetation less than 3m high

Native is a tract of land covered by trees native to New Zealand

Exotic is a tract of land covered by trees not native to New Zealand



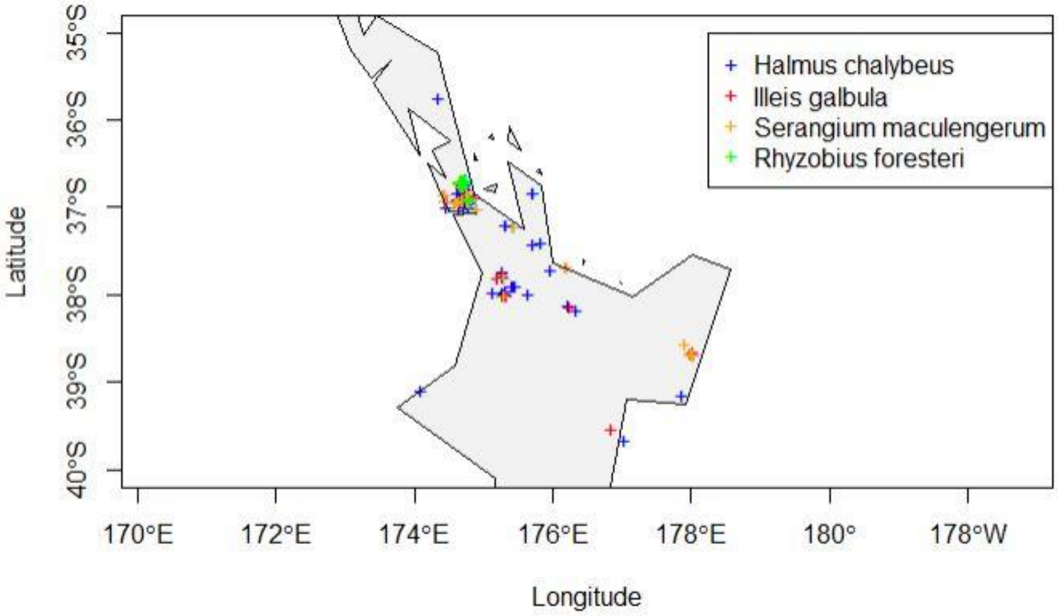
Supplementary figure 5.1 Length of the elytra (median ± IQ values) of each ladybird species

Supplementary table 5.2 Pairwise comparisons using t tests with pooled SD (P value adjustment method: BH) to compare elytra lengths between species

p values	*H. chalybeus*	*I. galbula*
I. galbula	<2e-16	
S. maculigerum	<2e-16	<2e-16

Supplementary table 5.3 One-way ANOVA and post-hoc paired t-tests (p value adjusted using Benjamini-Hochberg method) to compare elytra lengths between species

	Df	**Sum sq.**	**Mean sq.**	**F value**	**p**
Species	2	102.66	51.33	885	<2e-16
Residuals	106	6.15	0.06		



Supplementary figure 5.2 Distribution of four introduced species along the North Island of New Zealand (Data obtained from iNaturalist database)

8.4. Appendix E: Supplements for chapter 6

Supplementary table 6.1 Geographical and collection data on specimens collected in New Zealand used in this study

family	subfamily	species	origin (native/ introduced)	Log Code*	Area code †	locality	Date collected	method	DNA code
Coccinellidae	Coccinellini	*Coelophora inaequdlis*	introduced	AW001	AK	Bayview	25/12/2017	hand	COC001
Coccinellidae	Coccidulini	*Cryptolaemus montrouzieri*	introduced	HE008	AK	Glendhu scenic reserve	2/02/2018	beating	COC002
Coccinellidae	Coccinellini	*Cleobora mellyi*	introduced	HE010	AK	Ruru reserve	2/03/2018	beating	COC003
Coccinellidae	Coccidulini	*Rhyzobius*		HE027	AK	Albany	24/04/2018	hand	COC004
Coccinellidae	Chilocorini	*Halmus chalybeus*	introduced	HE027	AK	Albany	24/04/2018	hand	COC005
Coccinellidae	Coccidulini	*Rhyzobius*		HE018	AK	Long Bay	27/01/2018	beating	COC006
Coccinellidae	Coccidulini	*Adoxellus flavihirtus*	native	HE010	AK	Ruru Reserve	2/03/2018	beating	COC007
Coccinellidae	Coccinellini	*Harmonia antipoda*	native	AW001	AK	Bayview	4/03/2018	hand	COC008
Coccinellidae	Coccidulini	*Rhyzobius*		HE018	AK	Long Bay	27/01/2018	beating	COC009
Coccinellidae	Serangiini	*Serangium maculigerum*	introduced	HE010	AK	Ruru Reserve	2/03/2018	beating	COC010
Coccinellidae	Coccidulini	*Rhyzobius*		RL1766	NC	Craigieburn Recreation Area	20/01/2014	night beating	COC011

Coccinellidae	Coccidulini	*Veronicobius*	native	CA077		Campbell island			COC012
Coccinellidae	Coccidulini	*Rhyzobius minutulus*	native		AK	Kepu bush	20/04/2018		COC013
Coccinellidae	Diomini	*Diomus*	introduced	RL1991	CO	Lindis Pass	13/12/2017	sifting tussock litter	COC014
Coccinellidae	Coccidulini	*Rhyzobius rarus*	native	TB523	SD	Ruston Winch	13/03/2011	sifting leaf litter	COC015
Coccinellidae	Coccidulini	*Rhyzobius*		RL1049	OL	Mt Iron	11/01/2006	night beating	COC016
Coccinellidae	Coccidulini	*Rhyzobius*		TH036	TH	Threekings	9/11/2008	beating	COC017
Coccinellidae	Epilachnini	*Epilachna vigintioctopunctata*	introduced	HE028	AK	Landcare site	11/05/2018	hand	COC018
Coccinellidae	Coccinellini	*Illeis galbula*	introduced		AK	Auckland on silver beet	1/03/2004		COC019
Coccinellidae	Coccidulini	*Rhyzobius species 24*		RL458	BR	Lake Rotoiti	13/11/1999		COC020
Coccinellidae	Coccidulini	*Rhyzobius species 8*		RL1741	WD	Okarito, Trig Walk	17/01/2014	beating	COC021
Coccinellidae	Coccidulini	*Rhyzobius species 18*		TB630	SD	Long Island camp track	23/11/2011	sifting leaf litter	COC022
Coccinellidae	Coccidulini	*Rhyzobius species 25*		TB426	MB	Jacks Pass	9/01/2011	beating at night	COC023
Coccinellidae	Coccidulini	*Rhyzobius species 5*		RL1551	ND	Puketi forest	16/03/2011	fogging fallen dead	COC024
Coccinellidae	Coccidulini	*Rhyzobius species 4*		RL1754	WD	Chancellor hut	18/01/2014		COC025

Coccinellidae	Coccidulini	*Rhyzobius species 3*		RL1793	CO	Mt Benger nr summit	24/01/2014	sifting tussock litter	COC026
Coccinellidae	Coccidulini	*Rhyzobius species 2*		RL1605	KA	Okiwi bay	2012		COC027
Coccinellidae	Coccidulini	*Rhyzobius species 1*		RL1594	ND	Pukenui Forest	1/12/2011	beating	COC028
Coccinellidae	Coccidulini	*Rhyzobius species 20*		RL458	BR	Lake Rotoiti	13/11/1999		COC029
Coccinellidae	Coccidulini	*Apolinus lividigaster*	introduced			Rich's house	19/05/2018		COC030
Coccinellidae	Coccidulini	*Rhyzobius species 42_pink*							COC031
Coccinellidae	Coccinellini	*Harmonia axyridis*	invasive						COC032

*Log code is code indicated in the New Zealand Arthropod collection records for the specimens used in this study

†Area code is as indicated in (Crosby, Dugdale & Watt, 1998)

Supplementary table 6.2 Names, sequences and references of the primers used.

Gene	Primer	Sequence (5' – 3')	Reference
28S	28S ff	TTACACACTCCTTAGCGGAT	(Szawaryn, Bocak, Slipinski *et al.*, 2015)
	28S dd	GGGACCCGTCTTGAAACAC	
COI	Cl-J-1718	GGAGGATTTGGAAATTGATTAGTTCC	(Simon, Frati, Beckenbach *et al.*, 1994)
	TL2-N-3014	TCCAATGCACTAATCTGCCATATTA	

Supplementary table 6.3 Accession number, Guanine-Cytosine (GC) content and sequence lengths (seq. length) of COI and 28s sequences obtained from GeneBank used in the phylogenetic analysis

	COI			**28S**		
Name	**%GC**	**Accession number**	**Seq. Length**	**%GC**	**Accession number**	**Seq. Length**
Adalia decempunctata	32.60%	AJ313069	610	54.20%	FJ621324	288
Amida	28.80%	JF763619	650	54.70%	KP123118	633
Aphanocephalus	25.80%	KP829617	1239	49.60%	EU145687	768
Bucolus fourneti	27.00%	KP829612	1050	53.00%	FJ687745	755
Bulaea anceps	29.10%	KP829540	1218	53.90%	FJ687708	675
Calvia muiri	27.60%	GU073890	764	56.10%	GU073733	305
Cephaloscymnus	27.80%	JF763627	650	54.10%	KP829282	586

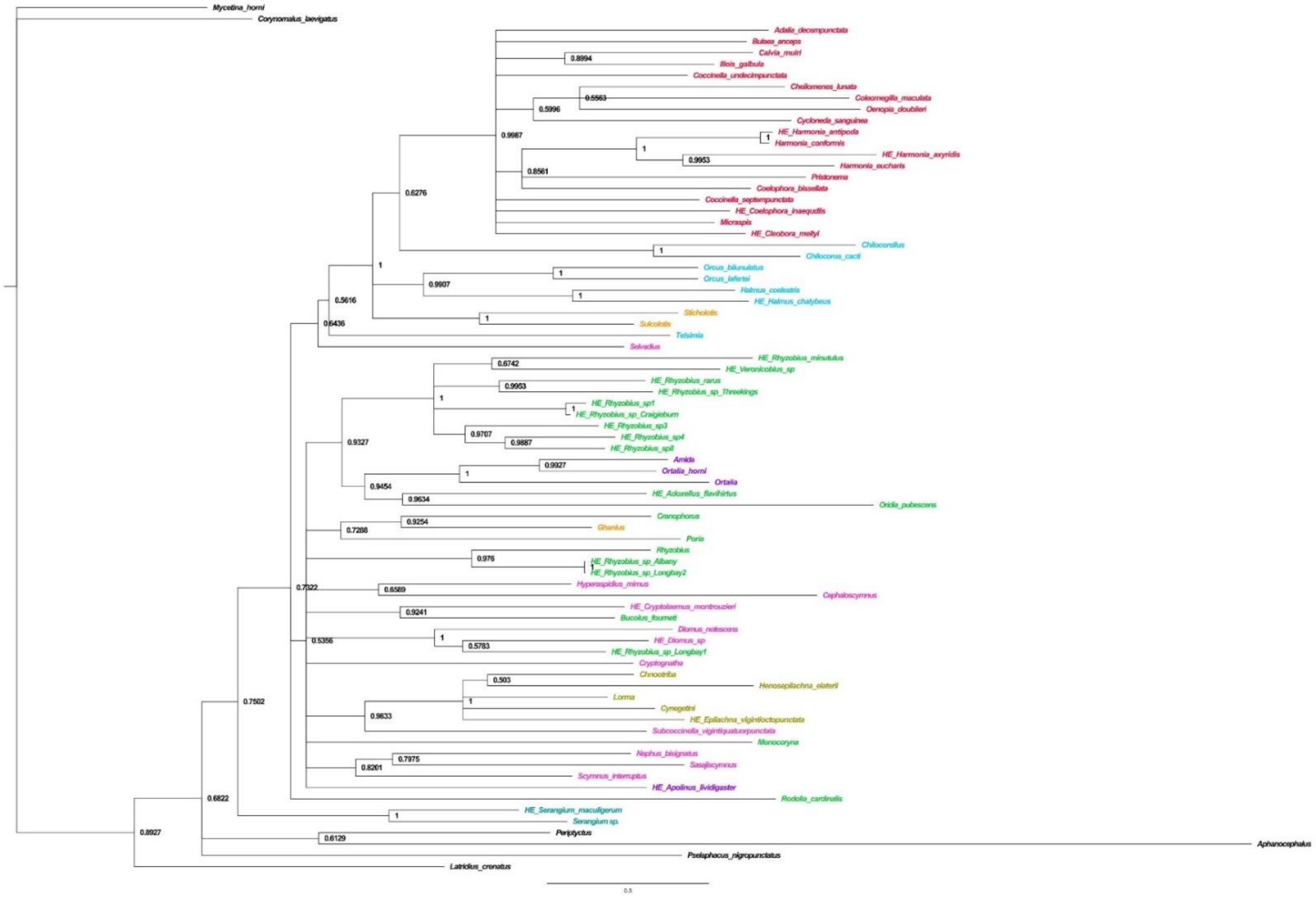
Cheilomenes lunata	27.00%	GU073891	764	54.60%	GU073734	293
Chilocorellus	24.80%	JF763628	650	54.00%	JF763540	561
Chilocorus cacti	26.90%	KP829574	1230	51.70%	KP419400	543
Chnootriba	28.90%	JF763631	650	55.10%	KP123160	633
Coccinella septempunctata	30.20%	JX508173	668	53.10%	DQ202668	639
Coccinella undecimpunctata	29.20%	KT445849	514	54.50%	FJ621327	288
Coelophora bissellata	29.50%	KP829578	1230	51.90%	KP829295	753
Coleomegilla maculata	28.30%	MF152799	750	51.40%	FJ687713	751
Corynomalus laevigatus	29.10%	EU164679	820	53.10%	EU164646	755
Cranophorus	28.00%	JF763635	650	53.30%	FJ687710	754
Cryptognatha	27.50%	JF763636	648	52.50%	JF763548	459
Cycloneda sanguinea	29.30%	KX755335.COX1	1311	52.50%	FJ687723	752
Cynegetini	31.20%	KP829570	1230	52.80%	KP829291	754
Diomus notescens	30.70%	KP829609	1230	53.20%	FJ687744	756
Ghanius	27.80%	JF763648	650	52.30%	KP829276	774
Halmus coelestris	29.30%	KP829577	1230	52.10%	FJ687728	756
Harmonia conformis	27.20%	MF152805	765	53.80%	GU073741	305
Harmonia eucharis	31.10%	KP829575	1197	52.10%	EU145672	752
Henosepilachna elaterii	30.40%	KP123237	773	54.20%	KP123121	633

Hyperaspidius mimus	28.00%	KP829571	1230	53.00%	FJ687725	756
Illeis galbula	29.70%	MF280634.1	827	52.20%	FJ687722.1	752
Latridius crenatus	33.20%	EU164685	820	52.30%	EU164654	765
Lorma	28.40%	KP123306	799	54.50%	KP123191	633
Micraspis	29.60%	KF022225	986	51.90%	FJ687720	749
Monocoryna	24.50%	JF763662	650	54.00%	JF763576	448
Mycetina horni	30.20%	GQ302320	1257	53.10%	EU145699	754
Nephus bisignatus	30.10%	GU073909	764	55.40%	GU073759	307
Oenopia doublieri	30.80%	GU073900	601	52.90%	GU073746	306
Orcus bilunulatus	29.90%	KP829604	1212	52.80%	FJ687740	755
Orcus lafertei	27.00%	KP829582	1230	52.80%	FJ687730	755
Oridia pubescens	30.80%	KP829593	1197	53.10%	FJ687734	754
Ortalia	28.40%	KP123333	803	54.80%	KP123218	633
Ortalia horni	29.30%	KP829579	1230	54.40%	KP829296	754
Periptyctus	30.60%	JX845096	1215	51.60%	JX845024	768
Poria	27.20%	JF763671	650	53.40%	FJ687733	753
Pristonema	27.30%	KP829539	1218	53.50%	FJ687706	715
Pselaphacus nigropunctatus	30.40%	EU164678	816	52.60%	EU164657	770
Rhyzobius	28.20%	MF152819	738	52.80%	KP829306	754

Rhyzobius lophanthae				52.50%	FJ687716	753
Rodolia cardinalis	27.00%	GU073916	764	56.00%	GU073776	309
Sasajiscymnus	32.70%	KP829580	1230	53.10%	EU145674	754
Scymnus interruptus	27.40%	GU073911	591	54.50%	GU073763	308
Scymnus loewii				54.90%	MK748238.1	366
Selvadius	28.30%	KP829607	1218	55.50%	JF763608	447
Serangium sp.	28.50%	KP829591.1	1218	52.70%	FJ687731.1	746
Sticholotis	26.90%	KP123325	803	53.10%	EU145673	754
Subcoccinella vigintiquatuorpunctata	30.40%	KP123326	803	54.80%	KP123211	635
Sulcolotis	29.50%	KP829613	1230	52.50%	FJ687746	754
Telsimia	28.90%	JF763694	650	52.90%	FJ687738	754

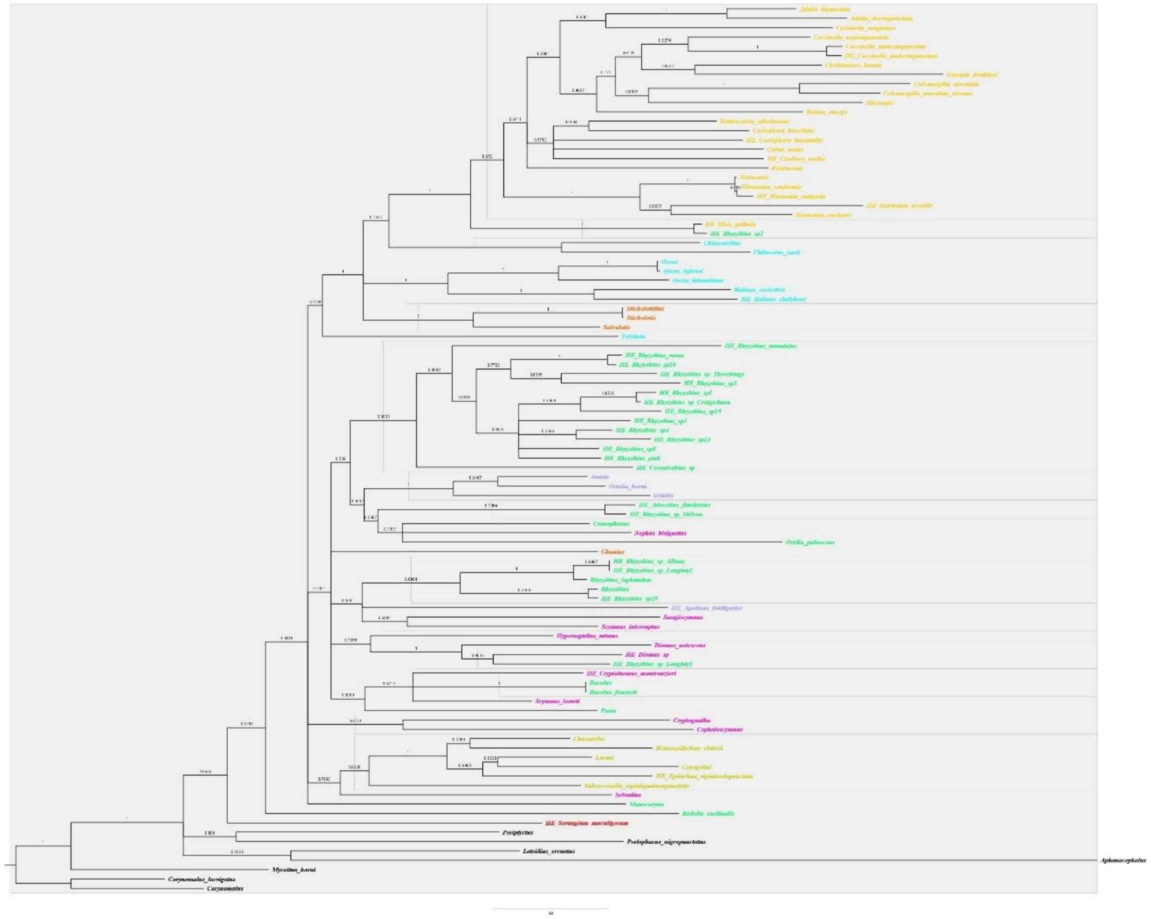
Supplementary table 6.4 BLAST output for 28S nucleotide sequence for *Illeis galbula* specimen collected from New Zealand

Name	**#Nucleotides**	**%Identical Sites**	**%Pairwise Identity**	**%GC**	**Accession**	**Bit-Score**	**Database**	**Grade**	**Organism**	**Query**
FJ687716	1128	99.50%	99.50%	52.70%	FJ687716	1026.01	nr	99.70%	Rhyzobius lophantae	HE_Illeis_galbula
FJ687741	1128	98.80%	98.80%	52.30%	FJ687741	1003.85	nr	99.40%	Rhyzobius sp.	HE_Illeis_galbula
KP123203	1129	97.70%	97.70%	54.20%	KP123203	970.611	nr	98.80%	Afissula sp.	HE_Illeis_galbula
KP123186	1129	97.70%	97.70%	54.20%	KP123186	970.611	nr	98.80%	Epilachna plicata	HE_Illeis_galbula
KP123182	1129	97.70%	97.70%	54.20%	KP123182	970.611	nr	98.90%	Afissula expansa	HE_Illeis_galbula
KP829278	1131	97.50%	97.50%	53.40%	KP829278	966.918	nr	98.80%	Nothocolus sp.	HE_Illeis_galbula
KP123189	1129	97.50%	97.50%	54.50%	KP123189	965.071	nr	98.80%	Epilachna sp.	HE_Illeis_galbula
KP123181	1129	97.50%	97.50%	54.20%	KP123181	965.071	nr	98.80%	Epilachna anhweiana	HE_Illeis_galbula
KP123219	1129	97.30%	97.30%	54.20%	KP123219	959.531	nr	98.70%	Epilachna flavicollis	HE_Illeis_galbula



Supplementary figure 6.1 Phylogenetic relationships among 77 Coccinellidae species inferred from mitochondrial (COI) sequences excluding *Illeis galbula* sample collected from New Zealand. One invasive, ten introduced and five native species collected in New Zealand are included in this phylogenetic analysis. Numbers at nodes are Bayesian posterior probabilities (PP) of the dataset.

Supplementary figure 6.2 Phylogenetic relationships among 88 Coccinellidae species inferred from nuclear (28s) sequences including *Illeis galbula* sample collected from New Zealand. One invasive, ten introduced and five native species collected in New Zealand are included in this phylogenetic analysis. Numbers at nodes are Bayesian posterior probabilities (PP) of the dataset.



Supplementary figure 6.3 Phylogenetic relationships among 95 Coccinellidae species inferred from mitochondrial (COI) and nuclear (28s) sequences including *Illeis galbula* sample collected from New Zealand. One invasive, ten introduced and five native species collected in New Zealand are included in this phylogenetic analysis. Numbers at nodes are Bayesian posterior probabilities (PP) of the dataset.

References

Crosby, T., J. Dugdale, and J. Watt. 1998. Area codes for recording specimen localities in the New Zealand subregion. New Zealand Journal of Zoology 25: 175-183.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America 87: 651-701.

Szawaryn, K., L. Bocak, A. Slipinski, H. E. Escalona, and W. Tomaszewska. 2015. Phylogeny and evolution of phytophagous ladybird beetles (Coleoptera: Coccinellidae: Epilachnini), with recognition of new genera. Systematic Entomology 40: 547-569.