Chapter 3

Genetic variation and heritability of parasitization traits in the parasitoid *L. heterotoma* attacking *D. suzukii*

Astrid Kruitwagen
Bregje Wertheim
Leo W. Beukeboom

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* Bregje Wertheim and Leo W. Beukeboom contributed equally to senior authorship
Chapter 3 | Genetic variation and heritability of parasitization traits

Abstract

Insight in the genetic variation and heritability of traits determining parasitization ability is important for understanding and predicting the evolution of host-parasitoid interactions. This knowledge can be applied to artificially select for enhanced parasitization traits for biocontrol. In this study we measured genetic variation of different traits that determine the final outcome of parasitization in European *L. heterotoma* parasitoids attacking the invasive pest *Drosophila suzukii*: (1) attack rate, (2) killing rate (3), lethal attack rate (killing efficiency) and (4) successful parasitism (offspring survival). By testing seven European lines, we found *L. heterotoma* to significantly reduce fly survival, with significant variation in attack rate and killing rate. However, offspring generally failed to successfully develop from the *D. suzukii* host. We crossed these European lines to create a genetically variable source population and performed a half-sib analysis to estimate genetic variance and heritability of these traits. Using a Bayesian animal model to partition the additive genetic variance, we found that attack rate and killing rate had a heritability of $h^2 = 0.2$ and killing efficiency had a $h^2 = 0.4$. These values increased with 0.1-0.2 when accounting for day-to-day differences in fly survival, indicating that environmental and/or host factors had a large effect on parasitization performances. We conclude that parasitoids can influence the population growth of invasive species such as *D. suzukii* in their non-native range. Moreover, the existence of genetic variation underlying host-killing allows for exploitation of this native parasitoid for biocontrol by artificial selection for increased parasitoid performance.

Key-words: biological control agents, genetic improvement, host-parasitoid interactions, invasive species, parasitoid, pest control, selective breeding, virulence, evolution
1. Introduction

Parasitoids are insects that are dependent on other insect hosts for reproduction: they lay their eggs in or on other insects who consequently die as a result of host feeding of the parasitic larvae (Carton, Bouletreau, Alphen, & van Lenteren, 1986; Godfray, 1994). The foraging environment of a parasitoid may however consist of a variety of host species that differ in suitability (Fleury et al., 2004; de Rijk, Dicke, & Poelman, 2013; Torné-Noguera, Arnan, Rodrigo, & Bosch, 2020). One important aspect of host suitability is host resistance, because this can limit the parasitoids’ host-range when its parasitization strategy does not enable it to cope with the host defences (Kraaijeveld, Van Alphen, & Godfray, 1998; Henry, Roitberg, & Gillespie, 2008; Desneux, Blahnik, Delebecque, & Heimpel, 2012). The parasitization strategy of females thus largely affects both parasitoid offspring and host survival.

The process of parasitization can be divided in different ‘steps’, namely, host finding, host inspection and selection, egg laying and offspring development and survival (Fig. 1A) and comprises a combination of behavioural, physiological, and morphological factors (Godfray, 1994; Roitberg, Boivin, & Vet, 2001; Fleury, Gibert, Ris, & Allemand, 2009). Hence, successful ‘completion’ of the steps of parasitization, results in emergence of parasitoid offspring and host mortality, i.e., reproductive host mortality. Clearly, the choices a parasitoid makes during parasitization, such as responding to host(-habitat) related cues and accepting hosts for oviposition, directly influence their reproductive success and fitness (Vet, Lewis, & Carde, 1995; Fatouros, Dicke, Mumm, Meiners, & Hilker, 2008). Strong selection is therefore expected on efficient host selection behaviour that allows for successful parasitoid offspring development. Yet, many studies have shown that mothers’ decisions are not always optimal for offspring survival, i.e., the so-called ‘mother knows best’ hypothesis is often not correct (Thompson, 1988; Henry, Gillespie, & Roitberg, 2005; Chesnais, Ameline, Doury, Le Roux, & Couty, 2015). Poor decision making may be due to insufficient information about the environment or changes in the environment of parents and offspring.

Another factor that can cause maladaptive host-choice decisions is the invasion of a host species exhibiting novel defences to which native parasitoids are vulnerable. Such an exotic host may disrupt the reliability of host-finding cues, resulting in attacking unsuitable hosts next to suitable hosts (Fox & Lalonde, 1993; Schlaepfer, Runge, & Sherman, 2002). Failure of parasitoids to successfully exploit the host can either result in survival of the host when the host escapes the attack or in host death, due to for example mechanical damage during host inspection or immune defence costs (Fig. 1A) (Abram, Brodeur, Urbaneja, & Tena, 2019).

To predict and determine whether adaptation to the invader could occur, insight is needed in the genetic variation of traits that influence the final outcome of parasitization (Fox & Lalonde, 1993; Schlaepfer et al., 2002; Strauss et al., 2006; Berthon, 2015). These traits include attack rate, the parasitoids’ ability and willingness to find and exploit hosts, killing rate, the parasitoids’ ability to reduce the survival rate of the attacked hosts and successful
parasitism, and the parasitoids’ ability to circumvent host resistance for offspring emergence, thus whether host killing results in parasitoid offspring (Fig. 1). Parasitoids might be able to find and attempt to exploit (novel) unsuitable hosts, even when this does not necessarily result in parasitoid offspring production. When these attacks and unsuccessful parasitization attempts kill the host, this is termed non-reproductive host-killing (Abram, Brodeur, Burte, & Boivin, 2016; Abram et al., 2019). This non-reproductive parasitism might therefore provide a starting condition for adaptation to the novel hosts when individuals appear whose parasitization strategy allows for offspring development (Abram et al., 2019). On the other hand, mismatch in host selection and offspring development might also result in evolution of host species discrimination to avoid oviposition in unsuitable host species (Schlaepfer, Sherman, Blossey, & Runge, 2005; Keeler & Chew, 2008). Hence, insight in genetic variation underlying these components of parasitization is central to understand host-parasitoid interactions, parasitoids’ reproductive success and the evolution of host range.

The study of genetic variation of parasitization performance in native parasitoids to attack invasive species is also important for biological control. Ideally, biocontrol agents are highly efficient in killing the invasive pest. Therefore, traits that influence parasitization are key for selection of candidate parasitoids for biocontrol application. In case of exotic pest species, finding and importing natural enemies from the invaders’ native range that are adapted to the pest is hampered by the Nagoya protocol (Cock et al., 2010; van Lenteren et al., 2011; Hajek et al., 2016). A potential alternative approach is the improvement of the biocontrol efficiency of non-adapted resident parasitoids by exploitation of natural genetic variation through selective breeding (Lommen et al., 2017; Kruitwagen, Beukeboom, & Wertheim, 2018). To explore the potential of this method, a crucial first step is to investigate the amount of intra-specific variation in parasitization performance and determine whether this variation has, at least partly, a genetic basis (Wajnberg, 2004b; Lommen et al., 2017; Kruitwagen et al., 2018).

In this study we investigate intraspecific variation in parasitization of the native parasitoid *L. heterotoma* on the non-native host *Drosophila suzukii*. This host was accidentally introduced in Europe and North America in 2008 and has become a major pest of soft fruits (Walsh et al., 2011; Cini et al., 2014; De Ros, Conci, Pantezzi, & Savini, 2015; Mazzi et al., 2017). Despite the relatively high virulence of this common European parasitoid wasp for native drosophilids, it has a low reproductive success when attacking *D. suzukii* (Chabert, Allemand, Poyet, Eslin, & Gibert, 2012; Poyet et al., 2013; Mazzetto et al., 2016; Knoll, Ellenbroek, Romeis, & Collatz, 2017). This is due to the strong immune response of the host to a wide range of parasitoid species (Kacsoh & Schlenke, 2012; Poyet et al., 2013; Iacovone, Ris, Poirié, & Gatti, 2018). This system therefore provides an interesting opportunity to investigate the genetics of traits that influence the final outcome of parasitization of a novel, highly resistant host. Moreover, study of the genetic basis of parasitization performance will provide more insight in the evolution of host-range and the potential of native parasitoids to be improved by artificial selection for use as biocontrol agents against *D. suzukii*. 
We first tested and compared seven European \textit{L. heterotoma} strains for four parasitization performance indices: (1) attack rate, (2) killing rate, (3) lethal attack rate (killing efficiency) and (4) successful parasitism (offspring survival) (Fig. 1, Table 1). As we found considerable variation among populations, indicating the presence of natural genetic variation, we next crossed these lines to produce a genetically variable population to further assess the genetic basis underlying these traits for genetic improvement for biocontrol. Key parameters to estimate the amount of genetic variation of a trait and its potential to respond to selection are additive genetic variance and narrow-sense heritability (Falconer & Mackay, 1996; Lommen et al., 2017). These are defined as the genetic effects that are independent of the genotype in which they occur (thus the main part on which selection acts) and the proportion of the total phenotypic variation due to additive genetic effects respectively. To estimate additive genetic variance and heritability values, we performed a half-sib analysis and used a Bayesian ‘animal model’ approach adapted to haplodiploids, to separate additive genetic effects from other sources of variation. We consider our results in the context of genetic improvement of this native parasitoid toward the invasive \textit{D. suzukii}.  

Figure 1 | The process of parasitization in parasitoid wasps. (A) Partitioned in different steps and outcomes for the host and parasitoid, and (B) translated to three parasitization traits. Note that the attack and killing rate include different mechanisms of parasitoid-induced host mortality, namely reproductive host killing – i.e., host mortality as consequence of successful parasitoid offspring development, and non-reproductive host killing – i.e., host mortality without successful parasitoid offspring development.
Table 1 | Description of parasitization performance indices used in this study. Each trait was also quantified by adjustment for average fly mortality of non-exposed flies, i.e., standardized (std) trait.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>Attack rate</td>
<td>Percentage of flies killed plus flies that survived parasitoid exposure but with encapsulated wasp egg</td>
</tr>
<tr>
<td>Killing rate</td>
<td>Percentage of flies killed out of a batch of 25 hosts</td>
</tr>
<tr>
<td>Lethal attack rate</td>
<td>Percentage of flies killed out of the total flies attacked</td>
</tr>
<tr>
<td>Successful parasitism</td>
<td>Percentage of killed flies that yielded wasp offspring</td>
</tr>
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2. Material and methods

Biological material

Seven strains of *Leptopilina heterotoma* were set up from different European locations: two from the Netherlands (collected from Vosbergen, NL-Vb, in 2012 and Wageningen, NL-Wa in 2016), two from Spain (Girona, SP-Gi in 2016 and Santa Christina d’Aro, SP-Sa in 2015) and three from France (Saint Etienne sur Chalaronne, FR-Sa, St Marcel Les Valence, FR-Sm and Bellegarde, FR-Be in 2012). Parasitoids were maintained on a relatively low-resistant *Drosophila melanogaster* host strain (WW) at 25°C, under a light-dark regime of 16:8. These flies were derived from wild flies collected near Leiden, the Netherlands, received in 2009, and kept as mass cultures at 20°C in quarter pint bottles containing 30 ml “WW” medium (agar (17g/L), yeast (26g/L), sugar (54g/L) and nipagine (16,7 ml/L)).

Parasitoids were tested and selected on *D. suzukii* collected from Westland, the Netherlands in 2016. *Drosophila suzukii* were reared in quarter pint bottles containing 30 ml cornmeal diet (“DS” medium) (agar (10 g/L), glucose (30 g/L), sucrose (15 g/L), heat-inactivated yeast (35 g/L), cornmeal (15 g/L), wheat germ (10 g/L), soya flour (10 g/L), molasses (30 g/L), propionic acid (5 mL/L), and Tegosept (2 g/L)).

Standardized parasitization performance test

A standardized test was used for measuring four parasitoid performance indices: attack rate, killing rate, lethal attack rate and reproductive success. Tested females were at least five days old before their performance was measured. To make sure larvae did not die due to “clumsiness” of inexperienced wasps (Samson-Boshuizen, Bakker, & van Lenteren, 1973), individual wasps were first given experience with *D. suzukii* larvae for several hours. Next, each individual female was placed in a vial for four hours with 25 late-first/second instar *D. suzukii* larvae on *Drosophila* medium. Experiments were done at 25°C, and insects had access to a honey droplet on the cotton plug. On each testing day at least 10 vials were maintained that were not exposed to parasitoids, to measure *D. suzukii* survival in the absence of a parasitoid. The number of emerging adult *D. suzukii* flies (*f*) and parasitoid offspring (*p*) were counted to quantify parasitization performances. To account for temporal fluctuations in *D. suzukii* survival, performances were corrected for average fly survival of non-exposed flies (control fly survival, *n*) on the same testing day. The attack rate was the percentage of flies that were parasitized, as estimated from the excess mortality in larvae due to wasp exposure and the number of flies that were attacked but survived (i.e., flies that successfully mounted an immune response (encapsulation)). Encapsulation of wasp eggs by
the host was quantified by squashing the flies between two object glasses and inspection under the microscope for presence of a melanized egg. Emerged flies were inspected under the microscope for presence of at least one encapsulated parasitoid egg to quantify the number of hosts that had been parasitized but successfully mounted an immune response (encapsulation) \((e)\) and those without capsules \((w)\). The percentage of flies that each wasp attacked, corrected for the mortality in non-exposed larvae, was then calculated as:

\[
\text{attack rate} = \frac{n - f - e}{n} = \frac{n - w}{n} \cdot 100\% 
\]

Each wasps’ killing rate was calculated as the percentage of flies killed in excess to the mortality in non-exposed flies:

\[
\text{killing rate} = \frac{n - f}{n} \cdot 100\% 
\]

When the killing rate was negative for an individual (i.e., \(f > n\)), it was set to zero (i.e., \(f\) was set to \(n\)). The efficiency at which flies were killed was calculated as the proportion of flies killed from the total number of “attacked” flies:

\[
\text{lethal attack rate} = \frac{\text{killing rate}}{\text{attack rate}} = \frac{n - f / n}{n - w / n} = \frac{n - f}{n - w}
\]

The proportion of killed flies that yielded wasp offspring was measured as indication of each individuals’ successful parasitism:

\[
\text{successful parasitism} = \frac{p}{n - f} \cdot 100\% 
\]

**Establishment of a genetically diverse line**

European populations of *L. heterotoma* were first tested and compared for their ability to parasitize *D. suzukii* following the standardized individual performance test. Next, a genetically diverse laboratory strain was created to estimate heritability of parasitization performances following a reciprocal crossing scheme using the seven European populations. This method ensured equal genetic contribution of all wasp strains and could potentially lead to new allelic combinations. Moreover, it enabled monitoring of potential masked effects of mating preferences, incompatibilities (e.g., due to *Wolbachia* presence) and deleterious effects of homozygotes. Unmated males and females from two different lines were put together (without hosts) for 3-5 days to assure mating. Next, females were placed on second instar *D. melanogaster* larvae to reproduce. No signs of unviability or high male/female biased sex ratio (>70%) were detected in the F2 except for crosses between FR-Sa and FR-Be which were highly male biased. These were therefore repeated to ensure their genetic contribution to the mix population.
Heritability – half sib design

Half-sib families were created by mating one male (sire) with three virgin females (dams), and three offspring of each female were tested for parasitization performances. First, parents (P) were randomly collected from the genetically diverse line by separation of pupae in individual vials prior to emergence of the adult wasps. Next, 1-3-day old unmated males were allowed to mate with three unrelated 1-3-day old virgin females by placement in vials with agar (simultaneously). They were provided with honey as food and kept at 25°C for 3 days. To generate a new generation (F1) each female was provided with 20-30 \textit{D. melanogaster} larvae on our standard WW rearing medium to parasitize for 24 hours. This setup ensures relatively high-quality hosts, as prior tests showed high host survival rate (>90%) in the absence of wasps (Kruitwagen et al. unpublished data). Three daughters per female were tested for parasitization performances on \textit{D. suzukii}. To reduce common environmental effects when comparing siblings (i.e., similarities within families due to shared environmental experience rather than genetic differences), F1 females were placed on three different host batches each for 24h. Female F2 offspring were allowed to mate with their brothers, by keeping offspring from each host-batch in each vial for at least 3 days after emergence. Next, from each host-batch, one F2 female was randomly selected and tested. Female offspring were tested for the four parasitization performance components. Due to practical restrictions, families were tested in five different blocks. Offspring performances of each block were tested on the same day.

Data analysis

All analyses were done in R (version 3.6.1) (R Core Team, 2020). Parasitization performance indices of European lines were analysed with generalized mixed models for binomial data by specifying a two-column matrix with the number of “successes” and “failures” using the \texttt{lme4} package (Bates, Machler, Bolker, & Walker, 2015). Parasitization performances were compared by correcting for day-to-day variation in fly survival. To this end, performances were standardized with the average fly survival of the control flies that were not exposed to wasps on the same testing day (n). Testing date and replicate line were included as random effects. When data were overdispersed, observation level random effect was added (Harrison, 2015). Significance of main effects was tested by comparing the full model to the model without the fixed effect by ANOVA. Post-hoc comparison of means was performed with Tukey tests, using the \texttt{emmeans} package (Lenth, 2020).

The additive genetic variance was estimated with an ‘animal model’ using the \texttt{MCMCglmm} R package (version 2.29). Unlike the ‘classical’ approach, which entails partitioning variance using a mixed effect model with nested random effects, this Bayesian approach can be applied to unbalanced pedigrees and generalized models when normality is violated (Wilson et al., 2010), which was the case in our study. Estimates of additive genetic variation are based on pedigree data describing all known individual relationships. To account for haplodiploid sex determination of the parasitoids, following Sheehan, Choo, and Tibbetts (2017), the genetic covariance matrix was estimated using the “\texttt{makeS}” function of the \texttt{nadiv} package (Wolak, 2012).
Each parasitization performance trait was analysed by specifying a two-column matrix with the number of “success” and “failures”, using the “multinomial2” family argument (Hadfield, 2010). As parasitization performances are also expected to be partly determined by the fitness of the fly hosts, models were also fitted by taking variation in fly survival into account between the five testing days by standardizing parasitization performances with the average fly survival of the controls that were not exposed to wasps. Animal ID was fitted as random effect to estimate the additive genetic variance. Moreover, measurement day and mother ID were taken as random effects to account for similarities between individuals measured on the same measurement day and for influence of the mother. One block was omitted because the average *D. suzukii* survival of the control group was about half of the other 4 blocks (10.8, vs 21.1, 20.0, 22.0, 20.0), suggesting a low-quality host batch, which makes estimation of parasitization performances of these wasps unreliable. From the models, we computed the quantitative genetic parameters: additive genetic variance and heritability. The narrow sense heritability ($h^2$) was estimated by dividing the genetic variance component by the total phenotypic variance ($V_a/V_{total}$). We added 1 to the denominator due to the probit link function.

Weakly uninformative priors were chosen. The residual variance ($V_r$) was fixed to 1 as for binomial-related families the residual variance is not identifiable (de Villemereuil, 2012). Note that $V_a$ scales with the value of $V_r$, meaning that heritability estimates are roughly non-sensitive to the actual value to which $V_r$ is fixed (Pierre de Villemereuil, personal communication). For the random effects (which includes $V_a$) the inverse-Gamma prior is advised and commonly used ($V=1$, $\nu=0.002$ in MCMCglmm) (Hadfield, 2010). However, as this places too much weight on 1 when estimating heritability in binomial traits, following de Villemereuil (2012) and de Villemereuil, Gimenez, and Doligez (2013), we used the Chi-square distribution with 1 degree of freedom ($V = 1$, $\nu = 1000$, $\alpha_\mu = 0$, $\alpha_V = 1$ in MCMCglmm). This improves the rate of convergence and shows a relatively close uniform distribution of heritability. Posterior distributions were sampled 910,000 times. Autocorrelation (<0.1) and effective sample size (>1000) were verified to increase confidence in parameter estimates and convergence was tested with the Heidelberg stationary test (Wilson et al., 2010; de Villemereuil, 2012).

### 3. Results

**Strain differences in parasitization performance**

Females of *L. heterotoma* of all seven European populations readily accepted *D. suzukii* hosts for parasitization (Fig. 2a). Parasitization by *L. heterotoma* significantly reduced *D. suzukii* survival (GLMM, $\beta = -0.97$, $\chi^2(1) = 5.77$, $p = 0.016$), and killed on average 37.4% ± 2.74 SE of the flies with a range of 0-100%. Populations differed in killing rate (i.e., the percentage of flies killed after adjustment for fly mortality of non-exposed larvae) (GLMM, $\chi^2(6) = 122.38$, $p < 0.01$) (Fig. 2b): the FR-Sm line had a significantly lower killing rate compared to NL-Wag, FR-Be, and SP-Gi, and the SP-GI population showed a higher killing rate than SP-Sa and NL-VB (Tukey’s post hoc test, $p < 0.05$). The differences in attack rate approached significance (GLMM, $\chi^2(6) = 12.28$, $p = 0.056$) (Fig. 2a), but the
proportion of attacked flies that were killed differed significantly (Fig. 2c) \(\chi^2(6) =19.52, p=0.003\), and ranged between 0 and 100% with an average of 49.3% ± 3.24 SE. The FR-Sm population was less efficient in host-killing compared to FR-BE, SP-Gi and NL-Wa (Tukey post hoc test, \(p<0.001\)). The percentage of killed flies that yielded offspring did not differ (GLMM, \(\chi^2(6)=3.03, p=0.8\)), and was nearly zero in all populations (Fig. 2d). Of the 2070 fly hosts that were exposed to parasitoids, only five yielded offspring (0.24%) (2 by SP-Gi, and 3 by FR-Be). In other experiments, parasitoids of FR-Sa and NL-Wa also occasionally successfully reproduced on *D. suzukii* (A. Kruitwagen, unpublished data).

**Figure 2** | Parasitization performances of seven European *L. heterotoma* strains attacking *D. suzukii*: (A) attack rate, (B) killing rate; (C) lethal attack rate, and (D) successful parasitism. Boxplots provide data for ten replicates per strain, each replicate containing 30 larvae that were exposed to two parasitoid females. Each performance index was standardized for fly mortality when not exposed to wasps; for trait description see Table 1. Abbreviations: FR, France; NL, The Netherlands; SP, Spain, for strain name abbreviations see Material & Methods. Horizontal lines represent median, top and bottom are 25th and 75th percentiles and points are outliers.

**Additive genetic variation and heritability of parasitization performances**

In total 68 sires, 122 dams and 357 offspring were analysed. Not each sire mated successfully with the three females provided: 33 sires mated with two dams and 28 with one dam. Offspring of each female were tested for their parasitization performances on *D.*
Table 2 | Parasitization performances of wasps from a genetically variable line of *L. heterotoma*. The line was generated by reciprocally crossing 7 European parasitoid strains. For each of the parasitization performance indices (in percentages), we provide the mean value, standard deviation (SD), standard error of the mean (SE), and minimum and maximum value. These measurements are based on *n* = 357 parasitoid wasps that were tested in four blocks.

<table>
<thead>
<tr>
<th>Trait (%)</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>Min</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Killing rate</td>
<td>48.78</td>
<td>24.561</td>
<td>1.299</td>
<td>8.0</td>
<td>100</td>
</tr>
<tr>
<td>Std. killing rate</td>
<td>38.15</td>
<td>28.898</td>
<td>1.529</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>Attack rate</td>
<td>4.18</td>
<td>23.318</td>
<td>1.234</td>
<td>12.0</td>
<td>100</td>
</tr>
<tr>
<td>Std. attack rate</td>
<td>68.57</td>
<td>28.234</td>
<td>1.494</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>Lethal attack rate</td>
<td>67.76</td>
<td>25.740</td>
<td>1.362</td>
<td>15.4</td>
<td>100</td>
</tr>
<tr>
<td>Std. Lethal attack rate</td>
<td>53.70</td>
<td>34.083</td>
<td>1.803</td>
<td>0.0</td>
<td>100</td>
</tr>
<tr>
<td>Successful parasitism</td>
<td>0.96</td>
<td>3.307</td>
<td>0.175</td>
<td>0.0</td>
<td>20</td>
</tr>
<tr>
<td>Std. Successful parasitism</td>
<td>1.68</td>
<td>6.090</td>
<td>0.322</td>
<td>0.0</td>
<td>50</td>
</tr>
</tbody>
</table>

suzukii (Table 1). Killing rate, attack rate and lethal attack rate all showed large intra-specific phenotypic variation ranging from 0 to 100% and related individuals exhibited comparable parasitization performance; this was not the case for successful parasitism (Table 2, Fig. 3). Animal models demonstrated significant additive genetic effects underlying attack rate, killing rate and lethal attack rate but not for successful parasitism which had (almost) zero genetic variance (Table 3). Day of measurement (families were tested in four different blocks) also influenced phenotypic variation, indicated by a significant effect of ‘testing day’ (Table 3), but ‘mother’ did not, which includes variance due to common maternal environment. This non-genetic factor thus also contributes to the observed phenotypic variation in parasitization performances. Heritability, i.e., the proportion of phenotypic variation due to additive genetic effects, was significantly higher than zero for killing rate, attack rate and lethal attack rate (Table 3). Heritability was about 0.1 – 0.2 larger when standardized for natural fly survival, indicating that environmental or host effects influenced trait value expression. Attack rate showed low to moderate heritability, for the standardized $h^2=0.22$ and for the non-standardized $h^2=0.44$. Heritability of host-killing was relative lower: for the standardized $h^2=0.15$ and non-standardized $h^2=0.28$. Standardized lethal attack rate (killing efficiency) had the largest heritability of 0.6 and successful parasitism the lowest, i.e., close to zero.

### 4. Discussion

In this study we investigated genetic variation and heritability of parasitization traits of the parasitoid wasp *L. heterotoma* on the non-native pest *D. suzukii* by quantification of four parasitization performance indices: attack rate, killing rate, lethal attack rate and successful parasitism. If parasitization is successful, it results in parasitoid offspring and host mortality (‘reproductive host mortality’). In line with previous studies (Chabert et al., 2012; Kacsoh & Schlenke, 2012; Mazzetto et al., 2016; Stacconi et al., 2017; Iacovone et al., 2018), our study revealed that European *L. heterotoma* populations exhibited a near zero reproductive success on this novel host, but significantly reduced fly survival. Such parasitoid induced host death without reproducing is known as non-reproductive host killing. This is thus an
additional source of host mortality due to parasitism, besides the host mortality from parasitoid offspring development. Interestingly, we also found large variation between European populations in attack rate and non-reproductive host killing, indicating intra-specific genetic variation. We then questioned to what extent the observed phenotypic variation in parasitization performances is determined by genetic effects and is heritable, which is essential to predict evolutionary potential and response to selection for genetic improvement (Falconer & Mackay, 1996; Wajnberg, 2004b; Lommen et al., 2017; Kruitwagen et al., 2018). We therefore crossed the European lines to create a genetically variable *L. heterotoma* line and constructed half-sib families to quantify the amount of genetic variance and heritability of traits that influence the final outcome of parasitization. There was no genetic variation in successful parasitism. However, ‘animal models’ showed significant additive genetic effects in attack rate, killing rate and lethal attack rate. Moreover, these traits had moderate to high heritability (0.2-0.6).

Heritability was larger for lethal attack rate (i.e., the proportion hosts killed out of the attacked ones) compared to the attack and killing rate. The latter two are likely mainly determined by behavioural traits (e.g., activity level, host finding ability), whereas killing efficiency may have a physiological basis (e.g., venom composition). This is in line with the general observation that behavioural traits have low heritability ($h^2 < 0.3$) (Mousseau & Roff, 1987; Stirling, Réale, & Roff, 2002; Dochtermann, Schwab, Anderson Berdal, Dalos, & Royauté, 2019). A low heritability can indicate low genetic variation underlying phenotypic variation, possibly due to strong selection eroding intra-specific genetic

Figure 3 | Performance per half-sib family (sire), ordered on median values of killing rate. Performances were standardized for the average fly mortality of non-exposed flies; for trait description see Table 1. Horizontal lines represent median, top and bottom are 25th and 75th percentiles and points are outliers.
Table 3 | Variance components and heritability of parasitization performances of *L. heterotoma* tested on *Drosophila suzukii* hosts from Bayesian animal models. Models were compared with and without standardization for average *D. suzukii* mortality on the same measurement day when not exposed to wasps. Point estimates are posterior mode and ranges are 95% credible interval of the posterior mode, i.e., the range of values that the parameter takes with 95% probability. Note that residual variance was set to 1 due to nature of the data (see material and methods).

<table>
<thead>
<tr>
<th>Variance components</th>
<th>Heritability ($h^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Additive genetic variance ($V_a$)</td>
</tr>
<tr>
<td>Killing rate</td>
<td>0.413 (0.256-0.688)</td>
</tr>
<tr>
<td>Std. killing rate</td>
<td>1.077 (0.7406-1.553)</td>
</tr>
<tr>
<td>Attack rate</td>
<td>0.658 (0.287-1.117)</td>
</tr>
<tr>
<td>Std. attack rate</td>
<td>1.886 (1.091-1.054)</td>
</tr>
<tr>
<td>Lethal attack rate</td>
<td>2.784 (1.819-3.831)</td>
</tr>
<tr>
<td>Std. lethal attack rate</td>
<td>4.656 (3.362-6.462)</td>
</tr>
<tr>
<td>Successful parasitism</td>
<td>0.01052 (2.172e-6 - 1.364)</td>
</tr>
<tr>
<td>Std. successful parasitism</td>
<td>0.004270 (2.274e-7 - 0.989)</td>
</tr>
</tbody>
</table>

variation (Falconer & Mackay, 1996). This would imply that attack and killing rate are under strong natural selection, whereas the lethal attack rate (the efficiency by which hosts are killed) is not. It can also indicate that these traits are influenced to a relatively large extent by environmental and non-additive genetic variance (Price & Schluter, 1991; Houle, 1992). As such, even when there is a relative low heritability, there might still be considerable natural genetic variation present and these traits might still respond to selection.
In our experiment we attempted to reduce environmental variation to improve our estimate of genetic parameters. Parasitoids were reared on low resistant hosts (*D. melanogaster*) in standardized larval densities and nutritious rich environment to ensure high quality hosts, and to reduce variance induced by the developmental host conditions (see also this thesis Chapter 4). Moreover, all hosts were similar in age and the parasitoids were tested at constant temperature and at the same time of day (afternoon). Yet, several environmental factors could have induced phenotypic variation. We found that the heritability estimates standardized for day-to-day differences in fly mortality were larger compared to the unscaled models. Standardized traits were scaled to average fly survival, thereby reducing variation induced by differences in fly condition. Hence, this suggests that either environmental and host factors also induced variation in parasitization performances. The *D. suzukii* hosts might differ in condition and behaviour, for example due to (small) differences in nutritional status, size, genotypes and their location within a patch (e.g., in or on the food medium) which could impair host finding. Furthermore, variation in parasitization could have been due to differences in the parasitoids’ status/condition, such as whether they had mated or not (Pompanon, Fouillet, & Bouletreau, 1999), their body size (this thesis Chapter 4) and fat reserves (Ellers, Van Alphen, & Sevenster, 1998).

It should be noted, however, that the estimates of additive genetic variance and heritability are influenced by the statistical model used (Wilson, 2008), the population of investigation and the conditions in which the individuals are tested (Falconer & Mackay, 1996; Wilson et al., 2010). Consequently, although heritability estimates are useful for predicting response to artificial selection in controlled conditions and detecting presence of genetic variation, the question is how to translate these predictions to the field (Weigensberg & Roff, 1996; Hoffmann, 2000; Blanckenhorn, 2002). Evolution in nature likely takes place under different (a)biotic conditions in which selection pressure might vary and change. Hence, care should be taken by extrapolating heritability estimates and predicting evolutionary trajectories. This also applies to our finding of genetic effects of non-reproductive host-killing. Attacking and killing hosts without reproducing is clearly maladaptive. This raises the question what maintains heritable variation in attack rate and non-reproductive host killing and how it influences the evolution of parasitization. The parasitoids’ parasitization strategy is likely adaptive for exploiting host species with other level/type of resistance. Non-reproductive host-killing might therefore be a ‘by-product’ of virulence when attacking unsuitable hosts, which could maintain non-reproductive host killing in nature. As there was a lack of ability in the parasitoids to overcome *D. suzukii* resistance, it would be expected that selection will act on host choice. As such, avoidance of unsuitable hosts might be more likely to evolve, resulting in host-range conservation rather than host-range expansion/shift. Overall, to predict the ecological and evolutionary consequences of an invader host species on resident parasitoids, more insight is needed in host-parasitoid population dynamics in nature (e.g., parasitoid encounter rates with different host types), fitness costs of attacking the novel hosts, and the genetic variation for parasitization traits.

In conclusion, although the reproductive success of *L. heterotoma* was nearly zero on *D. suzukii*, we found significant genetic variation in host killing. Lethal ovipositor probing or immune defence costs, for example, can negatively affect the hosts’ survival rate (Abram et
Therefore, even without being able to reproduce, parasitoids can still have considerable effects on the unsuitable host population, and thus be valuable for biological control (Abram et al., 2016; Kaser, Nielsen, & Abram, 2018). Interestingly, although this source of host-killing is often reported in literature, it has received little attention in the ecology and evolution of host-parasitoid interactions and for controlling pest populations (Abram et al., 2016). Moreover, genetic parameters have been measured for a large variety of traits, including proxies of parasitization (e.g., Geden, Smith, Long, & Rutz, 1992; Henter, 1995; Cronin & Strong, 1996; Olson & Andow, 2002; Andow & Olson, 2003; Henry, May, Acheampong, Gillespie, & Roitberg, 2010; Samková, Hadrava, Skuhrovec, & Janšta, 2019), but, to our knowledge, not explicitly for non-reproductive effects (but see Carton, Capy, & Nappi, 1989). Consequently, little is known about the sources of variation in parasitoid-induced host mortality without reproduction. We here report that genetic variation underlies the intraspecific variation in non-reproductive host killing. This opens possibilities for selecting resident parasitoids to control invasive pests, as part of pest management programs to develop and improve biocontrol agents.

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