THE ROLE OF HOST-PARASITOID INTERACTIONS, RAPID EVOLUTION, AND HOST SHIFTS IN THE BIOLOGICAL CONTROL OF INVASIVE INSECT PESTS

By

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ABSTRACT

THE ROLE OF HOST-PARASITOID INTERACTIONS, RAPID EVOLUTION, AND HOST SHIFTS IN THE BIOLOGICAL CONTROL OF INVASIVE INSECT PESTS

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This thesis focuses on two invasive species, spotted wing drosophila (*Drosophila suzukii*) and the brown marmorated stink bug (*Halyomorpha halys*). The first chapter investigates the potential of native parasitoids to increase their developmental success on *D. suzukii*. Two native parasitoids were subjected to 10 generations of selection on *D. suzukii* and fitness metrics were measured in generations 0, 3, and 10. Both parasitoid species responded rapidly to selection, reaching peak developmental success on the novel host within three generations, which they then maintained for seven additional generations. There was no increase in preference towards the novel host or changes in development time or body size associated with adaptation. The sex ratio became less female biased for both parasitoids after three generations of selection but rebounded in one species by generation 10. These results indicate that artificial selection can increase the performance of native parasitoids within a few generations and may be used to improve biocontrol when co-adapted natural enemies of invasive species are not available.

The second chapter investigates how the fitness of the adventive parasitoid of *H. halys*, *Trissolcus japonicus,* may change in response to intermittent or continuous exposure to a non-target native stink bug species. Acceptance of the native stink bug species did not increase with either intermittent or continuous exposure. However, reproduction on the native host resulted in a significant fitness cost for *T. japonicus* and compromised the fitness of the parasitoid on its original host species. These results suggest that non-target effects of *T. japonicus* may remain insignificant in nature given the fitness cost of attacking native species.

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Introduction

While there has been a rise in exotic insect introductions over the past century, the importation of natural enemies that attack exotic pests has declined (Cock et al., 2010; Huang et al., 2011). Importing an invasive species' natural enemy from its home range to control the invader is known as classical biological control. Worldwide, this method has provided some level of control for at least 226 invasive insect species since it was first used in 1888 (Heimpel & Cock, 2018). However, introductions have occasionally resulted in unforeseen ecological harm (<1% of all introductions), attracting public scrutiny (Heimpel & Cock, 2018). This has resulted in stricter regulations that increase the time and costs associated with classical biological control (Heimpel & Cock, 2018; Van Driesche et al., 2020).

Native natural enemies, such as parasitoids, could potentially provide control of exotic pests in the absence of classical biological control agents. However, native enemies are usually inefficient at attacking invaders due to the novelty of the exotic prey or host (Chabert et al., 2012; Kruitwagen et al., 2018). If there is positive selection benefit to adapting to the exotic host, native enemies may be able to increase their attack rate and developmental success. Adaptation can be advantageous if the invasive species becomes abundant enough to, for example, displace their native hosts, and if sufficient genetic variation is present in the local enemy population to respond to such selective pressures (Carroll et al., 2005; Strauss et al. 2006; Carlsson et al., 2009).

In the wild, adaptation of a parasitoid to novel hosts can be slow as it may require physiological and behavioral changes (Kawecki & Ebert, 2004; Strand & Obrycki, 1996; Strauss et al., 2006) to take place without strong directional selection given that parasitoids in the wild have a choice between the native and novel hosts. Even parasitoids that accept a novel host usually have lower developmental success on the novel host species compared to native species since they lack co-evolutionary history (Abram et al., 2017; Konopka et al., 2018; Kruitwagen et al., 2018; Costi et al., 2020;). While native parasitoids may be able to kill exotic hosts without reproductive success (Abram et al., 2016; Kaser et al., 2018; Kruitwagen et al., 2021), developmental success on the novel host is essential to sustain parasitoid populations and to pass on genes that advance adaptation to the novel host. However, gene flow between individuals that successfully develop on the invasive host and those that reproduce on native hosts can swamp emerging adaptations (Kawecki & Ebert, 2004).

In a laboratory environment, a directional selective environment can be created by restricting host choice and gene flow and thereby increasing the speed of adaptation (Kruitwagen et al., 2018; Rossbacher & Vorburger, 2020; Hopper et al., 2021). Using experimental evolution experiments to create “improved” biological control agents is not a novel concept, but according to a recent review, most studies have selected for pesticide resistance or temperature tolerance (Lirakis & Magalhaes, 2019). The same review identified only eight experimental evolution studies that selected for increased host range. Of those, few used replicated populations or controlled for phenotypic plasticity and maternal effects (via a common garden environment) in their experimental design (Kawecki et al., 2012; Lirakis & Magalhaes 2019).

Furthermore, selection experiments can be useful in evaluating potential evolutionary trade-offs that affect host range (Fry, 2003). Adaptation to a novel host can be impeded or enhanced depending on whether genes for fitness traits are positively or negatively correlated (Strauss et al., 2006). Such trade-offs are associated with adapting to a novel host (Agrawal, 2000; Henry et al., 2008). Life history traits such as body size or development time can be impacted by reproducing on a sub-optimal host (Strand & Obrycki, 1996). Parasitoids can also adjust their sex ratio depending on host quality and environmental conditions (King, 1987; Godfray, 1994; West & Sheldon, 2002). Selection experiments can be useful in untangling how such life history traits influence the speed or probability of parasitoids adapting to a novel host (Wang et al., 2016).

I tested whether two native parasitoids could improve their developmental success on an invasive host, spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), through experimental evolution. *Drosophila suzukii* is an invasive fly from Asia that causes significant damage in a variety of economically valuable soft fruit crops (Hauser, 2011; Walsh et al., 2011). I used two cosmopolitan pupal parasitoids, *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae), collected in the U.S. These parasitoids show inter- and intraspecific variation in their ability to parasitize *D. suzukii* (Chabert, 2012; Rossi-Stacconi et al., 2015). The native parasitoids do not have a recent co-evolutionary history with *D. suzukii* given its recent invasion into North America (Hauser, 2011) and thus, the parasitoids had relatively low initial developmental success on *D. suzukii* (Jarrett et al., 2022). Overall parasitism rates in the wild have remained under 10% after a decade of *D. suzukii* invasion (Lee et al., 2019).

I performed 10 generations of selection of both native parasitoids on *D. suzukii*. I evaluated developmental success and host preference at generations 0, 3 and 10. I hypothesized that selection would increase developmental success on *D. suzukii* and that the selected lines would increase their preference for *D. suzukii* as a host over a natal host. I also evaluated three potential evolutionary trade-offs associated with adaptation: sex ratio, development time, and body size.

Materials and Methods

Parasitoid and Host Species

I used two pupal parasitoids (*P. vindemmiae* and *T. drosophilae*) that are adapted to develop on *D. melanogaster* but for which *D. suzukii* represents a novel host. *Pachycrepoideus vindemmiae* has a wide host range, attacking at least 60 Dipteran species across a range of genera (Wang & Messing, 2004). Conversely, the host range of *T. drosophilae* is restricted to *Drosophila* species (Wang et al., 2016). Both species are found worldwide, but our laboratory colonies were initiated from North American populations as described in Jarrett et al. 2022. I used *D. melanogaster* as the co-evolved 'control' host since it has a >200-year history in North America (Jarrett et al., 2022). Additionally, I demonstrated that our two parasitoid species had high initial developmental success on *D. melanogaster* (Jarrett et al., 2022).

Parasitoid and Fly Rearing

Both the fly and parasitoid colonies were maintained at 25 ± 2 °C with a 16L:8D photoperiod and 85% relative humidity. *Pachycrepoideus vindemmiae* populations were founded by two wild-caught females. They were captured in 2018 in Michigan, USA (42.6749, -84.4897). *Trichopria drosophilae* populations were founded by 30 individuals and originated from a laboratory colony in California, USA.

Both parasitoid species were reared on *D. melanogaster* for 10 generations to build up large populations (n = 1,800 individuals for each species) prior to experiments. The flies were provided with the Drosophila Species Stock Center cornmeal diet in drosophila vials (2.5 x 9.5cm; Lab-Express, Ann Arbor, MI, USA) with foam stoppers (Genesee Scientific, San Diego, CA, USA). To rear the fly species, each vial for *D. melanogaster* was initiated with 10 individuals and for *D. suzukii* with 20 individuals. This resulted in similar numbers of pupae available for parasitism given the different fecundities of the fly species (Jarrett et al., 2022).

Experimental Evolution Experiments

To test how the two native parasitoid species would respond to selection on *D. suzukii*, I set up three replicated selection lines for both parasitoid species on *D. suzukii* and three replicated control lines on *D. melanogaster* (Kawecki et al., 2012; Jarrett et al., 2022). Starting populations size for each replicated selection and control line were 300 individuals (80% females, mimicking the observed sex ratio after rearing on *D. melanogaster*) (Jarrett et al., 2022). By generation 3, the sizes of replicated populations increased to 800-1000 individuals, and were capped at this size for the duration of the experiment.

To initiate the replicated populations, parasitoids (n = 300 per population) were randomly divided into 12 vials (~25 parasitoids per vial). The adult wasps were provided with honey and left to parasitize fly pupae for 48 hours. Parasitoids were transferred to fresh vials with fly pupae every 48 hours until a minimum of 40 vials contained parasitized pupae. Vials were monitored every other day for both fly and parasitoid emergence for 50 days. Emerging parasitoids were collected and provided with honey until 100 individuals had emerged. I then initiated the next discrete generation. I continued to randomly add emerging parasitoids until the population reached at least 800 individuals. Once the population reached 1000 individuals, no more parasitoids were added. Rearing continued in this manner for 10 generations.

Individual fitness measurements were taken at three different time points during the experiment: generation 0, 3, and 10. Following generations 3 and 10, 200 randomly chosen individuals were removed from each of the replicated selection and control lines of both parasitoid species and placed in a common garden host environment (*D. melanogaster*). All lines were reared on *D. melanogaster* for one generation following generation 3 and for two generations following generation 10. This was done to control for phenotypic plasticity and maternal effects (Kawecki et al., 2012). Individual females emerging from the common garden were randomly chosen from each replicate population to be used in assays. Before all assays, the female was placed in a vial with honey and at least one conspecific male for 48 hours to ensure she was mated.

Developmental Success and Trade-off Assays

Generation 0

I first tested the initial developmental success of the two founding parasitoid populations in generation 0 on both *D. suzukii* and *D. melanogaster* to establish baseline developmental success rates prior to selection. For both parasitoid species, initial developmental success rates were assessed by offering individual females pupae of either *D. melanogaster* or *D. suzukii*. This was done for each of the three populations before the parasitoids were separated into control and selection lines.

In all assays, individual mated females from the common garden were placed in a 60 x 15 mm Petri dish arena (Thermo Fisher Scientific, Waltham, MA, USA) with 10 host pupae. The female parasitoid was removed after 48 hours and discarded. The Petri dishes were monitored for emergent flies and parasitoids for 5 weeks. Additionally, the sex of the emergent parasitoids was noted. This measurement was used to track the evolution of sex ratio, defined as the proportion of emergent females to the total number of emergent parasitoids.

Generation 3

To test for adaptation, the developmental success of both the selection and control lines were assessed on *D. suzukii* and *D. melanogaster*, respectively, following the generation in the common environment (*D. melanogaster*) (Jarrett et al., 2022). I only assayed females on the species on which they evolved to address the main question of whether native parasitoids could adapt to an invasive host. Therefore, there were three replicates of the control line assayed on *D. melanogaster* and three replicates of the selection line assayed on *D. suzukii* using 15-20 individual females per replicate population. Assays were conducted using the same methods described above.

Generation 10

In generation 10, I once again assayed individual females from the selection and control lines on their respective host using the same methods as described above. In this generation, I also measured the development time for all offspring emerging during assays. Development time was defined as the number of days between the removal of the female wasp from the arena and the emergence of the offspring.

I also saved the individual female parents emerging from the common garden in 90% ethanol at -80^{o}C. These parasitoids were weighed to test for a trade-off between body size and adaptation to *D. suzukii*. Measurements were taken using a microbalance (Model XP 26, Mettler Toledo, Columbus, OH, USA).

Host Preference

After generations 0, 3, and 10, host preference was assessed by giving 25 randomly chosen individual females a choice between the novel and the ancestral hosts. Randomly chosen females emerging from the common garden were held with a conspecific male for at least 24 hours for mating. They were then provided with 10 *D. melanogaster* and 10 *D. suzukii* pupae in a 60 x 15 mm Petri dish arena. The *D. melanogaster* pupae were placed on one side of the arena and the *D. suzukii* pupae were placed on the other. A thin paper towel barrier was placed down the middle of the arena. This allowed the female to cross but prevented the two different host species from mixing. The female was removed after 48 hours, and the pupae were separated by host species to monitor the emergence of flies and wasps.

Statistical Methods

All analyses were performed in R 3.5.1 (R Core Team, 2020). Generalized linear mixed models and linear mixed models were constructed using the *lme4* package (Bates et al., 2015). All post-hoc pairwise comparisons were performed in the *emmeans* package (Lenth, 2021).

Developmental Success

For each parasitoid species, I compared the developmental success of the selection and control lines on their respective hosts in generations 0, 3 and 10. Two different metrics were used to assess developmental success. First, developmental success was coded as a binomial outcome: 1 if there were any emerging parasitoid adults in an assay trial or 0 if no parasitoids emerged. For the second model, only those replicates that had successful emergence were used. Here, I compared the proportion of parasitoids emerging from the different host species. I refer to this metric as ‘emergence rate’, which was calculated by dividing the number of adults emerging by the total number of pupae offered and as such the data is bounded between 0 and 1.

For both analyses, I used generalized linear mixed models with a binomial distribution. The fixed effects were the generation (0, 3, or 10), treatment (rearing host of *D. suzukii* for the selection lines and *D. melanogaster* for the control lines), and the replicate population nested within treatment (rearing host) was included as a random effect. I tested for an interaction between generation and rearing host using the *anova* function with a Chi-square test. The interaction was removed from models in which it was not significant. Post-hoc pairwise comparison was performed using the *emmeans* function to test for differences between generations and rearing host.

Sex Ratio

First, I assessed if treatment (rearing host) over time influenced whether at least one female emerged. Given that parasitoids have haplodiploid sex determination, unmated females only produce male offspring (coded as 0’s) and mated females produce both male and female offspring (coded as 1 when a least one female emerged from a trial). I were interested in how selection on *D. suzukii* influenced the sex ratio over generations 0, 3 and 10. For those trials with successful mating, I then asked how the proportion of emerging females (values bounded between 0 and 1) changed over time in the different treatments. For both analyses, I used generalized linear mixed models with a binomial distribution. The fixed terms in both models were the generation (0, 3, or 10), treatment (rearing host), and the replicate population nested in treatment was included as a random effect. I tested for interaction between generation and rearing host using the *anova* function with a Chi-square test. The interaction was removed from models in which it was not significant. Post-hoc pairwise comparisons using the *emmeans* function were used to compare differences between generation and rearing host.

Development Time

A linear mixed model was used to compare the development times of the parasitoids in the selection and controls lines in generation 10. The fixed terms in the model were treatment (rearing host) and the sex of the emerging parasitoid. The replicate population nested within treatment and the individual female parent were the random effects.

Body Size

A linear mixed model was used to compare the body size of the parasitoids after 10 generations of rearing on either *D. suzukii* or *D. melanogaster*. Treatment (rearing host) was the fixed term, and the random effect was the replicate population nested within treatment.

Host Preference

To test whether host preference evolved, I compared host choices of parasitoids from the different treatments in generations 0, 3, and 10. Preference was defined as the difference between the number of parasitoids emerging from *D. melanogaster* and *D. suzukii*. This creates a metric where zero indicates there was an equal number of parasitoids that emerged from *D. melanogaster* and *D. suzukii*. Numbers greater than zero indicate preference for *D. melanogaster* and the scale indicates the level of preference. Numbers less than zero indicate preference for *D. suzukii* and the scale indicates the level of preference. For all analyses, trials in which no parasitoids emerged from both host species were excluded. If some parasitoids emerged from *D. melanogaster* but none from *D. suzukii* or vice versa, those were included.

Given that in generation 0 the selection treatments had not been implemented yet to assess evolution of host preference over time (in generations 0, 3, and 10), the control and selection lines were analyzed separately. The fixed effect in both models was generation, and the replicate population was included as a random effect.

Results

Developmental Success

The probability that at least one *P. vindemmiae* adult would emerge successfully was not affected either by generation or treatment (all $P > 0.06$). The emergence rates of *P. vindemmiae* adults in the selection and control lines showed different trajectories over time (generation*treatment interaction: $z = 5.41$, $P < 0.001$) (Fig. 1.1a). For the selection lines, emergence rates on *D. suzukii* significantly increased between generations 0 (58.6 ± 3.8%) and 3 (71.8 ± 3.7) (pairwise comparison: $P = 0.0179$), and from generation 0 (58.6 ± 3.8%) to 10 (68.8 ± 3.8%) (pairwise comparison: $P < 0.001$). The initial variation in the performance of the replicate populations was reduced as selection proceeded (Fig. 1.2a). For the control lines, emergence rates on *D. melanogaster* were similar between generation 0 (77.4 ± 3.0%) and 3 (80.5 ± 2.6) ($z = -1.88$, $P = 0.41$), and then decreased significantly by generation 10 (58.5 ± 4.4%) compared to both generation 0 (pairwise comparison: $P = 0.0048$) and generation 3 (pairwise comparison: $P < 0.0001$). This decrease appears to have been driven by one of the replicate populations (Fig. 1.2b).

For *T. drosophilae*, the probability of at least one offspring emerging differed between treatments and over time (treatment*generation interaction: z = 2.697, P = 0.007). In the selection lines, emergence probability on *D. suzukii* increased from generation 0 (68.5 ± 1.1%) to generation 3 (97.6 ± 2%) (pairwise comparison: P = 0.003), but by generation 10, selection lines were not any more likely to produce offspring (89.8 ± 5.6%) compared either to generation 0 (pairwise comparison: P = 0.101) or to generation 3 (pairwise comparison: P = 0.481). In the control lines, there was no change over time in the probability that the females would produce at least one offspring on *D. melanogaster* (all P > 0.94). Emergence rates of *T. drosophilae* were overall higher on *D. melanogaster* compared to *D. suzukii* (treatment: z = -5.480, P < 0.0001) (Fig. 1.1b). Changes in emergence rates between the generations varied for the selection and control lines (generation 3*treatment interaction: z = 2.14, P = 0.0323; generation 10*treatment interaction: z = 2.66, P = 0.008). The pattern of emergence rates for the selection lines of *T. drosophilae* were similar to those of *P. vindemmiae* on *D. suzukii* showing an increase between generation 0 (29.1 ± 4.2%) and generation 3 (48.9 ± 4.9%) (pairwise comparison: P < 0.0001), and between generation 0 and generation 10 (40.9 ± 1.9%) (pairwise comparison: P = 0.006), but not between generations 3 and 10 (pairwise comparison: P = 0.136). In the control lines, emergence rates on *D. melanogaster* first increased between generation 0 (66.3 ± 4.4%) and generation 3 (75.3 ±0.04%) (pairwise comparison: P = 0.014), and then decreased between generation 3 and generation 10 (66.0 ± 5.0%) (pairwise comparison: P = 0.0197). This resulted in no change overall in emergence rates from generation 0 to 10 (pairwise comparison: P = 1.000) Variability among the three replicate populations appears to be higher for both the control and selection lines in *T. drosophilae* compared to *P. vindemmiae* (Fig. 1.2 c,d).

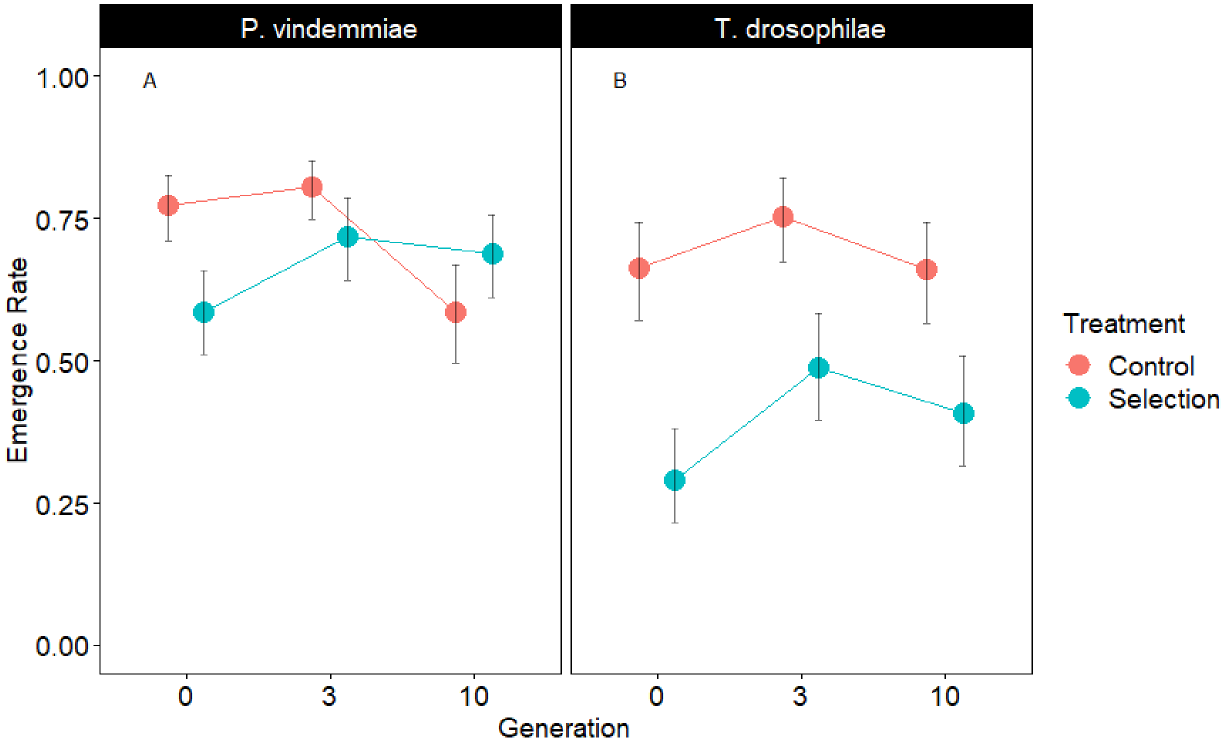

Figure 1.1. The proportion of either *D. suzukii* (selection treatment) or *D. melanogaster* (control treatment) pupae that had successful parasitoid emergence of either *P. vindemmiae* or *T. drosophilae* during experimental evolution over 10 generations. Means ± 1SE are shown for three replicated populations for each treatment in each generation. Both for *P. vindemmiae* and *T. drosophilae*, emergence rates on *D. suzukii* increased between generation 0 and 3, and generation 0 and 10, but not between generation 3 and 10. The emergence rate of the control populations of *P. vindemmiae* on *D. melanogaster* decreased between generation 3 and 10, and generation 0 and 10. The emergence rate of the control populations of *T. drosophilae* increased between generations 0 and 3, but then decreased between generations 3 and 10 resulting in no change overall from generation 1 to 10 (see results for details).

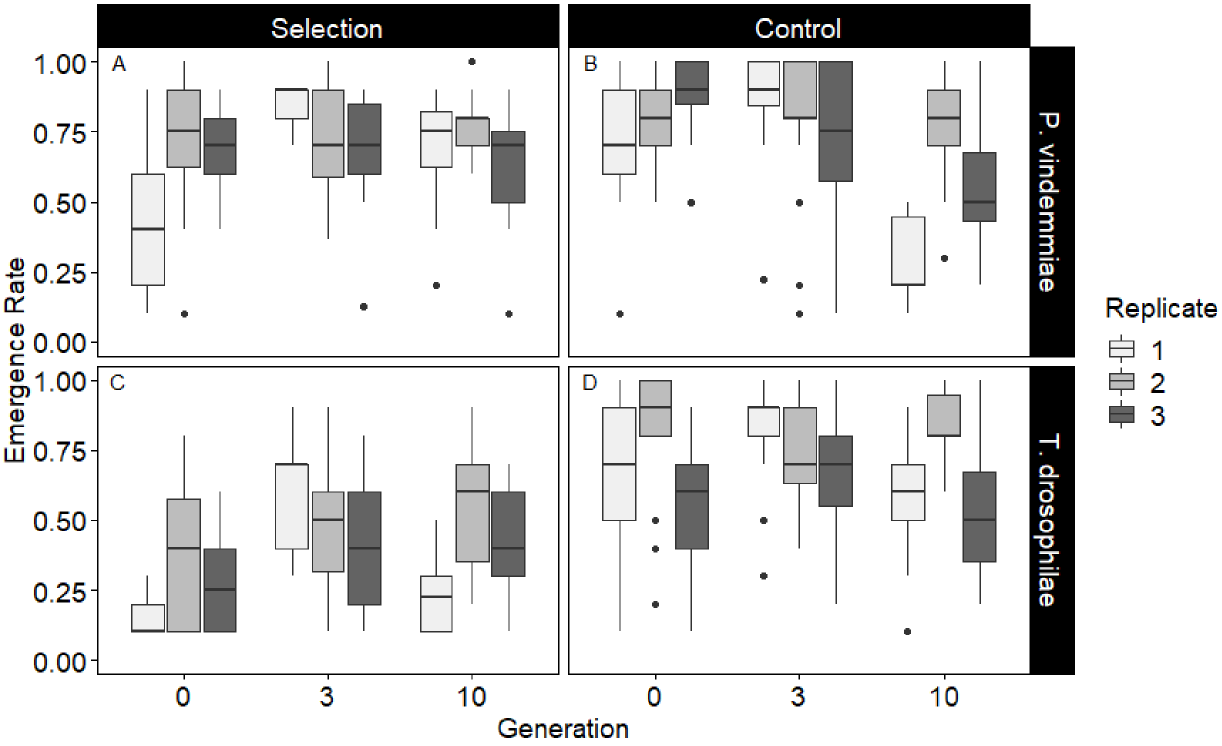

Figure 1.2. The proportion of either *D. suzukii* (selection treatment) or *D. melanogaster* (control treatment) pupae that had successful parasitoid emergence of either *P. vindemmiae* or *T. drosophilae* during experimental evolution over 10 generations. Replicates are shown in different shades of grey. Dots indicate outlier observations, the horizontal line indicates the median with the box representing the interquartile range, and vertical lines are 1.5 times the interquartile range.

Sex Ratio

For *P. vindemmiae*, the probability of at least one female emergingdecreased from generation 0 to generation 10 (z = -3.45, P < 0.0001) on both *D. suzukii* (pairwise comparison: P= 0.008) and on *D. melanogaster* (pairwise comparison: P = 0.008) (114 trials with no emergent parasitoids excluded). There was a significant interaction between generation and rearing host influencing the sex ratio for *P. vindemmiae* (z = -2.26, P = 0.02) that stemmed from the selection treatment showing a decrease in the proportion of females from generation 0 (81.9 ± 2.6%) to generation 3 (64.5 ± 3.9%) (pairwise comparison: P = 0.002) (Fig. 1.3a). However, by generation 10, the sex ratio on *D. suzukii* rebounded (78.8 ± 3.4%) and was similar to generation 0 (pairwise comparison: P = 0.978). For the control lines, there was no significant change in sex ratio for any generation (P > 0.76 for all pairwise comparisons of generations).

For *T. drosophilae*, the probability of at least one female emerging did not change over time (generation 3: z = 0.81, P = 0.417; generation 10: z = -0.9, P = 0.367) or on the different rearing hosts (treatment: z = -0.78, P = 0.0439) (55 trials with no emergent parasitoids excluded). There was a significant interaction between generation and rearing host influencing the sex ratio (z = -3.05, P = 0.002) (Fig 1.3b) that stemmed from a decline in the proportion of females in the selection lines from generation 0 (76.6 ± 4.1%) to generation 10 (58.9 ± 4.5%) (pairwise comparison: z = 3.02, P = 0.03). For the control lines, there was no significant change in the sex ratio between any generations (P > 0.89 for all generations).

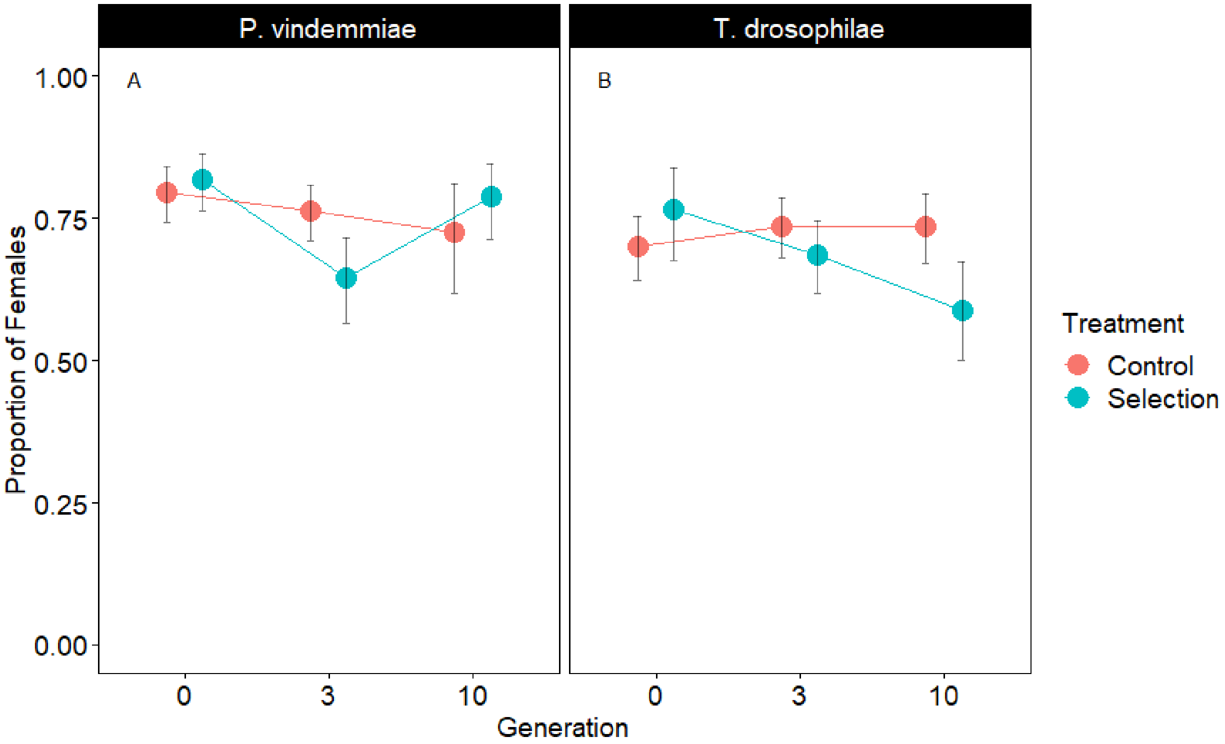

Figure 1.3. The proportion of females emerging from *D. suzukii* (selection treatment) or *D. melanogaster* (control treatment) for *P. vindemmiae* and *T. drosophilae* during experimental evolution over 10 generations. The mean ± 1SE of three replicated populations is shown for each generation and treatment. For *P. vindemmiae*, the proportion of females emerging on *D. suzukii* decreased between generations 0 and 3 but then rebounded in generation 10. For *T. drosophilae*, the proportion of females emerging on *D. suzukii* decreased from generations 0 to 10. For both *P. vindemmiae* and *T. drosophilae*, the proportion of females emerging on *D. melanogaster* did not change throughout the experiment (see results for details).

Development Time

There was no difference in development time between the selection lines and the controls lines for either *P. vindemmiae* (t = -0.87, P = 0.43) or *T. drosophilae* (t = 0.55, P = 0.61). As expected, males had a significantly shorter development time than the females in both *P. vindemmiae* (t = -5.14, P < 0.001) and *T. drosophilae* (t = -7.07, P < 0.001). For *P. vindemmiae,* the average development time for males was 15.3 ± 0.52 days in the control lines and 14.7 ± 0.51 days in the selection lines. For *P. vindemmiae* females, the average was 16.1 ± 0.53 days in the control lines and 15.5 ± 0.51 days in the selection lines. For *T. drosophilae,* the average development time for males was 18.5 ± 0.39 days in the control lines and 18.8 ± 0.40 days in the selection lines. For *T. drosophilae* females, the average was 19.6 ± 0.38 days in the control lines and 19.9 ± 0.40 days in the selection lines.

Body Size

There was no difference in body size between the selection lines and the control lines for either *P. vindemmiae* (t = 1.66, P = 0.18) or *T. drosophilae* (t = 0.24, P = 0.82). For *P. vindemmiae,* the mean female weight in the control lines was 0.16 ± 0.02 mg and 0.20 ± 0.02 mg in the selection lines. For *T. drosophilae,* average weight of the females was 0.12 ± 0.01 mg in both the control line and the selection line.

Host Preference

For *P. vindemmiae,* there was no change of preference in the selection lines (P > 0.32 for all generations) or the control lines (P > 0.14 for all generations) (Fig. 1.4a). In the *T. drosophilae* selection lines, females increased their preference towards *D. suzukii* from generation 0 to generation 3 (z = -4.51, P < 0.001) (Fig. 1.4b). However, this intermittent increase in preference towards the novel host disappeared by generation 10 (z = -1.64, P = 0.10). In the control lines, preference did not change between generations 0 and 3 (z = -0.62, P = 0.53).

In generation 10, the control lines preferred *D. melanogaster* slightly less in generation 10 compared to the ancestral state (z = -2.04, P = 0.04).

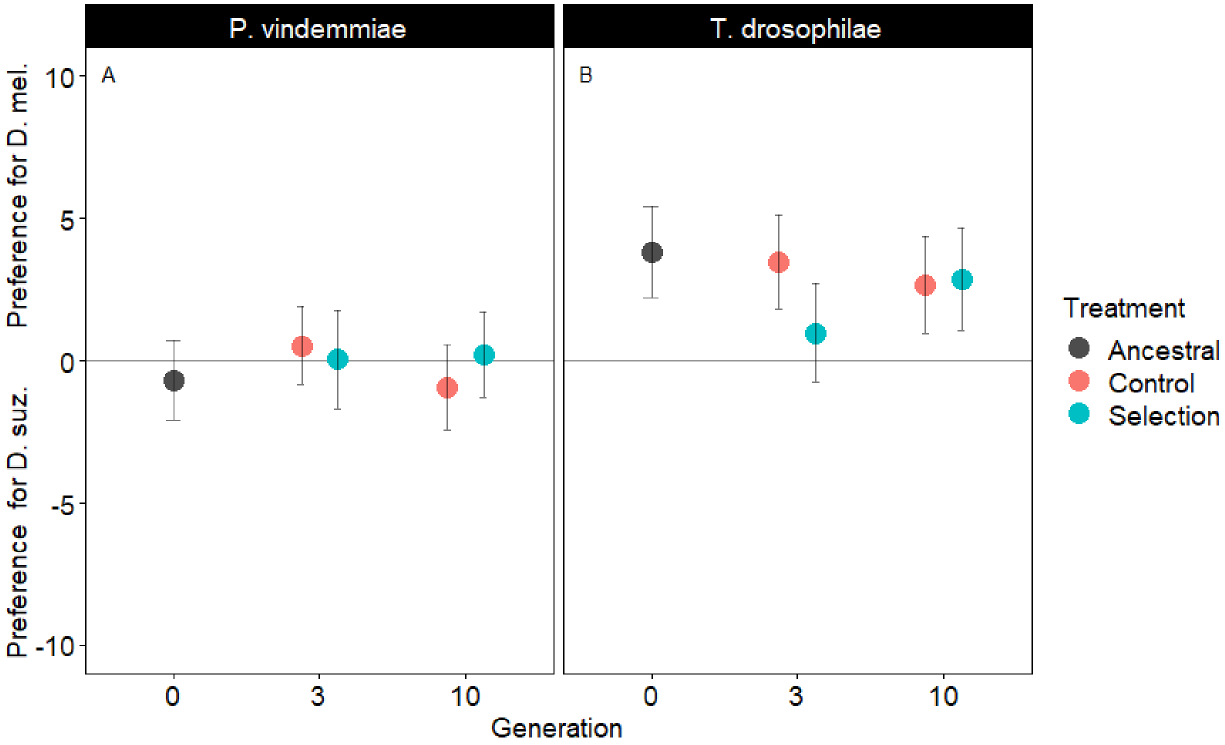

Figure 1.4 The preference of *P. vindemmiae* and *T. drosophilae* parasitoids reared on *D. suzukii* (selection) or reared on *D. melanogaster* (control) during multiple generations. Preference was defined as the number of parasitoids emerging from *D. melanogaster* minus the number of parasitoids emerging from *D. suzukii.* Hence, positive values indicate preference for *D. melanogaster* and negative values indicate preference for *D. suzukii.* The mean ± 1SE of three replicated populations is shown for each generation and treatment. For *P. vindemmiae,* preference did not change throughout the experiment in either the control treatment or the selection treatment. For *T. drosophilae,* preference did not change in the control treatment. For the selection treatment, *T. drosophilae* increased preference towards *D. suzukii* between

Figure 1.4 (cont'd) generations 0 and 3 but moved back towards increased preference for *D. melanogaster* in generation 10 (see results for details).

Discussion

I showed that both native parasitoids, *P. vindemmiae* and *T. drosophilae* adapted rapidly to a novel host, *D. suzukii*, increasing their developmental success in just three generations of selection, and then maintained this adaptation for seven more generations. This adaptation was not accompanied by increased preference towards the novel host and there were no trade-offs in development time or body size associated with increased developmental success on *D. suzukii*. Initially the sex ratio became more less female biased for both parasitoid species, but it recovered in *P. vindemmiae* over time.

While both parasitoid species had an overall increase in developmental success between generations 0 and 10, I did not see continual improvement between generations 3 and 10. There can be multiple factors that may limit the extent of adaptation and that could prevent achieving higher developmental success beyond a particular threshold. One factor could be the physiological ability of the parasitoid to develop in the host. Kruitwagen et al. (2021) evolved a native larval parasitoid, *Leptopilinia heterotoma* (Förster) (Hymenoptera: Figitidae), on *D. suzukii* for seven generations. While host killing rates increased, developmental success rates failed to do so. This could be due to the inability of the larval parasitoid to overcome the immune defenses of the host (Kacsoh & Schlenke, 2012; Poyet et al., 2013). However, this immune response from the host does not seem to be as prevalent in the pupal stage, making it easier for the pupal parasitoids in our study to adapt (Kacsoh & Schlenke, 2012). Reproduction on a particular host species may also be limited by behavioral factors. While a parasitoid may be able to physiologically reproduce on a host, it may be behaviorally more or less inclined to accept the host. Host acceptance behavior can be based on a variety of factors such as the morphology of the host as well as host kairomones (Vinson, 1976; Romani et al., 2002). Alternatively, slight changes in the experimental methods might have altered the rates of developmental success measured. Following generation 3, the parasitoids spent only one generation in the common garden but following generation 10, there were two common garden generations prior to assays. Therefore, the generation 3 developmental success rates could be inflated due to lingering maternal effects or plastic responses to the selective environment (Kawecki et al., 2012). The additional time spent in the common garden environment, however, adds confidence to the level of adaptation achieved over 10 generations.

The presence of evolutionary trade-offs may influence the host selection behavior of the parasitoid (Olaye et al., 1997). For example, parasitoid females may not lay eggs in poor quality hosts as it can have detrimental effects on the fitness of the offspring such as smaller body size and longer development time (Strand & Obrycki, 1996). Parasitoid females may also adjust the sex ratio of their brood based on the quality of hosts available (Charnov, 1982; Godfray, 1994).

I did not, however, find any trade-off between development time or body size associated with developing on *D. suzukii* for either parasitoid species. Body size is important in female fitness as it correlated with greater fecundity and longevity (Sagarra et al., 2007; Beukeboom, 2018). However, larger bodied parasitoids tend to take longer to develop (Harvey, 2005). Longer development time can be maladaptive as it increases the chances of predation (Doyon & Boivin, 2005). These fitness traits may be less malleable as parasitoids must balance maximizing body size whilst minimizing development time (Harvey, 2005).

On the other hand, increased parasitism on *D. suzukii* was correlated with a decrease in sex ratio on *D. suzukii* between generations 0 and 3 for both parasitoid species. Parasitoid females are able to adapt the sex ratio of their offspring and will do so in response to environmental factors (Hardy, 1994) and to host quality (Charnov, 1982; Godfray, 1994). Female biased sex ratios are generally favored among parasitoids as it reduces mate competition increases the overall productivity of the population (Hamilton, 1967; Hardy, 1994; West et al., 2005; Abe & Kamimura, 2012) Between generations 3 and 10, the sex ratio of *P. vindemmiae* rebounded, becoming more female biased while the sex ratio of *T. drosophilae* continued to become less female biased. This difference could be related to the initial host range of the parasitoid species. *Pachycrepoideus vindemmiae* is considered a generalist, attacking a wide range of Dipteran species (Wang & Messing, 2004) while *T. drosophilae* is considered a specialist only attacking *Drosophila* species (Wang et al., 2016). Specialists are more likely to suffer trade-offs when adapting to a novel host, perhaps explaining the trade-off between developmental success and sex ratio in *T. drosophilae* (Poisot et al., 2011). As a generalist, for *P. vindemmiae,* it may have been easier to overcome the barriers that prevented *T. drosophilae* from producing similar numbers of females on *D. suzukii* than on *D. melanogaster*.

Generalist and specialist strategies may also contribute to how preference evolves. Our results showed that *P. vindemmiae* demonstrated no preference for either host species throughout the experiment. Such a lack of preference could make it easier for generalists, in general, and for *P. vindemmiae,* here, to adapt to novel hosts as they are less likely to be deterred by low initial survival rates. However, this potential for adaptation may increase the risks of non-target effects (Zepeda-Paulo et al., 2013). *Pachycrepoideus vindemmiae* may be most useful in environments where the density of *D. suzukii* is much higher than the density of other host species since it may attack the host with which it comes in contact most (Kaçar et al., 2017). For specialists such as *T. drosophilae*, it may be more difficult for preference to shift (Poisot et al., 2011). Host selection behavior has been shown to correlate with survival probability (Kraaijeveld et al., 1995). Hence, low survival rates on *D. suzukii* relative to that on *D. melanogaster* may deter changes in preference for *T. drosophilae*. Preference is an important consideration when considering how laboratory results may translate to field condition. High specialization can be favorable as there is a smaller likelihood of non-target attacks. However, in the case of *T. drosophilae,* which has a clear preference for its co-evolved host, it may be unlikely to attack the target host, *D. suzukii,* in the presence of native Drosophilid hosts.

There are many pathways to adapting to a novel host depending on the genetic architecture of the species and the standing genetic diversity within a local population (Strand & Obrycki, 1996; Kraaijaveld & Godfray, 2001). In the laboratory, there is the added challenge of maintaining genetic diversity through many generations of rearing. For example, Woltering et al. (2019) reared *T. drosophilae* on *D. suzukii* for over 30 generations. The study did not find evidence for the adaptive evolution of parasitism rate. However, loss of genetic diversity due to long-term laboratory rearing could have hindered adaptation. A study investigating the long-term rearing of *Trichogramma brasicae* (Bezdenko) (Hymenoptera: Trichogrammatidae) found that the parasitoids were less active and foraged less after 20 generations of rearing with no added genetic diversity (Ghaemmaghami et al., 2022). The study from Woltering et al. (2019) also suggests that one generation is enough to increase parasitism rates on *D. suzukii*. However, it is impossible to untangle the roles of adaptive evolution, natural variation and phenotypic plasticity without a common garden or replicated populations.

Due to the complex nature of host range expansion, I cannot discount the role of natural variation in response to selection. The populations used in our study were founded by few individuals (two for *P. vindemmiae* and 30 for *T. drosophilae).* Population bottlenecks can reduce genetic diversity and limit adaptive potential (Barrett & Schuttler, 2008). Alternatively, population bottlenecks can increase additive genetic variation and thereby enhance rapid evolution (Goodnight, 1988; Willis & Orr, 1993; Van Heerwaarden et al., 2008). Our study demonstrated substantial adaption despite small founding sizes. However, low founding sizes also increase the likelihood for the replicated populations to diverge leading to different results (Rich et al., 1978). Within our own study, there was variation between replicated lines. For example, the emergence rates in control lines for *P. vindemmiae* decreased in generation 10. However, this seems to be driven by one population, and could have been caused by genetic drift due to the long-term laboratory rearing (Kawecki & Ebert, 2004).

Both *P. vindemmiae* and *T. drosophilae* have already been used in augmentative releases against *D. suzukii*. Releases of *T. drosophilae* have reduced *D. suzukii* populations by up to 50% (Rossi-Stacconi et al., 2019; Gonzalez-Cabrera et al., 2021). So far, augmentative releases of *P. vindemmiae* have had less success, but this may be due to the small release sizes (Hogg et al., 2022). Additionally, the studies releasing *T. drosophilae* reared the parasitoids on *D. suzukii* before release while the study from Hogg et al. (2022) reared *P. vindemmiae* on *D. melanogaster* prior to release. Our study suggests that rearing the parasitoids on the target host for at least three generations could make augmentative releases more effective but rearing beyond that is unlikely to yield additional benefits in terms of increasing levels of adaptation.

I showed that native parasitoids are able to adapt higher developmental success rates on a novel host and maintain those adaptations for several generations. As the importation of exotic biological control agents becomes more restricted, it is imperative to look at alternative methods to controlling exotic pest species (Cock et al., 2016; Van Driesche et al., 2020). Using experimental evolution to create “improved” strains of native parasitoids could make for a less costly and time-consuming method of biological control. However, it is yet to be determined if increases in developmental success rates in the laboratory will translate to field conditions. Our study is the first step towards evaluating the potential application of experimentally evolved native parasitoids in pest suppression.

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CHAPTER 2: NON-TARGET ATTACK OF THE NATIVE STINK BUG, *PODISUS MACULIVENTRIS* BY *TRISSOLCUS JAPONICUS* COMES WITH FITNESS COSTS AND TRADE-OFFS

Introduction

When exotic species invade new regions, their natural enemies are sometimes co-introduced with them (Talamas et al., 2015; Wheeler et al., 2017; Kaser & Heimpel, 2018; Stahl et al., 2018; Hogg et al., 2021). Even though these accidentally introduced natural enemies can potentially be beneficial by providing at least partial control of invasive pests (Rayamajhi et al., 2017; Wheeler et al., 2017; ; Kaser & Heimpel, 2018; Abram et al., 2019; Miksanek & Heimpel 2019), there is a concern regarding their non-target effects since they do not pass through formal host range testing as intentionally introduced classical biological control agents do (Heimpel & Mills, 2018).

Some of the accidentally introduced natural enemies prove quite host specific to the target invasive species (Wheeler et al., 2017; Girod et al., 2018; Daane et al., 2021), however, others can pose a threat to native species (Howard et al., 2006; Kaser & Heimpel, 2015; Girod et al., 2018; Power et al., 2020; Daane et al., 2021). For example, the unintentionally introduced parasitoid *Aphelinus certus* (Yasnosh) (Hymenoptera: Aphelinidae), which significantly reduced soybean aphid *Aphis glycines* (Matsumura) (Hemiptera: Aphididae) populations in agricultural fields in North America is known to readily colonize natural prairies and attack native aphid species (Kaser & Heimpel, 2018; Miksanek & Heimpel, 2019). Two adventive parasitoids of the invasive spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), have made it to North America following the recent invasion of the fly. In British Columbia, *Ganaspis brasiliensis* (Ihering) and *Leptopilina japonica* (Novković and Kimura) (Hymenoptera: Figitdae) were shown to have up to 66% parasitism rate on *D. suzukii* (Abram et al., 2019), which is the highest attack rate found outside the native range of this invasive fly (Abram et al., 2022). While *G. brasiliensis* appears quite specific to *D. suzukii*, *L. japonica* has a much wider host range that include several species native to North America and Europe (Girod et al., 2018; Daane et al., 2021).

Attack of native species by adventive parasitoids may also influence their effectiveness at controlling the invasive species they followed. Switching of adventive parasitoids to a native host could come at a cost of reduced fitness on the novel host or in trade-offs when switching back to the invasive host (Morris & Fellowes, 2002; Jones et al., 2015; Fors et al., 2016; Bertin et al., 2017; Slater et al., 2019; Woltering et al., 2019; Jarrett et al., 2022). When the generalist *Venturia canescens* (Gravenhorst) (Hympenoptera: Ichneumoniade) switched from their original host, *Plodia interpunctella* (Hubner) (Lepidotera, Pyralidae) to a novel host, *Ephestia kuehniella* (Zeller) (Lepidoptera, Pyralidae) their survival was reduced on the novel host (Jones et al., 2015). Host switching could also result in changes in preference between the ancestral and novel hosts. The parasitoid, *Asobara tabida* (Förster)(Hymenoptera: Braconidae) exhibited a change in preference, that depended on the rearing Drosophilid host (Rolff & Kraaijeveld, 2001). *Asobara tabida* reared on *D. melanogaster* developed a preference towards this host compared to *A. tabida* populations that had been reared on *Drosophila suboscura* (Collin) (Diptera: Drosophilidae) (Rolff & Kraaijeveld, 2001). Understanding when adventive parasitoids might attack native species and the fitness consequences of this is fundamental to predicting the biological control these parasitoids might exhibit.

To get a better understanding of this, I studied the exotic parasitoid, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) that followed the invasive brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) to North America. *Halyomorpha halys* was first detected in the USA in 2001 (Hoebeke & Carter, 2003) and adventive populations of *T. japonicus*, a coevolved natural enemy from the native range of *H. halys* (Talamas et al., 2015). *Trissolcus japonicus* is an oligophagous egg parasitoid that had been evaluated in quarantine as a candidate for introduction as a classical biological control agent since 2007 (Talamas et al., 2015). However, host specificity studies revealed that *T. japonicus* can successfully parasitize and develop on at least 13 native North American species (Botch & Delfosse, 2018; Hedstrom et al., 2017; Lara et al., 2019), which prevented its field release. Nevertheless, adventive populations of *T. japonicus*, first found in 2014 (Talamas et al., 2015), keep spreading and their presence is confirmed in at least 14 states as of 2021 (NE IPM, 2021). Given that *T. japonicus* has shown up to 85% parasitism rates of *H. halys* locally in the native range (Yang et al., 2009; Zhang et al., 2017), augmentative release programs are underway in several states to increase the distribution and population densities of this promising natural enemy of *H. halys* (Jentsch, 2017; Lowenstein et al., 2019).

Little is known about how *T. japonicus* may impact native Pentatomidae species since the laboratory no-choice host range tests only reveal the physiological host range of species, which is known to be broader than the ecological host range, the actual number of species they may utilize in nature (Van Klinken, 1999; Schaffner, 2001; Sabbatini-Peverieri et al., 2021). The few field studies find low (< 8%) or no attack of native stink bugs by *T. japonicus* (Milnes & Beers, 2017; Jarrett et al., 2019). One of the native species that may be the target of *T. japonicus* is *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), a beneficial predatory stink bug. There is one field study that found no attack (Jarrett et al., 2019). However, in Ontario, two *T. japonicus* individuals emerged from naturally laid *P. maculiventris* eggs that were co-parasitized by a native parasitoid, *Telenomus podisi* (Ashmead) (Hymenoptera: Platygastridae), in the field

(Gariepy & Talamas, 2019). The preference and suitability of *P. maculiventris* for parasitism by *T. japonicus* is not well known since laboratory experiments have shown large differences in parasitism rates (Hedstrom et al., 2017; Botch & Delfosse, 2018; Lara et al., 2019). In a study from Oregon, *T. japonicus* only parasitized 3% of *P. maculiventris* egg masses offered (Hedstrom et al., 2017), while in experiments in California 56% of egg masses offered were parasitized (Lara et al., 2019). These differences may stem from variable experimental designs and the differing characteristics of the *P. maculiventris* and *T. japonicus* populations used in experiments.

Host switching is one of the factors that can influence *P. maculiventris* parasitism rates by *T. japonicus* and the fitness of the parasitoid (Botch & Delfosse, 2018). In a laboratory study, acceptance of native stink bug eggs for parasitism by *T. japonicus* depended on the rearing host in the generation prior to testing (Botch & Delfosse, 2018). If *T. japonicus* was reared on *H. halys* it showed strong preference for *H. halys* eggs over native stink bug eggs, however, when the rearing host was *P. maculiventris,* acceptance of *H. halys* eggs decreased and oviposition in *P. maculiventris* eggs increased (Botch & Delfosse, 2018). The host switching resulted in decreased brood and adult sizes of *T. japonicus* on the native stink bugs (Botch & Delfosse, 2018). These results show that reproduction of *T. japonicus* on a novel host just for a single generation can incur fitness costs. I do not know how extended exposure to novel hosts or host switching over multiple generations may impact *T. japonicus* fitness and thus their potential effectiveness on the long term at attacking *H. halys* or native stink bugs.

In this study, I tested how intermittent host switching between the ancestral *H. halys* and the novel *P. maculiventris* hosts over a 10-generation period and continuous exposure to either host for three consecutive generations may impact the developmental success and parasitism rate of *T. japonicus*. In the first experiment, I created replicated *T. japonicus* populations that either had or did not have a history of host switching over 10 generations of rearing. Individuals from the ‘no host switching’ treatment were then reared for three generations on *H. halys* and those from the ‘host switching’ treatment on *P. maculiventris*. In the second experiment, I started with *T. japonicus* from our base colony and reared replicated *T. japonicus* populations for three generations continuously either on *H. halys* or *P. maculiventris* without a history of host switching. For both experiments, I measured developmental success and parasitism rates on both *H. halys* and on *P. maculiventris* in a reciprocal transplant experiment to assess any potential trade-offs associated with development on the different hosts. I hypothesized that prior exposure to *P. maculiventris* would result in better performance of *T. japonicus* on this novel host when forced to reproduce on it for several consecutive future generations; a scenario that could occur in nature as *H. halys* may become less common. I also assumed that without a history of host switching, there would be a cost for *T. japonicus* to attack the novel *P. maculiventris* host and to keep reproducing on this native species.

Materials and Methods

Rearing of Stink Bugs

Podisus maculiventris colonies were founded by 250 nymphs in 2018 (Idlewild Butterfly Farms, Louisville, KY, USA). The colony was later supplemented with 100 nymphs in 2020 (Rincon-Vita Insectaries, Ventura, CA, USA). *Halyomorpha halys* colonies were founded by >200 individuals from eggs shipped from the Philip Alampi Beneficial Insect Laboratory (New Jersey Department of Agriculture, Trenton, NJ, USA) in 2018 and then supplemented annually with additional eggs from the same source. Colonies of both *H. halys* and *P. maculiventris* were maintained in environmental chambers at 25 ± 2^{o}C with a 16L:8D light cycle and 50-75% relative humidity.

Podisus maculiventris egg masses were kept in 60 X 15 mm Petri dishes (Thermo Fisher Scientific, Waltham, MA, USA) lined with paper towel at densities of five egg masses per dish. Once the first instars emerged, they were provided with organic green beans. From the second instar to the third instar, stink bugs were held in a 1 L transparent container with mesh openings and a screw-top lid (Nalgene, Rochester, NY, USA). Densities were maintained at 50-60 individuals per container. Fourth instars, fifth instars, and adults were held in a 6 L transparent container with a mesh lid (Nalgene, Rochester, NY, USA). Densities for the fourth and fifth instars were maintained at 40-50 individuals per container. Adults were separated from the nymphs as they emerged and maintained at densities of 30 stink bugs per container. From the second instar forward, all stink bugs were fed organic green beans three times a week and wax worms (Top Hat Cricket Farm, Portage, MI, USA) every day. They were also provided with paper towel and water in a 60 ml deli cup (Gordon Food Service, Wyoming, MI, USA) with a cotton wick (Dynarex, Orangeburg, NY, USA). Adult containers were checked for egg masses daily. Egg masses were cut out from the paper towel with scissors or removed from the mesh lid with a paint brush. When possible, loose egg masses were glued back onto paper towel with Elmer’s glue.

Halyomorpha halys egg masses were kept in a 60 ml deli cup (one egg mass per cup). Once the first instars emerged, they were provided with a moistened cotton wick and organic green beans. From the second instar to the third instar, stink bugs were held in a 27 X 17.5 X 8 cm transparent plastic container lined with paper towel and covered with a mesh lid (Ziploc, Bay City, MI, USA). Densities were maintained at 20 stink bugs per container. Fourth instars, fifth instars, and adults were held in 30 X 30 X 30 cm mesh cages (Restcloud). From the second instar forward, all stink bugs were fed organic green beans, snap peas, bell pepper, broccoli, carrots, apples, and sunflower seeds three times a week. They were also provided with a 60 ml cup of water with a cotton wick. Adult cages were provided with a potted bean plant (*Phaseolus vulgaris*) and paper towel for oviposition. Cages were checked for egg masses daily.

Trissolcus japonicus colonies were founded by five (three females and two males) wild-caught individuals in Michigan, USA (Jarrett et al., 2019) and reared in environmental chambers at 22 ± 2^{o}C with a 16L:8D photoperiod and 25-50% relative humidity. To rear parasitoids, 20 adults were placed in 9-dram plastic vials (Thornton Plastic Co., Salt Lake City, UT, USA) with three *H. halys* egg masses (< 24 hours old) and a drop of honey for five days. Each *H. halys* egg mass contained on average 28 individual eggs and adult wasps emerged 20-30 days following parasitism. Asynchronously emerging parasitoids were removed from vials three times a week and placed in an environmental chamber at 10 ± 2^{o}C with a 16L:8D photoperiod and 25-50% relative humidity to slow down aging until the next generation was started. Parasitoids kept at 10^{o}C were placed to room temperature (22^{o}C) for one hour weekly to feed on honey drops provided on the lids of vials. This ‘chilling’ method was also used to build up large populations of *T. japonicus* for experiments, whereas adults emerging 1-3 months apart were chilled and then combined to start experiments once the required population sizes had been achieved. This short-term chilling did not have significant fitness consequences for *T. japonicus* performance in subsequent rearing (unpublished data).

Exp 1: History of Host Switching

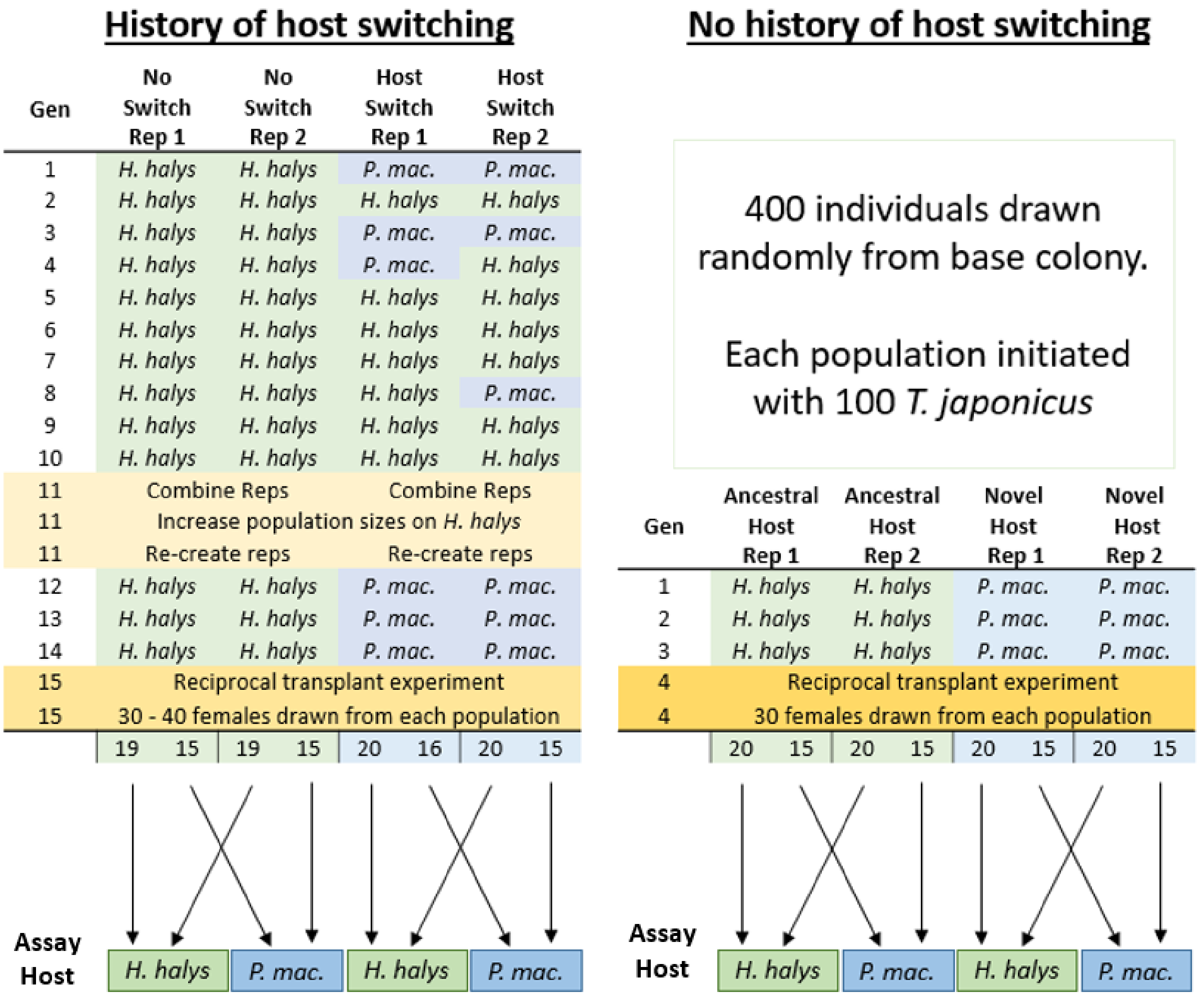
To test how variation in host availability may impact the fitness of *T. japonicus,* I created replicated populations that were either reared solely on *H. halys* ('no host switching') or on *H. halys* for seven generations and on *P. maculiventris* for three intermittent generations ('host switching') (Fig. 2.1). There were two replicated populations for both the 'no host switching' and 'host switching' treatments. All populations were started in the first generation by offering 150 adult *T. japonicus* individuals 15 *H. halys* or *P. maculiventris* egg masses for seven days to parasitize (observed sex ratio 60% female when reared on *H. halys*). Population growth rates differed between the treatments and among replications within the same treatment. The growth rate of populations in the 'no host switching' populations tended to be higher than those in the 'host switching' treatments. fFluctuations in growth rates often resulted in one or both replicated populations within a treatment to fall below replacement rate. There were also differences in the availability of both *H. halys* and *P. maculiventris* egg masses over time, hence there was some variation in the number of adult parasitoids starting each new generation and the number of stink bug eggs offered to them between generation 1 and 10 (Fig 2.2). However, in each generation more stink bug eggs were provided than what could be used by the parasitoids for oviposition (i.e., there was no egg limitation) (Fig. 2.2). For the 'host switching' treatment *P. maculiventris* was offered for three generations to the replicated *T. japonicus* populations during 10 generations of rearing. The timing of *P. maculiventris* exposure between generations depended on the growth rate of parasitoids and the availability of stink bug eggs. When the growth rate on *P. maculiventris* was decreasing (fewer adult wasps emerged than the starting population size) the emerging parasitoids were placed back on the ancestral host, *H. halys* to avoid further population decline and possible extinction (Fig. 2.3). This resulted in variable exposure of the two replicated populations in the ‘host switching’ treatment to *P. maculiventris* within the generations (Fig. 2.1).

Population sizes across all treatments and replications were low (< 30 individuals, except for the second replicate of the ‘no host switch’ treatment, which had >100 individuals) after 10 generations of rearing. To increase population sizes, 15 randomly chosen adult parasitoids emerging from generation 10 were combined for the two replications within each treatment (n = 30 individuals per vial). These parasitoids were offered 12 *H. halys* egg masses (ca. 330 individual eggs) in generation 11 by reusing the same individuals for parasitism. The emerging parasitoids were then randomly re-divided into two new replicated populations for the ‘no host switching’ and ‘host switching’ treatments to initiate generation 12 with 100 individuals starting each replicated population. Between generations 12 and 14 the parasitoids were reared continuously on *H. halys* for the ‘no host switching’ treatment and on *P. maculiventris* for the ‘host switching’ treatment (Fig. 2.1).

After three generations of continuous reproduction on either *H. halys* or *P. maculiventris*, a reciprocal transplant experiment was performed. For this, 30-40 *T. japonicus* females were drawn randomly from each replicated population from the two hosts. Half of the females from each replication and from each host were assayed on *H. halys* and the other half on *P. maculiventris* (Fig. 2.1). Females were kept at 10^{o}C for no longer than seven days and were placed at 25^{o}C with at least one male parasitoid and a honey droplet for 24 hours prior to fitness assays. Individual females were offered 15-19 eggs (< 24 hours old) in a 9-dram vial for 24 hours. Females were then removed and discarded. The developing eggs were placed in the 25^{o}C environment. Vials were monitored daily for 30 days for the emergence of both parasitoids and stink bugs. A minimum of 15 trials were conducted for each replicate population and host species host. Depending on egg availability, up to 20 trials were conducted.

Exp. 2: No History of Host Switching

To assess how continuous reproduction of *T. japonicus* on a native host may affect its fitness and if there may be any trade-offs associated with reproduction on a novel host, I conducted a separate experiment that had some common elements with the previous experiment. As in the previous experiment, I exposed replicated *T. japonicus* populations to either *H. halys* or *P. maculiventris*, but the parasitoids used in this experiment did not have a history of host-switching and did not go through 10 generations of isolation from the base population. I initiated replicated populations using 400 randomly chosen adult parasitoids (< 1 week old) from our laboratory colony that was at the time kept at > 5,000 individuals. Two replicated populations were initiated on *H. halys,* and two populations on *P. maculiventris* with 100 individuals each. The 100 founding individuals were divided into five vials (20 parasitoids per vial). Egg masses were combined so that a total of 79-87 eggs were available for parasitism in each vial (~ 6 *P. maculiventris* egg masses or ~ 3 *H. halys* egg masses). The adult wasps were provided with honey and left to parasitize eggs for 24 hours. Parasitoids were then removed and discarded. Vials with developing eggs were placed in an environmental chamber at 25 ± 2^{o}C with a 16L:8D photoperiod and 25-50% relative humidity. Vials were monitored daily for both stink bug and parasitoid emergence for 30 days. Rearing continued in this manner for three generations, initiating each new generation with 100 individuals. The same reciprocal transplant experiment was performed after generation 3 as described above for the previous experiment.



History of host switching

Gen	No Switch Rep 1	No Switch Rep 2	Host Switch Rep 1	Host Switch Rep 2
1	*H. halys*	*H. halys*	*P. mac.*	*P. mac.*
2	*H. halys*	*H. halys*	*H. halys*	*H. halys*
3	*H. halys*	*H. halys*	*P. mac.*	*P. mac.*
4	*H. halys*	*H. halys*	*P. mac.*	*H. halys*
5	*H. halys*	*H. halys*	*H. halys*	*H. halys*
6	*H. halys*	*H. halys*	*H. halys*	*H. halys*
7	*H. halys*	*H. halys*	*H. halys*	*H. halys*
8	*H. halys*	*H. halys*	*H. halys*	*P. mac.*
9	*H. halys*	*H. halys*	*H. halys*	*H. halys*
10	*H. halys*	*H. halys*	*H. halys*	*H. halys*
11	Combine Reps		Combine Reps	
11	Increase population sizes on *H. halys*			
11	Re-create reps		Re-create reps	
12	*H. halys*	*H. halys*	*P. mac.*	*P. mac.*
13	*H. halys*	*H. halys*	*P. mac.*	*P. mac.*
14	*H. halys*	*H. halys*	*P. mac.*	*P. mac.*
15	Reciprocal transplant experiment			
15	30 - 40 females drawn from each population			
	19 15	19 15	20 16	20 15

No history of host switching

400 individuals drawn randomly from base colony.

Each population initiated with 100 *T. japonicus*

Gen	Ancestral Host Rep 1	Ancestral Host Rep 2	Novel Host Rep 1	Novel Host Rep 2
1	*H. halys*	*H. halys*	*P. mac.*	*P. mac.*
2	*H. halys*	*H. halys*	*P. mac.*	*P. mac.*
3	*H. halys*	*H. halys*	*P. mac.*	*P. mac.*
4	Reciprocal transplant experiment			
4	30 females drawn from each population			
	20 15	20 15	20 15	20 15

Figure 2.1 Experimental design for the two experiments testing how a history of host switching (left panel) or lack of exposure to novel hosts (right panel) may influence the performance and fitness of replicated *T. japonicus* populations. In both experiments *T. japonicus* populations were reared for three consecutive generations on either *H. halys* or *P. maculiventris* (see generations 12-14 in the left panel and generations 1-3 in the right panel) and then individual females were assayed on both hosts in reciprocal transplant experiments. The difference between the two experiments is that in the first experiment the parasitoid populations had a 10 generation history that either included host switching or not, while in the second experiment only 'naïve'

Figure 2.1 (cont'd) parasitoids were used that were not exposed to any other host than *H. halys* and originated from a large base population.

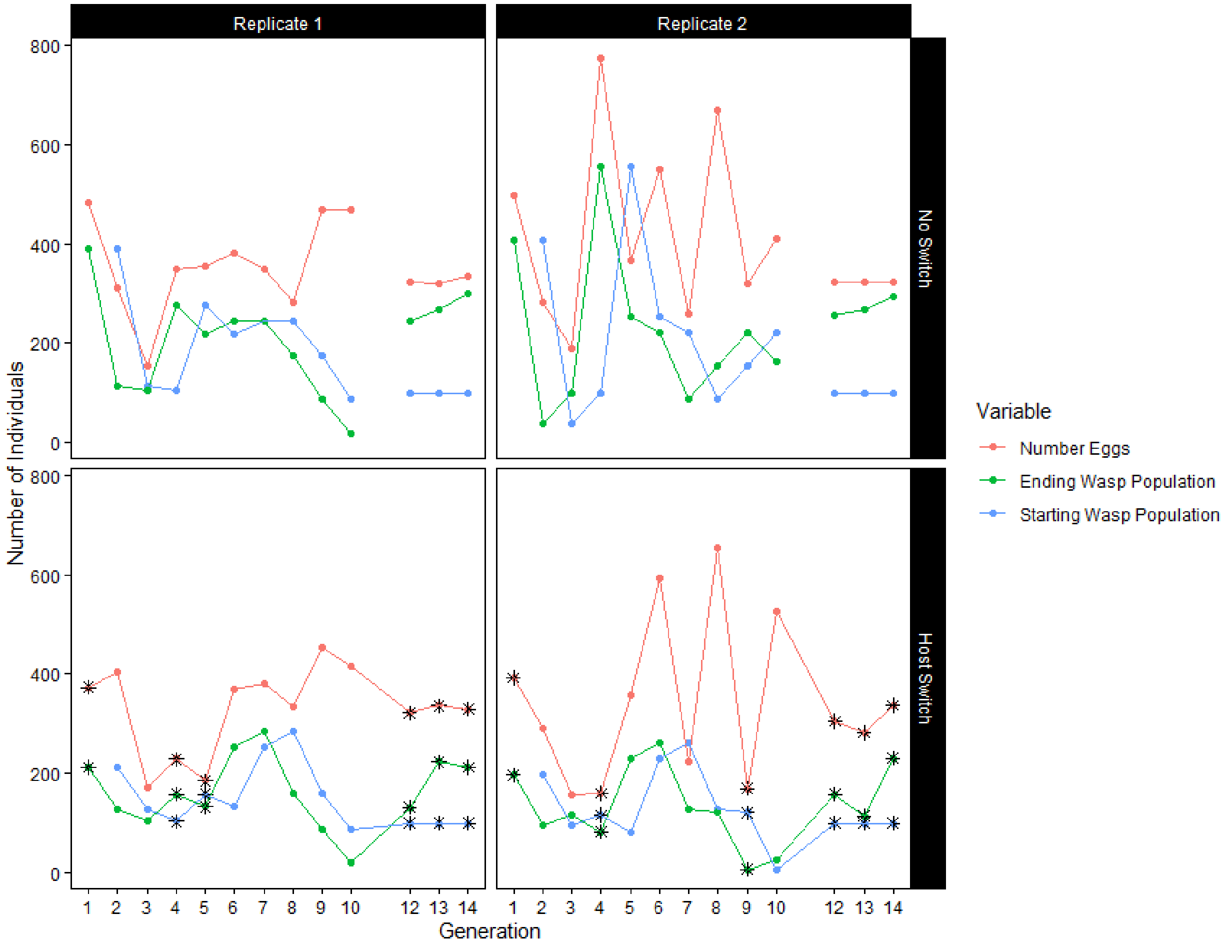


Figure 2.2 The number of adult *T. japonicus* parasitoids used to start each new generation (blue line), the number of *H. halys* or *P. maculiventris* (indicated by an asterisk) eggs offered (red line) and the number of emerging parasitoids (green line) for two replicated populations in a 'no host-switching' and a 'host-switching' treatment. Generation 11 was used to increase population sizes with a different protocol than prior generations, where the same parasitoids were re-used multiple times (indicated as a gap in the lines). For the final three generations the 'no host

Figure 2.2 (cont'd) switching' populations were reared on *H. halys* and the 'host switching' populations on *P. maculiventris*.

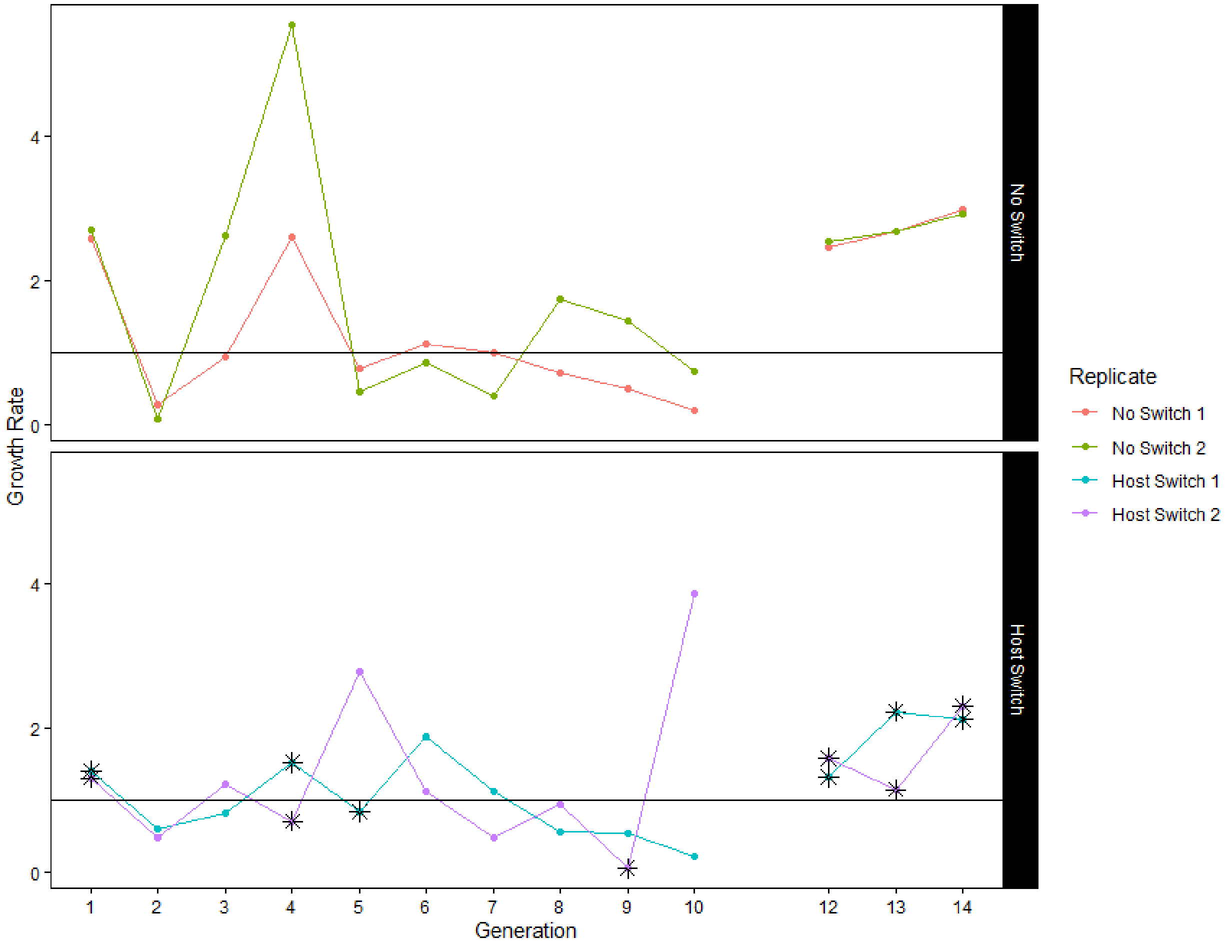


Figure 2.3 The growth rate (ending population size divided by starting population size) of two replicated populations in a 'no host switching' (top panel) and a 'host switching' treatment (lower panel). Generations in which parasitoids were offered *P. maculiventris* egg masses are indicated by an asterisk. All other generations were reared on *H. halys*. Generation 11 was used to increase population sizes with a different protocol than prior generations, where the same parasitoids were re-used multiple times (indicated as a gap in the lines). For the final three generations the 'no host switching' populations were reared on *H. halys* and the 'host switching'

Figure 2.3 (cont'd) populations on *P. maculiventris.* The solid line indicates a growth rate of 1 where the population would be stable.

Statistical Methods

All analyses were performed in R 3.5.1 (R Core Team, 2020). Generalized linear mixed models were constructed using the *lme4* package (Bates et al., 2015). All post-hoc pairwise comparisons were performed using the *emmeans* package (Lenth, 2021). For both experiments, the growth rate for each generation was calculated by dividing the number of emerging adult wasps by the number of wasps used for parasitism.

For experiment 1(history of host switching) only eight trials of the 139 performed in the reciprocal cross did not yield any offspring, therefore I could not run a separate analysis to compare the probability of successful emergence between treatments as for the second experiment. I compared emergence rates between treatments using data from all trials, including those eight with zero emergence. Emergence rates were calculated by dividing the number of emerging offspring by the total number of stink bug eggs offered for each trial. Values for this metric were bounded between 0 and 1, and thus a binomial distribution with a logit link function was used for the generalized linear mixed model (*glmer*). The fixed effect in the model was the rearing host treatment in the last three generations (*H. halys* – for the no host switch control treatment and *P. maculiventris* – for the host switching treatment) and the assay host in the reciprocal transplant (*H. halys* or *P. maculiventris*). The random effect was the replicate populations nested within treatment (rearing host).

For experiment 2 (no history of host switching), I evaluated how rearing of *T. japonicus* on either the ancestral (*H. halys*) or novel host (*P. maculiventris*) for three generations influenced developmental success of parasitoids on either host in the reciprocal transplant assays. First, I compared the probability of any offspring emerging from the individual female assays. For this analysis, individual trials that resulted in at least one offspring emerging from either *H. halys* or *P. maculiventris* were coded as 1 and those that did not yield emergence were coded as 0. For the second analysis, only those trials were used that yielded offspring and the emergence rates between treatments were compared. Both analyses used generalized linear mixed models (*glmer*) with a binomial distribution and logit link function. The fixed effects in both models were the rearing host treatment (*H. halys* or *P. maculiventris*) and the assay host (*H. halys* or *P. maculiventris*), and the random effect was the replicate populations nested within treatment (rearing host).

All models were tested with and without an interaction between rearing host and assay host using the *anova* function with a Chi-square test. Model estimates were converted to probabilities using the *emmeans* function. Post-hoc pairwise comparisons were conducted using the *emmeans* function with Tukey-adjusted P-value to test for differences between different combinations of rearing hosts and assay hosts.

Results

Exp. 1 - History of Host Switching

There was a significant interaction between the ‘no host switching’ and ‘host switching’ treatments and the assay host during the reciprocal transplant assays for emergence rates ($z = 3.01$, $P = 0.003$). This interaction stemmed from the lower emergence rates of *T. japonicus* from *H. halys* in the host switching treatment (75.3 ± 2.7%; mean ± SE) compared to the control populations that were only exposed to *H. halys* (85.7 ± 2.7%) (pairwise comparison: $z = 3.13$, P

= 0.010), while *T. japonicus* emergence rates were similar from *P. maculiventris* regardless of prior exposure to this novel host (‘host switching’: 62.1 ± 3.4% and ‘no host switching’: 64.2 ± 3.4%) (pairwise comparison: z = 0.43, P = 0.973) (Fig. 2.4). Emergence rates were higher overall when the final assay used the ancestral *H. halys* host compared to the novel *P. maculiventris*, regardless of prior exposure to the novel host (assay host: z = -8.2, P < 0.0001). There was a fitness cost to even a single generation of reproduction on *P. maculiventris* (64.2% compared to 85.7% emergence rate of the control populations from *P. maculiventris* and *H. halys*, respectively) (pairwise comparison: z = 8.2, P < 0.0001). Similarly, there was a fitness cost to development on *P. maculiventris* even if there was prior reproduction on this host (62.1% compared to 75.3% emergence rate of the ‘host switching’ populations from *P. maculiventris* and *H. halys*, respectively) (pairwise comparison: z = 4.8, P < 0.0001).

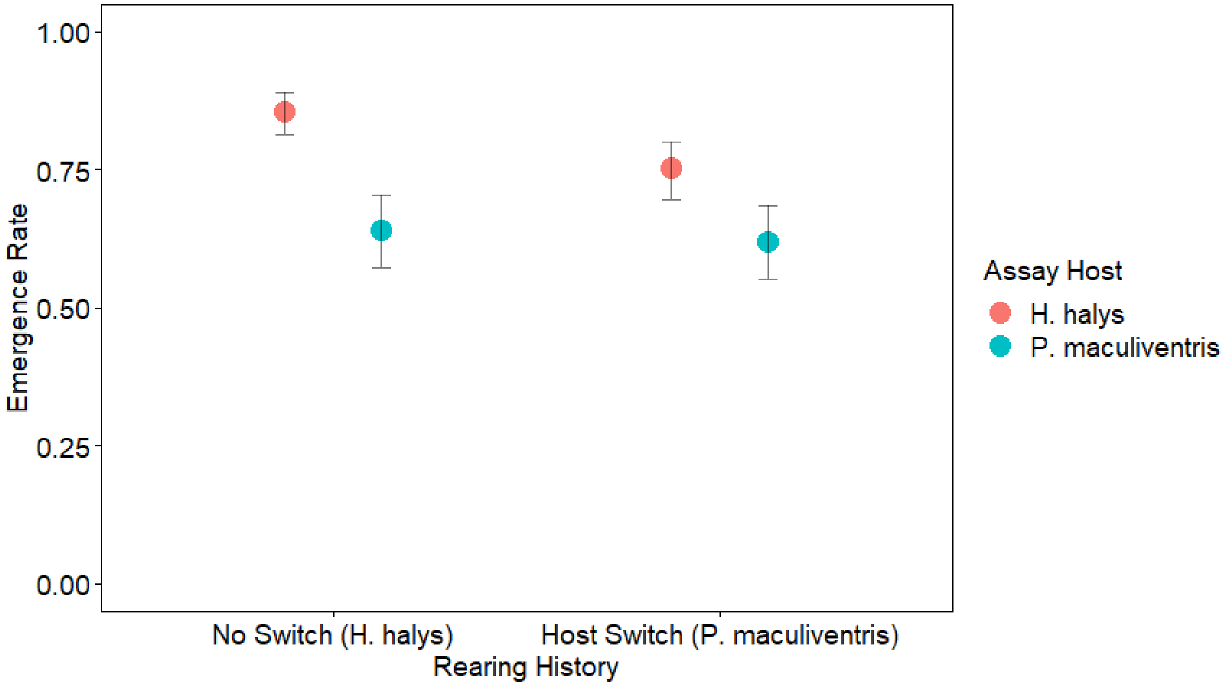


Figure 2.4 The proportion of parasitoids emerging from *H. halys* and *P. maculiventris* in the ‘no host switching’ and the ‘host switching’ treatments for the experiment with a history of host

Figure 2.4 (cont'd) switching. The mean ± 1SE for two replicated populations is shown for each treatment and host species. There was no significant difference in the emergence rate on *P. maculiventris* between the no switching and the host switching treatments (pairwise comparison: z = 0.43, P = 0.973). The emergence rate on *H. halys* was lower in the host switching treatment than in the no host switching treatment (pairwise comparison: z = 3.13, P = 0.010).

No History of Host Switching

Rearing *T. japonicus* for three consecutive generations on *P. maculiventris* reduced the probability of successful offspring emergence compared to rearing on *H. halys* (rearing host: z = -3.7, P = 0.000241) regardless of whether the final assay host was *P. maculiventris* or *H. halys* (assay host: z = 0.8, P = 0.42) (Fig. 2.5).

For the emergence rates comparisons 32 of the 140 total trials were excluded because no offspring emerged. *Trissolcus japonicus* emergence rates were overall lower when reared on *P. maculiventris* for three consecutive generations compared to rearing on *H. halys* (rearing host: z = -10.3, P < 0.0001) regardless of the host used for the final assay (assay host: z = 0.2, P = 0.863). Emergence rates measured on *H. halys* in the final assay declined sharply between treatments (61.6 ± 1.9% when reared on *H. halys* vs. 27.7 ± 2.3% when reared on *P. maculiventris* previously). This decline was less steep for emergence rates measured on *P. maculiventris* in the final assay (62.1 ± 2.3% when reared on *H. halys* vs. 49.8 ± 2.6% when reared on *P. maculiventris* previously), which resulted in a significant rearing host and assay host interaction (z = 4.7, P < 0.0001) (Fig. 2.6). In this experiment, a single generation of rearing on *P. maculiventris* did not result in lower performance (61.6% vs. 62.1% emergence rates) when prior to the final assay the sole rearing host was *H. halys* (pairwise comparison: z = -0.2, P = 0.1) as opposed to the previous experiment with a history of host switching (Fig. 2.4). The results of this experiment also differed from the previous one, in that, there was a cost to switching back to the ancestral host *H. halys* after being reared on the novel *P. maculiventris* for three generations (27.7% emergence rate on *H. halys* compared to 49.8% on *P. maculiventris* in the final assay) (pairwise comparison: z = -6.2, P <0.0001).

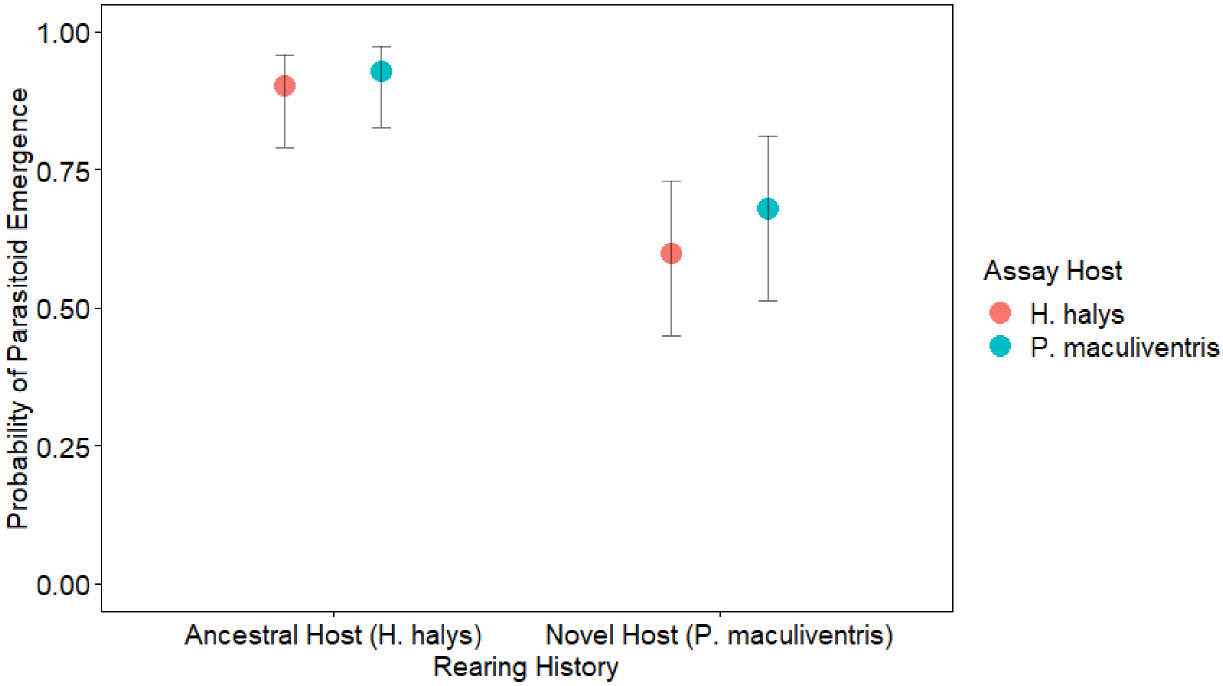


Figure 2.5 The probability of at least one parasitoid emerging from *H. halys* or *P. maculiventris* (assay hosts) eggs following three consecutive generations of reproduction on either *H. halys* (ancestral host) or *P. maculiventris* (novel host) in the experiment with no history of host switching. The probability ± 1SE is shown for two replicated populations for each rearing treatment and assay host species. The probability of parasitoid emergence was lower when the parasitoids were reared on *P. maculiventris* than when they were reared on *H. halys* (rearing host: z = -3.7, P = 0.000241).

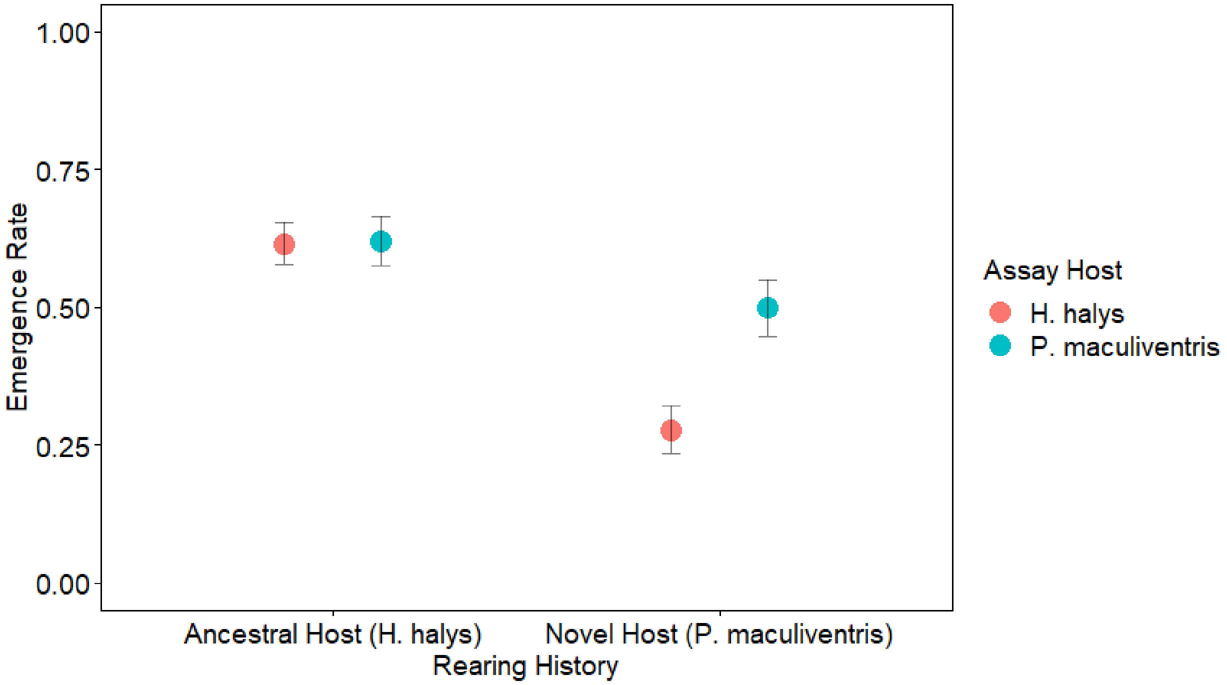


Figure 2.6 The proportion of parasitoids emerging from *H. halys* and *P. maculiventris* following three consecutive generations of reproduction on either *H. halys* (ancestral host) or *P. maculiventris* (novel host) in the experiment with no history of host switching. The mean ± 1SE for two replicated populations is shown for each treatment and host species. The emergence rate on *P. maculiventris* was lower when the parasitoids were reared on *P. maculiventris* (pairwise comparison: z=10.29, P < 0.001). Rearing on *P. maculiventris* also resulted in lower emergence rates on *H. halys* (pairwise comparison: z = 3.50, P=0.0026).

Discussion

I tested if the fitness of the adventive parasitoid *T. japonicus* changes in response to intermittent or prolonged exposure to a native host species. I simulated scenarios of variable host availability between their ancestral host, *H. halys*, which *T. japonicus* followed into North America, and a novel host, *P. maculiventris*, which is native to the region. Host switching between the ancestral and novel hosts might increase acceptance of native species, reducing host-specificity of *T. japonicus*, which could negatively affect native stink bugs. The biocontrol efficacy of *T. japonicus* on *H. halys* could also be compromised if reproduction on a novel host resulted in lower fitness on *H. halys.* I did not find evidence for increased performance on the novel host but found consistent fitness costs for *T. japonicus* to reproduction on the native *P. maculiventris* both with and without a history of host switching, and trade-offs when *T. japonicus* switched back to *H. halys* from *P. maculiventris*.

I hypothesized that occasional or prolonged host switching would increase fitness of *T. japonicus* on the native host. There is evidence that the maternal host environment can influence several life history traits, including host acceptance and offspring performance (Mousseau & Dingle, 1991; Mousseau & Fox, 1998; Hastings & Godfray, 1999; Daza-Bustamante et al., 2003; Saadat et al., 2014; Burke & Carroll, 2017; Botch & Delfosse, 2018), and thus a single generation spent on *P. maculiventris* could have potentially increased subsequent performance on this novel host. The aphid parasitoid, *Aphidius ervi* (Halliday) (Hymenoptera: Braconidae), that can attack several aphid species showed a preference towards the aphid species from which it emerged and the accompanying host plant on which the aphids fed upon (Daza-Bustamante et al., 2003). Similarly, the parasitoid *Bracon hebetor* (Say) (Hymenoptera: Braconidae) had higher preference and better performance on the host species on which it was previously reared compared to other suitable hosts (Saadat et al., 2014). Previous studies established that *T. japonicus* strongly prefers its natal host, *H. halys,* over native North American stink bug species (Botch & Delfosse, 2018; Boyle et al., 2020 Env. Ent; Boyle et al., 2020 Biol. Cont.). One study indicates that maternal effects may have a weak influence on the oviposition behavior and developmental success of *T. japonicus* (Botch & Delfosse, 2018). In choice experiments, *T.*

japonicus that had been reared on *P. maculiventris* showed increased selection of *P. maculiventris* eggs for oviposition (27.3%) compared to when the rearing host was *H. halys* (13.3%) and its emergence rates on the novel host increased slightly from 11.9% to 18.3% when reared on *P. maculiventris* in the generation prior to assays (Botch & Delfosse, 2018). However, in no-choice tests the acceptance of *P. maculiventris* egg masses did not increase after rearing on *P. maculiventris* (Boyle et al., 2020 Biol. Cont.). I found that a history of host switching followed by three continuous generations of rearing on *P. maculiventris* did not increase acceptance or developmental success of *T. japonicus* on this novel host (Fig. 2.4).

While maternal effects, that are a form of phenotypic plasticity, may impact the subsequent generation during host switching events, prolonged exposure to a novel host could result in adaptive evolution and have long-term effect on the genetics of the parasitoids. Parasitoids can adapt to a novel host in as little as three generations (Dion et al., 2011; Rossbacher & Vorburger, 2020; Hopper et al., 2021; Jarrett et al., 2022). For example, rearing of two Drosophilid parasitoids, *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae) continuously on the novel host *D. suzukii* for three generations increased emergence rates by 88% and 259%, respectively (Jarrett et al. 2022). Aphid parasitoids have also shown an ability to overcome novel host defenses within a few generations (Dion et al. 2011, Rossbacher & Vorburger 2020), and *Aphelinus rhamni* (Hopper & Woolley) (Hymenoptera: Aphelinidae) increased its parasitism rate on the novel and suboptimal host, *Rhopalosiphum padi* (Linnaeus) (Hemiptera: Aphididae) by eight-fold within three generations (Hopper et al., 2021). However, I did not find evidence of adaptation for *T. japonicus* on *P. maculiventris* despite three generations of continuous rearing on the novel host in experiments either with or without a history of host switching (Figs. 2.4-2.6).

Lack of adaptation could be related to fitness costs associated with reproducing on a suboptimal host. Various fitness traits may be positively or negatively correlated with adapting to a novel host and thereby may serve to advance or hinder adaptation (Strauss et al., 2006). One such trait may be body size, which has been shown to correlate positively with longevity, lifetime fecundity, offspring emergence rates and sex ratio (Sagarra et al., 2007; Beukeboom, 2018;). When an Ichneumonid parasitoid was reared on a novel host for three generations, adaptation to the novel host was accompanied with increased body size (Jones et al., 2015). In this experiment, the novel host was larger than the ancestral host (Jones et al., 2015). In our study, however, *P. maculiventris* eggs are smaller than *H. halys* eggs and accordingly, *T. japonicus* emerging from *P. maculiventris* are smaller (Botch & Delfosse 2018; Boyle et al., 2020 Biol. Cont.). I know that *T. japonicus* prefers to oviposit in larger eggs (Sabbatini-Peverieri et al., 2021), and thus the smaller egg size of *P. maculiventris* could present a significant obstacle for adaptation to this host. These results could differ with other native stink bug species whose eggs more closely resemble those of *H. halys.*

Besides having a fitness cost in terms of developmental success and emergence rate after one or more generations of reproduction on *P. maculiventris* (Figs. 2.4-2.6), there was also a trade-off for *T. japonicus* when switching back to *H. halys* from *P. maculiventris* (Fig. 2.6). There was a 22% drop in emergence rate when *T. japonicus* switched back to *H. halys* after three generations on *P. maculiventris,* which corresponded to a 34% decline in emergence rate compared to when *T. japonicus* developed on *H. halys* solely (Fig. 2.6). Similar trade-offs were observed for *A. rhamni* (Hopper et al., 2021) and *V. canescens* where there was a 31% decline in development success on the original host following three generations of rearing on a novel host (Jones et al., 2015).

While our two experiments cannot be compared quantitatively, I see some qualitative differences in emergence rates between the history of host switching and no host switching experiments. Overall, the emergence rates for both hosts and treatments were higher in the experiment with a history of host switching, especially for *T. japonicus* populations reared (85.7% vs. 61.6%) and/or assayed on *H. halys* (75.3% vs. 27.7%) (Fig. 2.4 vs. Fig. 2.6). Given that the rearing protocols were the same for the last three generations and then for the reciprocal transplant assays in both experiments, the differences likely stem from differences between the rearing history of parasitoids prior to the last three generations (Fig. 2.1). In the experiment with a history of host switching, the replicated populations spent 10 generations as relatively small, isolated populations (< 300 individuals) (Fig. 2.2) compared to the experiment with no history of host switching, which used parasitoids from our panmictic base colony of > 5000 individuals. I know that small populations are prone to genetic drift, loss of genetic variation and inbreeding (Ingvarsson, 2001; Fauvergue et al., 2012), which can lead to lower fecundity and oviposition rates in parasitoids (Henter, 2007; Tien et al., 2015). Thus, I would expect to see lower fitness in the populations with a history of host switching. However, population bottlenecks can sometimes lead to an increase in additive genetic variation and thereby enhance fitness (Goodnight, 1988; Willis & Orr, 1993; Van Heerwaarden et al., 2008). Alternatively, a phenomenon known as genetic rescue (where immigration to an inbred population increases the fitness of the population) could have occurred when the populations were mixed and re-divided in generation 11 (Ingvarsson, 2001; Tallmon et al., 2004; Whiteley et al., 2015;). This mixing event and the subsequent population growth could have restored genetic diversity and masked deleterious alleles, resulting in heterosis (Ingvarsson, 2001; Tallmon et al., 2004; Whiteley et al., 2015;). Possible heterosis in the history of host switching experiment could also explain why there was a higher fitness cost to reproduction on *P. maculiventris* and a more pronounced trade-off when switching back to *H. halys* from *P. maculiventris* in the no history of host switching experiment. In any case, the history of exposure to different hosts combined with demographic and genetic processes have likely influenced the results and their effects should also be considered in future studies.

While the arrival of *T. japonicus* to North America may be a welcome addition to the toolbox of controlling the invasive *H. halys*, there are valid concerns that it could negatively affect native stink bug populations (Hedstrom et al., 2017; Lara et al., 2019). Availability of *H. halys* and native stink bugs is likely to vary spatially and temporarily, particularly if additional control measures are taken against *H. halys* that reduce its populations (Abrams & Kawecki, 1999). This could result in occasional attack or more prolonged reproduction of *T. japonicus* on native species. Even though I found fitness costs for *T. japonicus* of attacking the native *P. maculiventris*, these costs may not be large enough to prevent non-target attack given the overall high reproductive success: 62-64% after a single generation of exposure and 50-62% emergence rate following three generations of continuous reproduction on *P. maculiventris*. Predatory stink bugs, such as *P. maculiventris*, may be particularly vulnerable to attack by *T. japonicus* since they begin oviposition earlier in the season than *H. halys* (Koch & Rich, 2015; Pezzini et al., 2019). Thus, reproduction on this alternate host could help maintain or boost *T. japonicus* populations until *H. halys* eggs become available, benefiting biological control. However, I saw a significant trade-off when *T. japonicus* switched back to *H. halys* from *P. maculiventris* that may counteract the potential benefit of attacking native species and could compromise its effectiveness as a biocontrol agent.

It is challenging to predict host-parasitoid dynamics in the field due to the myriad biotic, abiotic, temporal, and spatial factors that may affect it. Few studies try to assess non-target attack in the field (Milnes & Beers, 2017; Jarrett et al., 2019), and thus the ecological host range of *T. japonicus* in North America remains largely unknown. In this study, by simulating a few possible scenarios of host switching I aimed to better understand the outcomes of non-target attack and the accompanying fitness changes in *T. japonicus*. I find it somewhat encouraging that there was no improvement in performance of *T. japonicus* on *P. maculiventris* with continued exposure, and that there were fitness costs and trade-offs associated with reproduction on this native host. However, further studies are needed to evaluate how adventive populations of *T. japonicus* are affecting *P. maculiventris* and other native stink bug species in the field.

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CHAPTER 3: FUTURE DIRECTIONS

In the first chapter, I evolved two native, pupal parasitoids on an invasive fly, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). When the experiment was started, another native parasitoid, *Leptopilina heterotoma* (Förster) (Hymenoptera: Figitidae), was tested. This parasitoid attacked the larval stage of the fly rather than the pupal stage. In some populations, *L. heterotoma* can attack *D. suzukii* at low levels but the population captured in Michigan was not able to develop on *D. suzukii* and thus the selection experiments initiated with this species were unsuccessful. So far, there has not been success in improving the developmental success of native larval parasitoids on *D. suzukii* (Kruitwagen et al., 2021). *Drosophila suzukii* is known to have a strong immune response to parasitism in the larval stage. This may be a large hurdle for native larval parasitoids to overcome. When using native parasitoids, I would suggest that future studies use pupal parasitoids ((Kacsoh & Schlenke, 2012; Poyet et al., 2013). However, there are two larval parasitoids, *Ganaspis brasiliensis* (Ihering) and *Leptopilina japonica* (Novković and Kimura) (Hymenoptera: Figitdae), that are natural enemies of *D. suzukii.* Adventive populations of these Asian parasitoids have been found in North America. With their co-evolutionary history, these parasitoids are effective at attacking the larval stage of *D. suzukii* and could contribute to its control (Abram et al., 2019). Future studies could investigate the interactions and competition between exotic and native parasitoids.

Future studies should explore how experimentally evolved pupal parasitoids perform in field conditions. Chapter 1 demonstrated that the parasitoids have the physiological capability to improve their performance on *D. suzukii*. However, there are additional factors in the field that could affect their efficacy. For example, this experiment did not capture the foraging behavior of the parasitoids and their ability to locate the host in the field. Additionally, the flies were reared using an artificial diet. In nature, the flies would lay their eggs in fruit. The type and location of the fruit may influence the parasitoids' ability to locate and oviposit in the host.

In the second chapter, I exposed an exotic parasitoid, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) to a native host, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) both continuously and intermittently. I also attempted to evolve native parasitoids on *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) using methods similar to those described in Chapter 1. I tried *Trissolcus euschisti* (Ashmead) (Hymenoptera: Scelionidae), *Telenomis podisi* (Ashmead) (Hymenoptera: Platygastridae), *Ooencrytus sp* (Ashmead) (Hymenoptera: Encyrtidae)., and *Anastatus sp.* (Motchulsky) (Hymenoptera: Eupelmidae). These parasitoids were able to develop on *H. halys* at low levels, however I was not able to increase most native parasitoid populations high enough to establish colonies. I had the most success with *T. euschisti.* I started 'host switching' experimental lines with this species, analogous to the ones described in chapter 2 with *T. japonicus.* The host switching lines experienced large bottlenecks with as few as four parasitoids emerging in generations when it was reared on *H. halys.* One replicate went extinct after two generations of exposure to *H. halys* and the second replicate went extinct after three generations.

For experiments with *T. japonicus*, we only measured the emergence rate of the parasitoid after exposure to *P. maculiventris* (either host-switching or continuous). Additional metrics could be investigated to provide a greater insight into the evolutionary dynamics of *T. japonicus* reproducing on a sub-optimal host. For example, choice assays could be used to see if the fitness costs to reproducing on *P. maculiventris* affect the host selection behavior of the parasitoid. It may also be beneficial to investigate whether *T. japonicus* populations are able to regain their fitness on *H. halys* after suffering the fitness costs on *P. maculiventris.*

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