Chapter 7

General discussion

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Parasitoids must find, attack and lay eggs in or on hosts to provide their offspring with all the resources for their development. This entire process of parasitization is key for understanding and predicting their ecology and evolution, because it directly influences the parasitoids’ fitness and ecological niche (Godfray, 1994; Bailey et al., 2009). As such, parasitoids can have large ecological consequences by reducing population size of the host, which in return can have cascading effects via direct and indirect interactions on other species and influence the structure and dynamics of ecological species networks (Hassell & Waage, 1984; Thierry, Hrček, & Lewis, 2019). The parasitization process also determines the efficiency of parasitoids to control insect populations that are agricultural pests and is therefore important from a pest management perspective. Hence, the study of parasitization aids understanding and predicting the evolutionary ecology of parasitoids and can be important for development of biological pest control strategies. The parasitoids’ dependence on the host for their reproduction, however, also makes them vulnerable to changes in host species diversity and abundance. Parasitoids therefore cannot be understood in isolation, but only by their interaction with their hosts and the environmental factors they encounter.

In this thesis I investigated the evolutionary ecology of parasitization to establish how native parasitoids respond to a novel invasive host and to apply this knowledge for improvement of biological pest control. I combined experimental and computational methods integrating behavioural biology, quantitative genetics, microbiology and evolutionary biology of native parasitoids in response to a new potential host, the invasive pest Drosophila suzukii.
Chapter 7 | General discussion

**Linking evolutionary ecology and biological pest control**

The establishment and spread of exotic species outside their native range can be facilitated by a release from natural enemies. Even though the exotic species may encounter generalist natural enemies in the invaded range that could potentially exploit the exotic species, these enemies did not co-evolve with the invader, which may make them inefficient or even incompatible. Interestingly, native natural enemies in the invaded range might exhibit natural genetic variation in traits that influence exploitation of the invader e.g., their willingness and efficiency to find and attack the novel species. Consequently, although the immediate performance of natural enemies in the invaded area may be inefficient, evolution might occur towards higher exploitation efficiency when there is heritable genetic variation in traits relevant for exploitation of the invader.

Presence of genetic variation for parasitization behaviour and performance can be used for development of biological pest control strategies. For the effective exploitation of this variation, however, insight is needed in the fundamental biology of the natural enemy, the pest and the ecological conditions of the target area for release. We therefore proposed a four-step approach to investigate the potential of natural enemies such as parasitoids to control (invasive) pests, and to design a method to optimize their efficiency for augmentative biocontrol based on utilization of natural variation within and between species (Figure 2, Chapter 3). This four-step approach comprises the determination of variation (1) between species to choose the most promising candidate able to parasitize the pest. When the candidate species exhibits suboptimal performance, the next steps are to determine (2) variation within species in parasitization traits, and assess the (3) genetic and environmental factors that shape phenotypic variation to predict the efficiency in the target area for biological control and to (4) design a breeding program to further exploit variation to optimize their performance. Presence of heritable variation allows for the selection of the best genotypes to optimize performance for controlling the pest. Artificial selection in particular can speed up and direct evolution by selecting for lower or higher values in those traits that could be useful in the biocontrol practises.

Following the proposed four-step approach, I investigated the potential of this method for optimization of parasitoids for controlling *D. suzukii*. These results, together with an individual-based model study, allowed me to examine how native natural enemies in the invaded range might respond to invasive species by evolution.

**Parasitoids vary in their ability to parasitize *D. suzukii*: inter-specific variation**

Parasitoid species show large variation in the range of hosts species they can use for reproduction. The ability to exploit a novel host, such as *D. suzukii*, depends on the parasitoids’ parasitization strategy. Pre-requirements to include a novel host into their repertoire are the ability to localize the host, to oviposit, and to circumvent host resistance for successful offspring development (Chapter 3, Fig 1). Studies on host resistance mechanisms towards parasitoids have shown that the host immunological defensive barrier is an important factor restricting parasitoids to use a wide range of hosts. Survival of immature fruit flies mainly relies on their ability to form a capsule around the wasp egg to
sequester it and prevent it from developing (Carton & Nappi, 1997). These are formed by specialized circulating cells called haemocytes (Russo, Dupas, Frey, Carton, & Brehelin, 1996; Lavine & Strand, 2002). Interestingly, *Drosophila* species and populations exhibit large variation in haemocyte load (Eslin & Prevost, 1996; Gerritsma et al., 2013), and *D. suzukii* has a relative high constitutive number of circulating haemocytes enabling them to readily kill parasitoid eggs (Kacsoh & Schlenke, 2012; Poyet et al., 2013). Hence, the ability to circumvent the host encapsulation response is a crucial factor for establishment of a novel *D. suzukii*-parasitoid interaction.

We reviewed all known parasitoid species that have been found and tested in field and laboratory studies, and that occur in the invaded area of *D. suzukii* in Europe and North America. Our analysis revealed that from the 17 investigated parasitoid species, only 3 were able to use *D. suzukii* for reproduction (Kruitwagen et al., 2018). Among them are two pupal parasitoids, *Trichopria drosophilae* and *Pachycrepoideus vindemmiae*, and one larval parasitoid *Leptopilina heterotoma*. Interestingly, whereas the first species is rarely reported in temperate regions of Europe, the latter two parasitoid species were also found during field work at soft fruit farmers in the surroundings of Wageningen in the Netherlands in 2016 (unpublished results, A. Kruitwagen, A. Panel and J. Alkema). Pupae of *D. suzukii* have no/limited resistance against parasitoids and are thus more vulnerable to parasitization than the larval stage. The wide host-range of *P. vindemmiae* and their ability to parasitize other parasitoids (i.e., to hyperparasitize), however, makes this species not a good candidate for biological control due to high risk of non-target effects. We therefore focused on *L. heterotoma* to further investigate its potential to adapt to *D. suzukii* and be used as biocontrol agent.

**Parasitoids vary in their ability to parasitize *D. suzukii*: intra-specific variation**

Following the step-wise approach proposed in Chapter 2, I next estimated the amount of intra-specific variation in *L. heterotoma* to parasitize *D. suzukii*. I designed a standardized parasitization performance test and examined seven European *L. heterotoma* populations. In line with previous studies (Chabert et al., 2012; Kacsoh & Schlenke, 2012; Mazzetto et al., 2016; Stacconi et al., 2017; Iacovone et al., 2018), parasitoids were unable to circumvent host resistance and exploit *D. suzukii* for reproduction: of the 2070 fly hosts that were exposed to parasitoids, only five yielded offspring. Interestingly, parasitoids were able to attack and kill *D. suzukii*, and attack and host-killing rate varied significantly between populations, indicating intra-specific genetic variation (Chapter 3). This parasitoid-induced host mortality without reproduction is thus an additional source of host mortality, next to host mortality due to offspring development. As such, these results show that *L. heterotoma* could influence population growth of *D. suzukii* in their invaded range. We hypothesized that this can therefore be an interesting target trait for biological control.

We limited our research on the direct effects of parasitoid attack on host survival. Yet, other fitness consequences could also contribute to the impact of native natural enemies on invasive pests. For example, *Drosophila* that survived parasitoid attack but carry an encapsuled wasp egg can suffer reduced fecundity (Fellowes, Kraaijeveld, & Godfray, 1999) and higher vulnerability to desiccation and starvation (Hoang, 2001). To further
assess the impact of native larval parasitoids on the invasive host population, one could focus on sublethal effects next to host-killing: to what extent do flies that survived parasitoid attack suffer? This could be done by testing the fitness consequences of capsule bearing flies by comparing the survival, reproduction and dispersal abilities of flies with and without an encapsulated wasp egg.

**Causes of variation in parasitization in L. heterotoma**

In Chapter 3, I reported large intra-specific variation in various traits involved in parasitization. For evolution to occur, however, at least part of that variation needs to have a genetic basis that selection can act upon. Hence, the third step to investigate the evolutionarily potential of the parasitoids is to answer the question, “what causes the large phenotypic variation in parasitization?” (Chapter 2). To answer this question, I conducted two experiments focussing on genetic and environmental factors.

First, I crossed seven populations to form a genetically variable population and performed a half-sib analysis to measure two indices of genetic variation: additive genetic variance ($V_a$) and heritability ($V_a/V_{total}$) of different traits that determine the outcome of parasitization (Figure 1, Chapter 3). These traits include (1) attack rate, the parasitoids’ ability and willingness to find and exploit hosts, (2) killing rate, the parasitoids’ ability to reduce the survival rate of the attacked hosts, (3) lethal attack rate, the efficiency by which the attacked hosts are killed, and (4) successful parasitism, the parasitoids’ ability to circumvent host resistance for offspring emergence, thus whether host killing results in parasitoid offspring. Using a Bayesian animal model and pedigree data describing all known relationships, I found that the attack and killing rate had a heritability $h^2 = 0.2$, lethal attack rate had a $h^2 = 0.4$ and successful parasitism $h^2 = 0.0$. Although multiple studies have estimated genetic variation in traits that influence parasitism (Cronin & Strong, 1996; Olson & Andow, 2002; Henry et al., 2010), this study is one of the first to explicitly estimate and compare the heritability of different traits that influence the final outcome of parasitization, and to show genetic variation in non-reproductive host killing. The results of this study provided evidence that selective breeding can be used to improve traits in *L. heterotoma* that enhance their potential as biocontrol agents (step 3, Chapter 2), in particular through increased efficiency of non-reproductive host killing (Chapter 4). It also inspired the modelling study to predict how non-reproductive host killing influences host-range evolution (Chapter 6).

The quantitative genetic analysis showed that heritability of host-killing was $h^2 = 0.2$ (Chapter 3), indicating that this trait is also for a large part influenced by non-genetic factors. Therefore, we tested the influence of two environmental factors known to influence parasitoids’ fitness: (1) the developmental host quality by manipulation of host nutrition and (2) the endosymbiont *Wolbachia*, releasing the parasitoid from the physiological costs of carrying the endosymbiont.

By providing parasitoids with different quality hosts, we demonstrated that the developmental host has a significant influence on the parasitoids’ phenotype: wasps cultured on relatively low-quality hosts produced fewer offspring, these offspring were
smaller and showed a lower killing rate of *D. suzukii*. This study thus confirmed that the often-reported positive relationship between size and fitness in insects can also be applied to non-reproductive host killing. It should be noted, though, that killing without reproduction is not a direct measure of evolutionary fitness (see also below). Nonetheless, manipulation of developmental hosts could be used to develop more efficient biocontrol agents by using (mass-)rearing techniques increasing the quality of hosts for parasitoid development. Moreover, these results indicate that variation in host quality might be an important factor inducing plastic changes in the parasitoids’ phenotype in the field. Parasitoids for example encounter hosts that occur in different micro-habitats (e.g., climatic conditions, resources) influencing their ‘quality’ for the parasitoid. Hence, such variation within hosts may sustain intra-specific variation at the higher trophic level, i.e., the parasitoids.

Host-induced changes in the magnitude of non-reproductive host killing may also influence host-range evolution in parasitoids. The model presented in Chapter 6 shows that the likelihood that parasitoids will adapt to a novel initial unsuitable host, such as *D. suzukii*, is influenced by the magnitude of non-reproductive host killing. If, for example, a genotype appears in a parasitoid population that is able to exploit *D. suzukii* by exhibiting non-reproductive host killing, this genotype has a low chance to ‘invade’ the parasitoid population. In other words, killing unsuitable hosts can hinder specialisation on a novel host, resulting in host-range conservation (Chapter 6). On the other hand, host-induced plastic changes might in theory also increase the parasitoids’ success in a novel habitat, creating conditions for adaptation. This could occur for instance when parasitoids developing in ‘high quality hosts’ show an increased likelihood to successfully reproduce on *D. suzukii*. The results presented in this thesis indicate however that this scenario is unlikely to occur in *L. heterotoma*.

Three successive generations of heat-shock treatment did not remove *Wolbachia* in *L. heterototoma*, indicating that *Wolbachia*-parasitoid interaction is tolerant to heat stress. This makes it impossible to infer whether or not *Wolbachia* infection contributes to the phenotypic variation observed in the investigated parasitization traits, and whether it reduces the wasp’s ability to parasitize *D. suzukii* successfully. To further test the influence of *Wolbachia* on the parasitoids’ fitness, antibiotics could be used as an alternative method to remove the endosymbiont. This would be interesting as studies have shown that *L. heterototoma* has reduced fitness when they harbour *Wolbachia* (Fleury et al., 2000; Fytrou et al., 2006), suggesting that this bacterium might be an important factor hampering *L. heterototoma*’s success to parasitize *D. suzukii* and its efficiency as biocontrol agent.

From an ecological perspective, this study suggests that heat-tolerance, next to reproductive manipulation (cytoplasmatic incompatibility)(Mouton et al., 2005), might enable *Wolbachia* to persist in *L. heterototoma* populations. Yet, multiple factors can influence the response of the *Wolbachia - L. heterototoma* association to temperature, such as the parasitoids’ genotype and microbiome and the temperature “regime”, e.g., low or high temperature and exposure time (Mouton et al., 2007; Ross et al., 2017). To further investigate whether and how the *Wolbachia - L. heterototoma* interaction is influenced by
temperature, the next step would be to test different parasitoid strains over a large range of temperature regimes and measure Wolbachia titer and fitness-related traits. This will give more insight in the parasitoids’ ecology under different climatic conditions and how it may respond to global warming.

The results presented in Chapter 3 and 4 show that variation in the final outcome of parasitization is influenced by both genetic and environmental factors. Other sources of variation that could be important to understand the mechanistic basis of (non-)reproductive host killing are the parasitoids’ venom composition and quantity (Colinet et al., 2013; Cavigliasso et al., 2019), the parasitoids’ response rate to host(habitat) cues (Wiskerke, Dicke, & Vet, 1993; Vet et al., 1995; Wertheim, Vet, & Dicke, 2003) and experience of host finding and host preference (Papaj & Vet, 1990; Vet et al., 1995). This can give more insight in the fundamental aspects of parasitoid biology, such as what determines host specificity, and it can help to improve efficiency of parasitoids as biocontrol agents.

Artificial selection for non-reproductive host killing in *L. heterotoma* on *D. suzukii*

Parasitoids that kill hosts without getting the benefit from offspring development have less time and fewer eggs left for finding and parasitizing suitable hosts. Non-reproductive host killing is therefore clearly a maladaptive trait and is not expected to be selected and evolve in nature. Yet, as it (1) augments the parasitoids’ ability to control host population sizes and (2) it is influenced by genetic factors (Chapter 3), we hypothesized that we could artificially select on host-killing performance for optimization of parasitoids for biocontrol (step 4, Chapter 4). There are several ways of studying how to improve host-killing performance. For example, one could choose the best strain(s) available for their biocontrol efficiency or apply a “quick and dirty” experimental evolution approach by placement of parasitoid and *D. suzukii* populations together and just selecting the parasitoids able to reproduce each generation or follow the parasitoids dynamics over several generations without interference to see whether and which parasitoids will be selected (Tom Groot, personal communication; Lommen et al., 2017; Kruitwagen et al., 2018). There are several reasons why we chose for artificial selection on non-reproductive host killing instead. Firstly, by artificially selecting on host-killing we might select on a suite of underlying traits. For example, we might indirectly also improve the wasps’ search efficiency, host-preference and offspring survival. Secondly, crossing of different genotypes might result in new allelic combinations at various loci, enabling to substantially improve host-killing and circumvent host resistance. Thirdly, artificial selection also gives insight in the fundamental aspects of host-range evolution and establishment of novel host-parasitoid relationships. Although these factors are also a good argument for running experimental evolution, it has to be taken into account that experimental evolution is complex when two species interact over multiple generations to ensure their survival and thus host and parasitoid population persistence. As humans (the experimenters) are the selective agents in artificial selection, the strength and consistence of selection can be controlled. This is required in particular in this system due to low of variation and near zero heritability of offspring survival in *D. suzukii* (Chapter 3). Hence, if one would attempt to run experimental evolution, the parasitoid population size would be reduced significantly after already one generation,
losing any potential beneficial alleles quickly. This makes it almost impossible to establish a self-sustaining host-parasitoid system in which parasitoid evolution can take place. Hence, we tested whether killing rate could be improved by artificial selection as a proof of principle.

In Chapter 4, we attempted to improve parasitization traits by selective breeding for biological control. Despite additive genetic variance in killing rate (Chapter 3), we found a low and inconsistent response to selection indicating that phenotypic change might also have occurred due to drift rather than selection. Moreover, realized heritability was 0.17 after four generations, but zero after seven generations of selection. We concluded that non-reproductive host killing is sensitive to the environmental conditions in the laboratory, and in our artificial selection experiment, it was most likely affected by improving D. suzukii fitness over the course of the experiment. As such, presence of genetic variation does not guarantee a response to selection, even under fairly strong selection. Interestingly, we did find a consistent and strong correlative response in the attack rate (15% improvement relative to control lines), suggesting that evolution did occur and that this trait was less influenced by environmental factors.

Selection on host-killing did not improve successful parasitism, confirming a lack of genetic variation in offspring developmental success, as also found in the half-sib analysis (Chapter 3). In other words, parasitoids accept D. suzukii as a host, but do not possess adequate mechanisms to circumvent host resistance (e.g., venom composition). These results thus confirm the hypothesis that the relative high host resistance (haemocyte load) is a key characteristic that enables D. suzukii to escape from larval parasitoids present in the invaded range now (Kacsoh & Schlenke, 2012; Poyet et al., 2013) and that - in the case of parasitoid L. heterotoma - this will also not happen in its “evolutionary future”. In other words, the results of the half-sib analysis and selective breeding of L. heterotoma demonstrate that it is unlikely that this species will include D. suzukii in its range of hosts on which it can reproduce. It has to be noted, however, that the results are based on seven European populations. Sampling and testing of other populations would be required to further investigate whether a novel viable parasitoid-host interaction could establish. This is relevant as populations can show large genetic variation in traits that influence parasitization process.

A next step to understand which traits influence host-range evolution would be to link the underlying genetic, behavioural and physiological mechanisms. This will ultimately give insight whether and how such evolution could occur and thus the organisms’ adaptive capability. This information can also help to find key traits that determine parasitization success in different hosts and aid selecting of parasitoids for biological control. For example, we found that parasitoids exhibit genetic variation in attack rate, i.e., ability and willingness of L. heterotoma to find and exploit D. suzukii (Chapter 3). It is known that L. heterotoma uses sensilla in their antennae (Vet, Jong, Giessen, & Visser, 1990) and chemoreceptors on the tip of their ovipositor during parasitization to decide whether they accept a host (van Lenteren et al., 2007; Ruschioni, van Loon, Smid, & van Lenteren, 2015). It would therefore be interesting to compare the expression of olfactory and
chemoreceptor genes in these tissues in *L. heterotoma* strains and other parasitoids that differ in host range and their ability and willingness to parasitize *D. suzukii*, to test the role of sensory perception in change in host recognition and acceptance. Similar approaches have been taken in the parasitoids *Nasonia vitripennis* (Pannebakker, Trivedi, Blaxter, Watt, & Shuker, 2013) and *Chouioia cunea* (Zhao et al., 2016).

Another promising trait that could play an important role in host-range evolution is the parasitoids’ venom. Venom is known to play a key role in parasitization by suppression of host immunity (Rizki & Rizki, 1990), yet little is known about its capacity to evolve and influence host-range evolution in *L. heterotoma* and closely related species. Interestingly, Mathe-Hubert et al. (2019) found that venom composition differs between and within *L. heterotoma* and *L. boulardi*, of which the latter is a much more specialized species towards a few closely related *Drosophila* host species while *L. heterotoma* is considered a rather broad generalist able to use a wide range of *Drosophila* hosts. Moreover, Wey et al. (2020) found that venom contains virus-like particles that are encoded in the wasps’ genome and can thus be vertically transmitted. These results, together with the genetic variation in non-reproductive host killing presented in this thesis, questions which venom factors are involved in exploitation of different hosts, whether venom composition can evolve in response to host resistance and whether and how venom composition influences the magnitude of non-reproductive host killing. The available *L. heterotoma* genome, transcriptome and proteome data (Goecks et al., 2013; Heavner et al., 2013; Wey et al., 2020) together with the results presented in this thesis paves the way to further investigate these questions. Moreover, if a genetic variant of *L. heterotoma* is found able to reproduce on *D. suzukii*, it would be highly interesting to compare the role of venom composition in the transition from non-reproductive to reproductive host killing by comparing different *L. heterotoma* lines.

**Influence of non-reproductive host killing on host-range evolution**

It seems surprising that parasitoids exhibit genetic variation in host-killing without reproduction as this does not give parasitoids a direct fitness benefit. Non-reproductive host killing therefore is likely a by-product of evolution on parasitizing of other hosts. However, the invasive pest can act as a ‘trap’ when native parasitoids such as *L. heterotoma* attempt to exploit it without being able to circumvent host resistance. The invasion of a novel species like *D. suzukii* can therefore have a direct negative effect on native species: when the encounter rate with the “trap” is high, it can endanger the viability of native parasitoid populations.

Presence of genetic variation in traits that influence parasitization might enable native parasitoid populations to persist and be “rescued” by evolution. As such, this raises the question how natural enemies will respond in the field to invasion of novel host species. As little is known about the ecological and evolutionary consequences of these maladaptive host choices, I investigated how genetic variation in host preference and virulence would influence the response to an evolutionary trap, and what conditions would favour avoidance and adaptation. I explored these questions using an individual-based model, simulating the evolution of host-preference and performance in response to invasive species acting as
evolutionary trap under different conditions. With this model I show that non-reproductive host killing can hamper adaptation resulting in host-range conservation. Moreover, host-search efficiency and genetic variation in host-preference play a key role in the likelihood that parasitoids will include the suboptimal host in their host range or will evolve behavioural avoidance resulting in specialization and host-range conservation respectively. Invasive species might thus change the evolutionarily trajectory of native species and might ultimately drive speciation when parasitoid populations specialize by changing host-preference and performance.

To increase our understanding of host range evolution, and to predict these evolutionary processes in nature, the next step would be to link theoretical models with field data. Laboratory and theoretical studies have greatly advanced our knowledge to understand and make such predictions. Yet, field experiments are challenging but crucial, as the results of experiments in the laboratory do not always uphold under field conditions. Parasitoids will, for example, encounter and interact with other species in the field that are not present in the lab and are exposed to fluctuating and unpredictable climatic conditions. To this end, environmental, genetic, ecological and demographic data could be used to parameterize theoretical models to simulate biological systems under relevant conditions. Specifically, predicting the parasitoids’ evolutionary trajectory in this host-parasitoid system would require insight in factors that influence the selection pressure in nature such as host-species diversity and abundance in time and space (Chapter 6, Kraaijeveld & Godfray, 1999; Fleury et al., 2004). Moreover, the results presented in Chapter 6 indicate the importance of parasitoids’ foraging efficiency and host preference strategy. Yet relatively little is known about these behaviours, in particular in different complex environmental conditions in the field. For example: what is the parasitoids’ movement pattern and host-patch encounter rate in different environments, do parasitoids prefer certain hosts (or patches) and is there genetic variation in host preference? These questions could for example be tested in semi-field experiments using different spatial arrangement of host patches (Kostenko et al., 2015; Aartsma et al., 2019), integrated with mark-release-recapture techniques (Hagler, Jackson, Henneberry, & Gould, 2002). Moreover, sensor and computer technologies could potentially be used (in the future) to gain more insight in insect behaviour and movement such as camera-equipped traps in the field for automatic insect detection and monitoring (Preti, Verheggen, & Angeli, 2020), harmonic radar monitor and tracking methods (Chapman, Reynolds, & Smith, 2004; Chapman, Drake, & Reynolds, 2011; Daniel Kissling, Pattemore, & Hagen, 2014) or integrating 3D tracking and virtual reality to quantify insect behavior by mimicking field conditions (Fry, Rohrseitz, Straw, & Dickinson, 2008; Kaushik & Olsson, 2020).

**Biological control of D. suzukii**

*Drosophila suzukii* lays its eggs in a wide range of crop and non-crop fruits (Burrack, Fernandez, Spivey, & Kraus, 2013; Poyet et al., 2015; Kenis et al., 2016). Most soft and stone fruit growers use integrated pest management (IPM), aiming to keep insecticide use to a minimum to prevent residues on their end products and prevent negative effects on natural enemies (Herman Helsen, personal communication; Daane, Vincent, Isaacs, &
Ioriatti, 2018; Rodriguez-Saona, Vincent, & Isaacs, 2019). The introduction of *D. suzukii* however challenges IPM, due to lack of non-chemical control options (Haye et al., 2016). The release of natural enemies (i.e. augmentative biocontrol) and stimulating resident enemies in the surroundings (i.e. conservation biocontrol) may contribute to development IMP strategies for soft and stone fruits.

*Leptopilina heterotoma*

The results presented in Chapter 3 and 4 show that *L. heterotoma* might participate in the field in controlling *D. suzukii*. The parasitoid kills on average 30% of *D. suzukii* in a standardized laboratory trial, but this can reach even up to 100% (Chapter 3,4). Furthermore, the species was found in areas where the pest is active and causes fruit damage (Kruitwagen et al., unpublished results). We therefore next aimed to quantify the parasitoids’ efficiency to control *D. suzukii* populations by releasing parasitoids that were selected on killing ability of *D. suzukii* in cages under greenhouse conditions. These results showed, however, that *L. heterotoma* had no effect on blueberry infestation and *D. suzukii* population size (Bierman et al., unpublished results). Moreover, based on inspection of *D. suzukii* flies emerging from the infested berries, the parasitoids’ attack rate was low, as <2% (n=694) of the emerged flies contained an encapsulated wasp egg. One factor that could have reduced the wasp’s efficiency was their low motivation to search for hosts. The experimental conditions of this experiment may have been suboptimal, due to practical constraints: the parasitoids were relatively old (>2 weeks), they had no access to food (sugar) during their two-week stay in the cages and were exposed to fluctuating temperatures that reached below their optimum (<20 °C).

Hence, the value of *L. heterotoma* for controlling *D. suzukii* in the field and as part of biocontrol program is not yet clear and more research is needed to test the parasitoids’ efficiency to control the pest. This should focus in particular on (the improvement of) the parasitoids’ motivation and ability to detect presence of the hosts from a distance in greenhouses and/or in open field (e.g., orchards and vineyards). This could be done by testing whether the supplementation of sugar (e.g., honey) and the release of young (<1 week) and relatively large wasps (Chapter 3) increases the “quality” of *L. heterotoma* as biological control agent. Another future research direction would be to investigate whether the motivation and host finding ability might be improved. Previous research has shown that the parasitoids’ foraging success is influenced by infochemicals. Parasitoids for example use aggregation pheromones of adult *Drosophila* to detect presence of the host from a distance (Wiskerke et al., 1993; Wertheim et al., 2003). Dekker et al. (2015) however found that *D. suzukii* pheromone composition differs from other closely related *Drosophila* species. This might thus infer with localization of *D. suzukii* by *L. heterotoma*. It would be interesting to investigate whether and how the motivation and host finding ability might be improved by exposure to infochemicals pre- or post-release known to increase the parasitoids’ foraging success to lure them to the pest (Dicke, van Lenteren, Boskamp, & Van Dongen-Van Leeuwen, 1984; Papaj & Vet, 1990; Wertheim et al., 2003; Kruidhof, Kostenko, Smid, & Vet, 2019).
As *L. heterotoma* is not able to reproduce on *D. suzukii*, parasitoids could be released in large number for a fast and immediate control. Yet, from a growers’ perspective, it would be more profitable when biocontrol agents can provide control over multiple generations. To this end, a ‘banker system’ could be used consisting of a suitable host species. This however requires that the wasp does not have a high preference for its adapted/suitable hosts such as *D. melanogaster*. Hence, there are several open questions that need to be explored, including host finding and preference and whether and how host finding and killing can be further improved for biological control (see also above). Another point is to investigate the extent of host-killing under different field conditions (e.g., climate, crop and species community) because the level of resistance of *D. suzukii* of laboratory reared flies might differ from those in the field. Moreover, the vulnerability of the pest to parasitoids might vary between locations as environmental conditions such as temperature, host-plants and intra-specific genetic variation can influence their level of resistance (reviewed in Chapter 2). The magnitude of non-reproductive host killing could be measured by the use of molecular diagnostic tools to detect whether hosts died due to parasitoid attack (Abram et al., 2019) and by including controls inaccessible to natural enemies. Moreover, field data of host-parasitoid population dynamics and the impact of parasitization in laboratory estimates under relevant conditions could be combined and integrated in theoretical studies to quantify and predict parasitoid induced host mortality under field conditions.

**Other natural enemies**

Besides *L. heterotoma* there are several other natural enemies that are currently being investigated for biological control of *D. suzukii*, including European pupal parasitoids (*T. drosophilae* and *P. vindemmiae*) (Da Silva, Price, & Walton, 2019; Stacconi, Grassi, Ioriatti, & Anfora, 2019), Asian larval parasitoids (*Asobara japonica, L. japonica* and *Ganaspis brasiliensis*) (Girod et al., 2018; Giorgini et al., 2019), earwigs (*Forficula auricularia*) (Bourne, Fountain, Wijnen, & Shaw, 2019) and rove beetles (*Dalotia coriaria*) (Renkema & Cuthbertson, 2018). These alternative natural enemies not only differ in their ability to kill *D. suzukii*, but also in other characteristics important for biological control (Chapter 2), such as pest localization ability at various densities of the pest, attack of species other than *D. suzukii* and potential harmfulness to the fruits themselves (e.g., earwigs), and climate conditions under which they perform best. Yet, soft and stone fruit cultivation is highly diverse, with a wide range of crops and cultivation methods (e.g., under glass, plastic and in open ground) (Table 1). There might therefore not be a single species of natural enemy that performs best for controlling *D. suzukii* but, instead, this suite of natural enemies represents an array of possibilities that could be used (together) to develop solutions tailored to the specific (a)biotic growing conditions of each crop.
Table 1 | Soft and stone fruit cultivation in the Netherlands, 2019. Source: CBS

<table>
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<th>fruit open in field</th>
<th>ha</th>
<th>fruit under glass/plastic</th>
<th>ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>blueberries</td>
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<td>blackberries</td>
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<td>32</td>
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<tr>
<td>raspberries</td>
<td>252</td>
<td>raspberries</td>
<td>30</td>
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<tr>
<td>redcurrant</td>
<td>324</td>
<td>other</td>
<td>39</td>
</tr>
<tr>
<td>blackcurrant</td>
<td>206</td>
<td>total</td>
<td>590</td>
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<tr>
<td>plums</td>
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<tr>
<td>sweet cherries</td>
<td>529</td>
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<tr>
<td>sour cherries</td>
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<tr>
<td>strawberries</td>
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<tr>
<td>grapes</td>
<td>160</td>
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<tr>
<td>other</td>
<td>818</td>
<td></td>
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</tr>
<tr>
<td>total</td>
<td>5478</td>
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</table>

**Biological control and IPM**

The efficiency of these natural enemies has mostly been studied in isolation but not in the context of other natural enemies and control measures. The next step would be to investigate (1) whether these natural enemies could complement each other by release of multiple species and (2) how biological control can be combined with other control measures such as push-pull (Alkema, Dicke, & Wertheim, 2019), cultural control (e.g., exclusion netting) (Leach, Van Timmeren, & Isaacs, 2016), landscape diversity to promote natural enemies (e.g., conservation biocontrol) (Schmidt et al., 2019) and resistant crops (Kinjo, Kunimi, Ban, & Nakai, 2013; Entling, Anslinger, Jarausch, Michl, & Hoffmann, 2019) to ultimately develop sustainable integrated pest management strategies for season long protection. To this end, it is crucial to take into account the biology of the pest. Field monitoring shows, for example, that winter conditions in temperature climates are a bottleneck for *D. suzukii* due to cold temperature and low host plant availability (Grassi et al., 2018; Panel et al., 2018), and seasonal population build-up seems to be mainly facilitated by overwintered females (“winter morphs”) (Panel et al., 2018). Moreover, studies show that the fly moves between different crops and wild plants in the surrounding areas throughout the season (Klick et al., 2016; Tait et al., 2018; Delbac, Rusch, & Thiéry, 2020). These results indicate that IPM strategies to control *D. suzukii* (1) should already start in autumn/winter to reduce the number of overwintering females and slow down population build up and (2) could benefit by focussing on surrounding areas (e.g., non-crop plants) to prevent their dispersal and use non-crop plants as refuge before colonization of commercial plants. This could be done by promotion of natural enemies, placement of bait-sprays and removal of wild host plants in surrounding areas (“field margins”).
Prospects for selective breeding to improve biocontrol agents

The presence of natural standing genetic variation allows for optimization by selective breeding of trait values that are desirable for biocontrol. This method has high potential as multiple studies show that traits important for biocontrol exhibit genetic variation (Chinchilla-Ramírez, Pérez-Hedo, Pannebakker, & Urbaneja, 2020) and can be improved by selecting over multiple generations to shift the average trait value of the population up or downwards (Lommen et al., 2017; Lirakis & Magalhães, 2019). Yet, I show that, despite the presence of significant heritable variation, this does not ensure a response to selection (Chapter 5). Because artificial selection can be a time-consuming and labour-intensive method, the results of this thesis underline that it is crucial that several factors need to be considered and preferably investigated in advance.

The potential and feasibility of selective breeding depends on whether the trait can be efficiently and accurately be measured and compared among large numbers of individuals. In particular for traits that are difficult to record (e.g., behaviour and life-history traits), it would therefore be a great advantage to increase measurement ease by, for example, the use of cameras for high-throughput phenotyping of relevant traits, automatic recording of traits involved in parasitization behaviours (see also above and Chapter 2), or use of molecular markers linked to biocontrol trait values of interest (see below) (Lommen et al., 2017; Kruijtewagen et al., 2018; Leung et al., 2020). The natural enemy should have a relative short generation time and should be easily reared in the laboratory. The latter can be challenging as it might require rearing of the pest species as well. For example, in our system, the fecundity and survival rate of D. suzukii is significantly lower compared to D. melanogaster. To this end, rearing could then be done on an alternative host (“factitious hosts”) suitable for the parasitoid. Yet, rearing of the pest species remains necessary for selective breeding for some traits, such as pest killing ability and this can significantly slow down selective breeding of the parasitoid. Moreover, species might adapt to the rearing conditions (e.g., host species, abiotic conditions) which can consequently reduce the efficiency of the natural enemy under field conditions. The natural enemy should therefore be tested under relevant conditions. Also, it is helpful to know which non-additive genetic factors influence variation of the target trait (Chapter 2). Traits can for example be greatly influenced by dominance and epistatic components of genetic variance (Lommen et al., 2017) and non-genetic factors including temperature, daily and/or seasonal rhythms, artificial diet or host factors (e.g., susceptibility and quality) (Chapter 3,4). As such, these factors should be taken into account for the design of selective breeding method and maintained constant as much as possible to secure response to selection.

Intra-specific variation could also be exploited by comparing and selecting the strain/population/family with the best trait values for biological control. This is in particular useful if for example one population already meets the requirements for biocontrol, there is little genetic variation available to select upon, when it is difficult to design a method to efficiently measure a particular phenotype, and/or when the time and resources available to conduct selective breeding are limited. Technological advances also make it possible to develop methods to estimate breeding values using genetic and genomic...
data (methods reviewed by Leung et al., 2020). When the genetic architecture of biocontrol trait is known for example, molecular markers can be used to improve the accuracy and speed of selection process (i.e. marker assisted selection)(Lande & Thompson, 1990). This is in particular interesting when the assessment of individual phenotypes is laborious.

**Conclusion**

*How will organisms respond to something they do not know and what are the consequences?*

There are only a few ways by which organisms can respond to a novel prey or host in their habitat/environment: avoidance or exploration. Predicting which strategy results in high survival and increases in fitness requires insight in the organisms’ phenotypic, genetic and the ecological conditions it encounters. The natural enemy, *L. heterotoma*, readily explored the new invasive pest species *D. suzukii*, but they do not gain direct fitness benefits as they are unable to exploit the new host for reproduction. Their strategy therefore seems ‘foolishly optimistic’. Mothers are supposed to know what is best for their offspring, but they might not always have all required information to act optimally. Yet, although their suboptimal host choices can have detrimental effects for population persistence and can hamper adaptation towards the pest, their ‘foolishly optimistic’ behaviour can be artificially selected for the development of an optimized native biological control agent. Hence, this thesis demonstrates the importance of evolutionary biology to halt the impact of invasive species: (1) for development of sustainable control methods and (2) for the conservation and promotion of genetic diversity between and within species to foster resilience of species to changing environments. Moreover, this thesis shows how fundamental and applied research questions can complement and inspire each other to find new solutions to improve agriculture and to generate new insights in the ecology and evolution of species interactions.