

Plasticity of Body Growth and Development in Two Cosmopolitan Pupal Parasitoids

Xingeng Wang¹, Brian Hogg², Antonio Biondi³, and Kent Daane⁴

¹USDA ARS

²USDA Agricultural Research Service

³Universita degli Studi di Catania

⁴University of California, Berkeley

September 28, 2020

Abstract

1. *Pachycrepoideus vindemiae* and *Trichopria drosophilae* are cosmopolitan pupal parasitoids of *Drosophilidae* that attack the invasive *Drosophila suzukii*. This study investigated one aspect of their phenotypic plasticity – host acceptance and offspring fitness on 25 *Drosophila* species in a phylogenetic framework. Each parasitoid's key biological and ecological traits were compared among the different host species. 2. Results demonstrate that both parasitoids successfully parasitized and developed from all tested host species. Although the parasitoids' efficiency and offspring fitness varied among host species, effects on life-history characteristics or ecological traits appeared to be unrelated to the phylogenetic position of tested host species. 3. Both parasitoids benefited from attacking larger hosts, with body size of emerging progeny positively correlated to host size and an increased fecundity (mature egg load) of female wasps. Achieving larger body size came at no significant costs in immature development time. 4. The results show remarkable levels of phenotypic plasticity in the parasitoids' body growth and development, which is discussed with respect to their potential for host range expansion.

1. Introduction

Phenotypic plasticity has been discussed with respect to invasive species success and native species adaptation to environmental disturbances such as climate change (e.g., Wilson, Mullen & Holway, 2009; Davidson, Jennions & Nicotra, 2011; Hoffmann, Chown & Clusella-Trullas, 2013; Merila & Hendry, 2014; Kingsolver & Buckley, 2018). Plasticity in host specialization can also impact the success and effectiveness of insect parasitoids that provide ecosystem services (Desneux, Blahnik, Delebecque & Heimpel, 2012; Biondi, Desneux, Amiens-Desneux, Siscaro & Zappala, 2013). An example is a parasitoid's ability to utilize phylogenetically related host species that differ in quality; the ability to use multiple hosts is likely to increase the persistence and abundance of parasitoids (Bribosia, Bylemans, Migon & Van Impe, 2005), but may affect a parasitoids' key life-history characteristics and ecological traits (Godfray, 1994; Harvey, Harvey & Thompson, 1994). This is particularly true for parasitoids that attack non-growing host stages, such as pupae, as their offspring must develop on the host resources present at the time of oviposition (Ueno, 1998; Chen et al., 2018). Within the narrower focus of classical biological control, natural enemies that exhibit plasticity to environmental tolerances are sought after whereas natural enemies that exhibit host species plasticity are rarely purposely released (Heimpel & Cock, 2018; Hoddle, Lake, Minter & Daane, 2020).

Here, we report on the phenotypic plasticity of two insect parasitoids with respect to host size, which is often positively correlated with parasitoid fitness (Visser, 1994; Kazmer & Luck, 1995; Ellers, van Alphen & Sevenster, 1998; Eijs & van Alphen, 1999; Jervis, Ferns & Heimpel, 2003). Within the range of acceptable host sizes, a parasitoid population often benefits when female wasps oviposit into larger hosts (Stephens &

Charnov, 1982; King & Lee, 1994; Otto & Mackauer, 1998; Teder, Tammaru & Pedmanson, 1999). For example, Wang and Messing (2004b) showed that *Dirhinus giffardii* Silvestri, a pupal ectoparasitoid, prefers to attack larger tephritid host species, with numbers of emerged offspring, progeny size, and adult progeny searching efficiency all positively correlated to host size. However, the fitness gain from being larger can come at the cost of other traits, such as developmental time, which often increases with body size (Sequeira & Mackauer, 1992; Harvey, Harvey & Thompson, 1994; Petersen & Hardy, 1996; Harvey & Strand, 2002). In some cases, host quality may not increase with host size (e.g., Wang & Messing, 2004a), therefore, a parasitoid's plasticity to host size may depend on the degree of physiological and nutritional compatibility between parasitoid and host.

The plasticity of the drosophilid parasitoids *Pachycrepoideus vindemiae* (Rondani) (Hymenoptera: Pteromalidae) and *Trichopria drosophilae* (Perkins) (Hymenoptera: Diapriidae) were studied as part of a biological control effort against the invasive spotted wing drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), which has become a serious pest of various small fruit crops (Asplen et al., 2015). Quarantine studies to determine a parasitoid's suitability for release are, to some extent, an investigation of its plasticity and here the focus was on the host range plasticity of *P. vindemiae* and *T. drosophilae*. The former species oviposits into host pupae, immediately paralyzing the host (typical idiobiont), and then develops as a solitary ectoparasitoid enclosed by a protective puparium formed from the fly's hardened exoskeleton (Wang & Messing, 2004c). The latter species also oviposits into host pupae but develops as a solitary endoparasitoid that kills the host after the parasitoid larva begins feeding (typical idiobiont) (Wang et al., 2016b). Like other ectoparasitoids, *P. vindemiae* produces relatively few large eggs, whereas *T. drosophilae* produces high numbers of small eggs and is thus generally more efficient than *P. vindemiae* under suitable laboratory conditions (Rossi Stacconi et al., 2015; Wang, Kaçar, Biondi & Daane, 2016a; Kaçar, Wang, Biondi & Daane, 2017). *Pachycrepoideus vindemiae* appears to be more of a generalist than *T. drosophilae* and can act like a hyperparasitoid of other primary fruit fly parasitoids (Wang & Messing, 2004a), whereas the host range of *T. drosophilae* is thought to be more limited (Carton et al., 1986).

To date, *P. vindemiae* and *T. drosophilae* are among the few resident parasitoid species that successfully attack *D. suzukii* in the invaded regions of the Americas and Europe (Lee et al., 2019). Both species are efficient parasitoids of *D. suzukii* in laboratory settings (e.g., Kaçar et al., 2017; Wang et al., 2018; Wolf, Boycheva-Woltering, Romeis & Collatz, 2020); however, naturally occurring parasitism of *D. suzukii* populations has been generally low, likely due to a lack of host specificity in these parasitoids. Recent studies have reported the performance of *P. vindemiae* and *T. drosophilae* against *D. suzukii* and the common host *D. melanogaster* or a few other species (Wang, Kaçar, Biondi & Daane, 2016b; Chen et al., 2018; Woltering, Romeis & Collatz, 2019; Yi et al., 2020). Information is lacking, however, on the performance of these two parasitoids across a wide range of *Drosophila* species within a phylogenetic framework – basically, at the fringe of host species acceptance for parasitoids exhibiting plasticity is there an impact on offspring fitness. Therefore, this study sought to use these parasitoids as model species to investigate the more theoretical issue of body-size plasticity in parasitoids. From an applied point of view, this study also sought to determine potential host use to aid in field studies of *D. suzukii* biological control.

2. Materials and methods

2.1 Insects

All bioassays and insect rearing were conducted under controlled conditions ($23 \pm 1^\circ\text{C}$, 16L:8D, 40–60% RH) at the University of California (UC) Kearney Agricultural Research Center in Parlier, California, USA. As described in Wang et al. (2016b), colonies of *D. suzukii*, *P. vindemiae* and *T. drosophilae* were initiated from parasitoids field-collected from nearby farms, with new material periodically introduced to maintain colony vigor. Adult flies were held in Bug Dorm cages (BioQuip Products Inc., Rancho Dominguez, CA), while adult parasitoids were held in screened cages ($30 \times 30 \times 30$ cm) (Mega View Science Co. Ltd., Taichung, Taiwan); all insects were supplied with a 20% honey-water solution (*ad libitum*) as food. Parasitoids were reared on *D. suzukii* pupae, whereas fly larvae were reared on a cornmeal-based artificial diet.

2.2 Collection of *Drosophila* species

There are over 2,000 described *Drosophila* species worldwide (Markow & O’Grady, 2006); we selected 25 representative species from two subfamilies, seven genera, nine subgenera and 20 species groups (Table S1). Except for *D. suzukii*, all species were purchased from the UC San Diego *Drosophila* Stock Center and then reared for two generations on artificial diet (Table S1). Among them, 22 species were originally collected within the USA, one was from American Samoa and one was from Japan. All species except *Gitona americana* (subfamily Steganinae) belong to the subfamily Drosophilinae. The Japanese species (*Scaptomyza elmoi*) was selected as a close representative of endangered Hawaiian drosophilids. Host species selection considered phylogenetic relatedness, potential sympatry with *D. suzukii*, geographic distribution and occurrence in North America, and ecological diversity (e.g., breeding substrates) (Table S1). Host species phylogenetic relationship was constructed based on available COI gene sequences from the NCBI database. Because gene sequences of *Scaptomyza elmoi* and *Gitona americana* were not available, we completed a DNA extraction using DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD), amplifying the COI gene using polymerase chain reaction (PCR) with primer pair LepF1-short/LepR1-short (LepF1-short: 5’-ATTCAACCAATCATAAAGATAT-3’ and LepR1-short: 5’-TAAACTTCTGGATGTCCAAAAA-3’). Following target COI amplification, samples were treated with ExoSAP (0.5µl Exonuclease I, 0.5µl Shrimp Alkaline Phosphatase, 1.0 µl 10X Exonuclease Reaction Buffer, and 5.0µl PCR product), and run at 37 °C for 15 min, then at 80 °C for 15 min. Samples were then sequenced using a ABI 3730xl DNA Sequencer (Thermo Fisher Scientific).

2.3 Host acceptance, parasitoid fitness and foraging efficiency

No-choice assays were conducted to ascertain the innate potential of *P. vindemiae* and *T. drosophilae* to parasitize a specific host, which could be masked in choice assays by host preference. All tests used 3-6 day old mated female parasitoids and 2-3 day old fly pupae. For each treatment replicate, 10 pupae were placed on a wet tissue paper in a plastic petri dish (1.5 cm high, 8.5 cm diameter) and exposed to a single female wasp for 24 h. Tests had 25-30 replicates for each host species and parasitoid combination, except for *S. elmoi* that had only 5 replicates due to difficulties rearing this species. For each *Drosophila* species, 5 additional replicates of pupae were set-up and that were not exposed to the parasitoid to serve as a control for natural mortality of unexposed pupae. All treated and control pupae were checked twice daily, when the gender and developmental time of emerged adults were recorded. After emergence ceased, all dead pupae were reconstituted in water for 1 day and then dissected under a microscope to determine the presence or absence of recognizable fly or parasitoid cadavers (pharate adults or larvae). Percentage parasitism or host mortality due to parasitism was corrected based on the Schneider-Orelli formula:

$$P = (b - k) \times 100 / (1 - k)$$

where P is corrected mortality resulting from parasitism, and b and k are host mortality in the treatment and control, respectively. Mortality of unexposed host pupae in the controls varied among the various hosts ($F_{24,195} = 4.82$, $P < 0.001$), but was $< 5\%$ for most of tested species, with the exception of two species (*G. americana* and *D. tripunctata*) which had about 30% mortality (Figure S1). The number of parasitized hosts was estimated based on the corrected mortality, while offspring mortality was estimated based on developed offspring and the total number of parasitized hosts. Offspring sex ratio was estimated based on emerged adult parasitoids.

To estimate the effect of host species on parasitoid fitness, a subsample of 2-5 parasitized pupae were randomly selected from each replicate and their pupal length and width were measured ($\pm 0.001\text{mm}$), and they were then individually reared in gelatin capsules. Each emerged wasp was monitored for the immature developmental time. Emerged female wasps were held in vials (8 cm high, 2 cm diameter) streaked with 20% honey water and then killed 2 days later and dissected to record the mature egg load. The hind tibia length of each dissected female was also measured to serve as a proxy of female’s body size. Unemerged pupae were also dissected as described above and these data were included in the calculation of parasitism and offspring survival (i.e., proportion of parasitoids that successfully emerged from hosts). For these measures,

a subsample of 18 host species were measured. A total of 30-50 parasitized individual pupae were tracked for the entire process from exposure to the emergence of wasps for each host and parasitoid combination. Because host shape of different species was slightly different (Figure S2, we estimated puparium volume as a measure of size using the formula:

$$V = 4/3\pi [(l/2)^2 (w/2)]$$

where V is the volume of a prolate ellipsoid puparium with length l and width w (Wang & Messing 2004b).

To determine the effect of natal host species and female body size on the parasitoids' relative efficiency on *D. suzukii*, a sub-sample of 10-20 female wasps that emerged from various host species were tested for their foraging efficiency by providing each female with twenty 2-3 day old *D. suzukii* pupae using the same methods as described above. After a 24 h exposure, all tested females were killed in the freezer and dissected to measure their hind tibia length and all exposed pupae were reared to determine the number of hosts parasitized.

2.4 Data Analysis

All values are presented as mean \pm SE. Because host size varied even within the same species, depending on the rearing conditions and because bioassays for both parasitoids were conducted separately, data were analyzed separately for each parasitoid species. Moran's I was used to test whether fitness parameters of the parasitoids were associated with fly phylogeny, using the PhyloSignal function in the PhyloSignal package of R version 4.0.2 (Team 2020). Moran's I varies from -1 to +1, with more positive values indicating higher similarity between closely related taxa than expected by chance, and is insensitive to tree size and tree balance, and is not based on an evolutionary model (Munkemüller *et al.* 2012). All subsequent analyses were performed using JMP^(r), Pro 14 (SAS Institute Inc., Cary, NC, 1989-2019). The number of hosts parasitized, offspring survival and sex ratio on each host species was compared separately for each parasitoid using one-way ANOVA; prior to comparisons percentage data were logit transformed to normalize the variance. If a significant difference was detected, the mean values were further compared by Tukey HSD test. Body size was analyzed using Generalized Linear Model (GLM) with a normal distribution and an identity link function, considering the effects of natal host species, host size, parasitoid sex and the interaction between host species and host size, while the developmental time was analyzed also using GLM by considering the effect of natal host, wasp size and sex. Both the mature egg load and relative foraging efficiency of female wasps were analyzed using GLM with a Poisson distribution and a logit identity link function, considering the effect of natal host, female body size and their interaction. The relationship between the mean size of host species and mean number of hosts parasitized, mean offspring survival, or mean sex ratio were analyzed using linear regression. The relationship between host size and female body size or between female body size and mature egg load were analyzed using linear regression by pooling all measured female wasps for each parasitoid species.

3. Results

Both *T. drosophilae* and *P. vindemiae* attacked all 25 tested host species (Fig.1). Pupal size varied among the measured *Drosophila* species for *T. drosophilae* (pupal length: $F_{17,647} = 288.0$, $P < 0.001$, width: $F_{17,647} = 97.5$, $P < 0.001$, volume: $F_{17,647} = 174.4$, $P < 0.001$) and *P. vindemiae* (pupal length: $F_{17,629} = 148.2$, $P < 0.001$, width: $F_{17,629} = 64.4$, $P < 0.001$, volume: $F_{17,629} = 99.3$, $P < 0.001$). Large-body species were *D. robusta*, *D. hydi*, *D. immigrans*, *D. montana*, and *D. funebris* ($> 0.32 \text{ mm}^3$), small-body species were *D. putrida*, *D. willistoni*, *H. duncani*, *D. busckii*, and *D. melanogaster*, *D. simulans*, and *D. cardini* ($< 0.16 \text{ mm}^3$); the other species ranged from 0.16-0.19 mm^3 (Tables S2, S3).

Tests using Moran's I uncovered no associations between any of the parasitoid fitness parameters and fly species phylogeny, except for offspring survival of *T. drosophilae* (Table 1). The number of hosts parasitized varied among host species for *T. drosophilae* ($F_{24,590} = 5.3$, $P < 0.001$) and *P. vindemiae* ($F_{24,723} = 33.5$, $P < 0.001$). Overall, more hosts were parasitized by *T. drosophilae* (6.7 ± 0.1) than by *P. vindemiae* (3.0 ± 0.1) ($F_{1,1361} = 538.9$, $P < 0.001$). Offspring survival was also affected by host species for *T. drosophilae* (F

$24,584 = 11.0, P < 0.001$) and *P. vindemiae* ($F_{24,487} = 5.2, P < 0.001$). The survival rate of *T. drosophilae* was similar across the various hosts ($> 50\%$), except on *D. tripunctata* (1.5%), *G. americana* (30.3%) and *S. elmoi* (33.3%) (Fig. 2). There was wide variation in the offspring survival of *P. vindemiae* among the different host species; the lowest survival rate was observed in *D. persimilis* (4.9%), *S. elmoi* (5.6%) and *D. tripunctata* (8.8%) (Fig. 2). Overall, offspring survival was higher for *T. drosophilae* ($62.3 \pm 1.3\%$) than for *P. vindemiae* ($46.0 \pm 1.9\%$) ($F_{1,1119} = 100.7, P < 0.001$). Offspring sex ratio was similar across all tested species for *T. drosophilae* ($F_{24,511} = 2.3, P < 0.001$), but varied among various hosts for *P. vindemiae* ($F_{23,275} = 4.2, P < 0.001$) (Fig. 3). Across all species, offspring sex ratio was similar between *T. drosophilae* ($64.0 \pm 1.4\%$) and *P. vindemiae* ($76.0 \pm 1.8\%$) ($F_{1,834} = 3.7, P = 0.052$).

Body size was affected by host species, host size, sex and the interaction between host species and size (Tables 1, S2, S3). Developmental time was affected by sex for *T. drosophilae* but was not affected by host species or host size for either species or by sex for *P. vindemiae* (Tables 2, S2, S3). Consistent with the regression analyses, mature egg load was affected by host species and female body size and/or their interaction (Tables 2, S2, S3). The foraging efficiency of parasitoids (i.e., number of parasitized *D. suzukii* within a 24 h exposure) was not affected by body size but was affected by the natal host species and/or the interaction between the natal host and female's body size (Tables 2, S2, S3).

Linear regressions found that the body size of emerged female parasitoids increased positively with the size of host pupa (Fig. 4), and that the number of mature eggs increased positively with the body size of female wasps (Fig. 5). However, there were no significant relationships between mean host size (volume, measured only for 18 species) and the number of hosts parasitized (*P. vindemiae* : $F_{1,17} = 2.6, P = 0.128$), offspring survival (*T. drosophilae* : $F_{1,17} = 1.8, P = 0.202$; *P. vindemiae* : $F_{1,17} = 0.1, P = 0.776$) or sex ratio (*T. drosophilae* : $F_{1,17} = 3.9, P = 0.064$; *P. vindemiae* : $F_{1,17} = 3.8, P = 0.067$), except that the number of hosts parasitized by *T. drosophilae* increased with host size ($F_{1,17} = 8.8, P = 0.009$).

4. Discussion

Body size is a central element in theories of life-history evolution (Fox & Czesak, 2000; Lafferty & Kuris, 2002) and for parasitoid-host size models there often exist trade-offs for the use of smaller or larger hosts (King & Charnov, 1988; Teder, Tammaru & Pedmanson, 1999; Sagarra, Vincent & Stewart, 2001). In this study, both *P. vindemiae* and *T. drosophilae* were able to parasitize and develop from all 25 tested *Drosophila* species. Although parasitism efficiency and offspring fitness varied among the different host species, the parasitoids showed remarkable levels of phenotypic plasticity in body growth and development. Overall, host species-related effects on the key life-history traits (survival, development time, sex ratio and body size) of both parasitoids appeared to be unrelated to the phylogenetic position of tested host species.

The quality and quantity of different host species available to a parasitoid will inevitably vary, and host suitability may depend on a parasitoid's ability to adjust to variation in host resources. Here, there were positive correlations between host and parasitoid-progeny size. Previous studies showed that *T. drosophilae* reared from the larger *D. suzukii* (Woltering, Romeis & Collatz, 2019) or *D. hydei* (Chen et al., 2018) had a larger body size than those reared on the smaller *D. melanogaster*, and *T. drosophilae* showed a preference for *D. suzukii* over *D. melanogaster* in choice tests (Woltering, Romeis & Collatz, 2019; Yi et al., 2020). Wang and Messing (2004a) showed that *P. vindemiae* successfully developed on several tephritid hosts, including *Bactrocera cucurbitae* (Coquillett) and *B. latifrons* (Hendel), as well as on puparia of these species that had been parasitized by their primary tephritid parasitoids, although *P. vindemiae* reared from these secondary hosts were smaller than those reared from the tephritid hosts or from *D. melanogaster*. It was observed that *P. vindemiae* could only consume a small part of the host resources when attacking a tephritid host (Wang & Messing, 2004a), but in the current study parasitoid larvae consumed almost all the host resources prior to pupation. There seems to be no obvious physiological constraint on the maximal use of *drosophila* host resources by these parasitoids.

According to the "adult size-fitness hypothesis", fitness increases with body size (Kazmer & Luck, 1995). A positive relationship between body size and fitness has been found in both vertebrates and invertebrates,

including parasitoids (e.g., Ellers & Jervis, 2003; Jervis et al., 2003; Segoli & Rosenheim, 2015). The results of the current study are in line with these past findings. Although the 24 h fecundity of female wasps was not significantly affected by the female's size in either parasitoid, female body size of both parasitoids was positively correlated with host size and was in turn associated with increased mature egg load. Chen et al. (2018) similarly reported that *T. drosophilae* females reared from the larger *D. hydei* had a higher mature egg load, percentage of female offspring and longevity than those reared from the smaller *D. melanogaster*. It was suspected that smaller females would suffer more from egg limitation than larger females in terms of life-time fecundity (Wang & Messing, 2004b), as was recently shown for *T. drosophilae* where large females had higher life-time fecundity and longevity, possibly due to larger energy reserves (Chen et al., 2018). Also, *P. vindemiae* reared from the tephritid host *M. domestica* were larger and had higher attack rates than those reared from *D. melanogaster* (Morris & Fellowes, 2002).

Importantly, we did not observe trade-offs between body size and other fitness traits. Although body size of both parasitoid species was correlated with host size, and both offspring survival and sex ratio varied among host species, this variation was unrelated to host size or the host's phylogenetic position, with the exception of offspring survival for *T. drosophilae* (Figures 2-3; Table 1). Furthermore, offspring developmental time was not affected by host species in either parasitoid. Often, the fitness gain from being large comes at the cost of prolonged developmental time (Sequeira & Mackauer, 1992; Harvey & Strand, 2002). The lack of a relationship between developmental time and body size in both parasitoids in the current study suggests that they grow faster on larger host species, as predicted for parasitoids developing in a fixed resource system (Otto & Mackauer, 1998). This reflects the plasticity of body growth in both parasitoids, suggesting that host quality seems to be correlated with host size in both parasitoids, and that both can adjust their growth rates to maximize body size. These results suggest that larger hosts are likely to be more suitable for mass-rearing programs.

Pachycrepoideus vindemiae and *T. drosophilae* are clearly generalist parasitoids, which has both advantages and disadvantages for aspects of ecosystem services (Stiling & Cornelissen, 2005). A lack of physiological immunity against these pupal parasitoids may explain, in part, their broad host ranges (Kacsoh & Schlenke, 2012). Physical immunity may influence host susceptibility to the parasitoids, however; for successful parasitism, *P. vindemiae* and *T. drosophilae* must drill through the host puparial wall before injecting venom or laying eggs. Thus, host acceptance may be contingent on the puparial wall thickness, which likely varies among drosophilid species. We found that *T. drosophilae* accepted all tested host species relatively equally, whereas there was wide variation in hosts parasitized by *P. vindemiae*. The larger body size of *T. drosophilae* relative to *P. vindemiae* might make it easier for the former to penetrate the puparial wall of some host species. Larger *P. vindemiae* may be more able to drill through the thicker protective outer layer of some host pupae (Morris & Fellowes, 2002). Host acceptance might then be a dynamic process (Hopper, Prager & Heimpel 2013), as oviposition into larger hosts results in larger progeny that might be more capable of ovipositing into larger hosts.

The "host-ecology hypothesis" of host range evolution (Shaw, 1994) assumes that parasitoid species can broaden their host ranges by recruiting new hosts that exist within the parasitoids searching niche. Parasitoids may attack hosts that are closely phylogenetically related and that share similar physiological properties and defense mechanisms, and more importantly, overlapping niches (Godfray, 1994). Therefore, phylogenetic concordance would be expected if host ranges evolve according to the host-ecology hypothesis, as appears to be the case for larval parasitoids of *D. sukii* (KM. Daane, unpubl.). In contrast, the two pupal parasitoids tested in the current study are physiologically capable of developing from various host species regardless of phylogenetic position. Phenotypic plasticity towards host species and host size in *P. vindemiae* and *T. drosophilae* indicates their potential for host range and geographic range expansion. In nature, these pupal parasitoids likely switch from one host species to another, as a consequence of temporal or spatial variation in host community composition, or they may colonize new hosts via novel associations. It seems plausible that the lack of fitness costs associated with parasitizing larger hosts would select for larger progeny. However, the diverse array of *Drosophila* species in various habitats (Gleason, Roy, Everman, Gleason & Morgan, 2019) might counterbalance selective forces. Host availability for parasitoids may vary

stochastically, as they typically sample only a few patches in their lifetime (Ellers & Jervis, 2003). Although a narrow host range makes a parasitoid from the pest’s native range an attractive candidate for importation in classical biological control, the ability to switch between hosts is likely to increase the persistence and abundance of generalist parasitoids (Bribosia et al., 2005).

As generalists, these pupal parasitoids might contribute to *D. suzukii* control by being more common in a diverse range of habitats, as generally appears to be the case for natural enemies that move across habitat boundaries (Tscharntke et al., 2007). *D. suzukii* has been shown to utilize a wide variety of host plants and habitats (Poyet et al., 2015; Haye et al., 2016; Kenis et al., 2016; Santoiemma, Trivellato, Caloi, Mori & Marini, 2019). The apparent lack of costs associated with being large in *P. vindemiae* and *T. drosophilae* could further increase their ability to disperse between host patches and habitats. Larger parasitoids may possess superior dispersal ability at the local and/or landscape scale; they may be able to move more efficiently between host or habitat patches and be less vulnerable to changes in environmental conditions among habitats and over time (Abram, Parent, Brodeur & Boivin, 2016).

Large size may not be advantageous under all conditions, however, and body size plasticity in *T. drosophilae* and *P. vindemiae* could actually improve biological control by increasing variation in parasitoid body sizes. Although host-limited environments may favor larger individuals that can move more efficiently between widely separated host patches (Ellers et al., 1998), smaller individuals may have an advantage in host-rich environments where dispersal capacity is less important, and may escape predators more easily when predation pressure is high (Ellers, Bax & Van Alphen, 2001). In the field, environmental conditions such as host density and stochasticity will ultimately determine the realized fitness of different parasitoid sizes, and the relative frequencies of parasitoid sizes are likely to vary over time as conditions change (Ellers et al., 2001).

In this study we show that *P. vindemiae* and *T. drosophilae* are likely to attack multiple drosophilid species in invaded regions, but it is still unknown if they will host shift in response to increasing or decreasing *D. suzukii* numbers. Field surveys in Asia found that specialized larvae parasitoids had a greater impact on *D. suzukii* (Daane et al., 2016; Girod et al., 2018; Giorgini et al., 2019). The parasitoids’ ecosystem service impact will be influenced not only by their plasticity to hosts but by environmental limitations such as temperature tolerances, habitat location, and host searching behaviors.

Acknowledgements

We thank Valeria Hochman-Adler, Pahoua Yang, May Yang and Robert Straser (University of California, Berkeley) for laboratory assistance, and Kei-Li Ooi (University of California, Berkeley) for the collection of COI gene sequences for two species and the construction of the phylogeny of all tested host species. Funding was supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture Specialty Crops Research Initiative under Agreement No. 2015-51181-24252, the California Cherry Board, and the University of California’s Agricultural and Natural Resources Competitive Grants Program. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer. We confirm

that we have no conflicts of interest.

Authors’ contributions

AB, XG and KMD conceived the ideas and designed methodology; KMD obtained funding; AB and XG collected the data; XG and BNH analysed the data; XG, BNH and KMD led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data Availability Statement

Data available from the Dryad Digital Repository (to be provided on acceptance of manuscript).

References

- Abram, P.K., Parent, J.P., Brodeur, J. & Boivin, G. (2016). Size-induced phenotypic reaction norms in a parasitoid wasp: an examination of life-history and behavioural traits. *Biological Journal of the Linnean Society* , 117 , 620-632. <https://doi.org/10.1111/bij.12658>
- Asplen, M.K., Anfora, G., Biondi, A., Choi, D.S., Chu, D., Daane, K.M., Gibert, P., Gutierrez, A.P., Hoelmer, K.A., Hutchison, W.D., Isaacs, R., Jiang, Z.L., Karpate, Z., Kimura, M.T., Pascual, M., Philips, C.R., Plantamp, C., Ponti, L., Vetek, G., Vogt, H., Walton, V.M., Yu, Y., Zappala, L. & Desneux, N. (2015). Invasion biology of spotted wing drosophila (*Drosophila suzukii*): a global perspective and future priorities. *Journal of Pest Science*, 88 , 469-494. <https://doi.org/10.1007/s10340-015-0681-z>
- Biondi, A., Desneux, N., Amiens-Desneux, E., Siscaro, G. & Zappala, L. (2013). Biology and developmental strategies of the Palearctic parasitoid *Bracon nigricans* (Hymenoptera: Braconidae) on the Neotropical moth *Tuta absoluta* (Lepidoptera: Gelechiidae). *Journal of Economic Entomology*, 106 , 1638-1647. <https://doi.org/10.1603/ec12518>
- Bribosia, E., Bylemans, D., Migon, M. & Van Impe, G. (2005). In-field production of parasitoids of *Dysaphis plantaginea* by using the rowan aphid *Dysaphis sorbi* as substitute host. *Biological Control* , 50 , 601-610. <https://doi.org/10.1007/s10526-004-5526-2>
- Carton, Y., Bouletreau, B., van Alphen, J.J.M. & van Lenteren, J.C. (1986). The *Drosophila* parasitic wasps. *The Genetics and Biology of Drosophila* (eds M. Ashburner, H.L. Carson & J.N. Thompson), pp. 347-394. Academic Press, London.
- Charnov, E.L., Losdenhartogh, R.L., Jones, W.T. & Vandenassem, J. (1981). Sex ratio evolution in a variable environment. *Nature*, 289 , 27-33. <https://doi.org/10.1038/289027a0>
- Chen, J.N., Zhou, S.C., Wang, Y., Shi, M., Chen, X.X. & Huang, J.H. (2018). Biocontrol characteristics of the fruit fly pupal parasitoid *Trichopria drosophilae* (Hymenoptera: Diapriidae) emerging from different hosts. *Scientific Reports*, 8 , e13323. <https://doi.org/10.1038/s41598-018-31718-6>
- Chen, W., He, Z., Ji, X.L., Tang, S.T. & Hu, H.Y. (2015). Hyperparasitism in a generalist ectoparasitic pupal parasitoid, *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae), on its own conspecifics: when the lack of resource lead to cannibalism. *Plos One*, 10 , e0124305. <https://doi.org/10.1371/journal.pone.0124305>
- Daane, K.M., Wang, X.G., Biondi, A., Miller, B., Miller, J.C., Riedl, H., Shearer, P.W., Guerrieri, E., Giorgini, M., Buffington, M., van Achterberg, K., Song, Y., Kang, T., Yi, H., Jung, C., Lee, D.W., Chung, B.K., Hoelmer, K.A. & Walton, V.M. (2016). First exploration of parasitoids of *Drosophila suzukii* in South Korea as potential classical biological agents. *Journal of Pest Science*, 89 , 823-835. <https://doi.org/10.1007/s10340-016-0740-0>
- Davidson, A.M., Jennions, M. & Nicotra, A.B. (2011). Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters*, 14 , 419-431. <https://doi.org/10.1111/j.1461-0248.2011.01596.x>
- Desneux, N., Blahnik, R., Delebecque, C.J. & Heimpel, G.E. (2012). Host phylogeny and specialisation in parasitoids. *Ecology Letters*, 15 , 453-460. <https://doi.org/10.1111/j.1461-0248.2012.01754.x>
- Eijs, I.E.M. & van Alphen, J.J.M. (1999). Life history correlations: why are hymenopteran parasitoids an exception? *Ecology Letters*, 2 , 27-35. <https://doi.org/10.1046/j.1461-0248.1999.21045.x>
- Ellers, J., Bax, M. & van Alphen, J.J.M. (2001). Seasonal changes in female size and its relation to reproduction in the parasitoid *Asobara tabida* . *Oikos* , 92 , 209-314. <https://doi.org/10.1034/j.1600-0706.2001.920213.x>
- Ellers, J. & Jervis, M. (2003). Body size and the timing of egg production in parasitoid wasps. *Oikos*, 102 , 164-172. <https://doi.org/10.1034/j.1600-0706.2003.12285.x>

- Ellers, J., van Alphen, J.J.M. & Sevenster, J.G. (1998). A field study of size-fitness relationships in the parasitoid *Asobara tabida*. *Journal of Animal Ecology*, *67* , 318-324. <https://doi.org/10.1046/j.1365-2656.1998.00195.x>
- Foray, V., Desouhant, E. & Gibert, P. (2014). The impact of thermal fluctuations on reaction norms in specialist and generalist parasitic wasps. *Functional Ecology*, *28* , 411-423. <https://doi.org/10.1111/1365-2435.12171>
- Fox, C.W. & Czesak, M.E. (2000). Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology*, *45* , 341-369. <https://doi.org/10.1146/annurev.ento.45.1.341>
- Gao, S., Tang, Y., Wei, K., Wang, X., Yang, Z. & Zhang, Y. (2016). Relationships between body size and parasitic fitness and offspring performance of *Sclerodermus pupariae* Yang et Yao (Hymenoptera: Bethyridae). *PloS One* *11* , e0156831. <https://doi.org/10.1371/journal.pone.0156831>
- Giorgini, M., Wang, X.G., Wang, Y., Chen, F.S., Hougardy, E., Zhang, H.M., Chen, Z.Q., Chen, H.Y., Liu, C.X., Cascone, P., Formisano, G., Carvalho, G.A., Biondi, A., Buffington, M., Daane, K.M., Hoelmer, K.A. & Guerrieri, E. (2019). Exploration for native parasitoids of *Drosophila suzukii* in China reveals a diversity of parasitoid species and narrow host range of the dominant parasitoid. *Journal of Pest Science*, *92* , 509-522. <https://doi.org/10.1007/s10340-018-01068-3>
- Girod, P., Borowiec, N., Buffington, M., Chen, G.H., Fang, Y., Kimura, M.T., Peris-Felipo, F.J., Ris, N., Wu, H., Xiao, C., Zhang, J.P., Aebi, A., Haye, T. & Kenis, M. (2018). The parasitoid complex of *D. suzukii* and other fruit feeding *Drosophila* species in Asia. *Scientific Reports*, *8* , e11839. <https://doi.org/10.1038/s41598-018-29555-8>
- Gleason, J.M., Roy, P.R., Everman, E.R., Gleason, T.C. & Morgan, T.J. (2019). Phenology of *Drosophila* species across a temperate growing season and implications for behavior. *Plos One*, *14* , e0216601. <https://doi.org/10.1371/journal.pone.0216601>
- Godfray, H.C.J. (1994). *Parasitoids: Behavioral and Evolutionary Ecology* . Princeton University Press, Princeton, New Jersey.
- Harvey, J.A., Harvey, I.F. & Thompson, D.J. (1994). Flexible larval growth allows use of a range of host sizes by a parasitoid wasp. *Ecology*, *75* , 1420-1428. <https://doi.org/10.2307/1937465>
- Harvey, J.A. & Strand, M.R. (2002). The developmental strategies of endoparasitoid wasps vary with host feeding ecology. *Ecology*, *83* , 2439-2451. <https://doi.org/10.2307/3071805>
- Haye, T., Girod, P., Cuthbertson, A.G.S., Wang, X.G., Daane, K.M., Hoelmer, K.A., Baroffio, C., Zhang, J.P. & Desneux, N. (2016). Current SWD IPM tactics and their practical implementation in fruit crops across different regions around the world. *Journal of Pest Science*, *89* , 643-651. <https://doi.org/10.1007/s10340-016-0737-8>
- Heimpel, G.E. & Cock, M.J.W. (2018). Shifting paradigms in the history of classical biological control. *Biocontrol*, *63* , 27-37. <https://doi.org/10.1007/s10526-017-9841-9>
- Hoddle, M.S., Lake, E.C., Minter, C.R. & Daane, K.M. (2020). Chapter 2, Importation Biological Control. *Biological Control: A Global Initiative* (eds P.G. Mason & N. Dennis). CSIRO Publishing, Canberra, Australia.
- Hoffmann, A.A., Chown, S.L. & Clusella-Trullas, S. (2013). Upper thermal limits in terrestrial ectotherms: how constrained are they? *Functional Ecology*, *27* , 934-949. <https://doi.org/10.1111/j.1365-2435.2012.02036.x>
- Hopper, K.R., Prager, S.M. & Heimpel, G.E. (2013). Is parasitoid acceptance of different host species dynamic? *Functional Ecology*, *27* , 1201-1211. <https://doi.org/10.1111/1365-2435.12107>

- Jervis, M.A., Ferns, P.N. & Heimpel, G.E. (2003). Body size and the timing of egg production in parasitoid wasps: a comparative analysis. *Functional Ecology*, 17 , 375-383. <https://doi.org/10.1046/j.1365-2435.2003.00742.x>
- Kacar, G., Wang, X.G., Biondi, A. & Daane, K.M. (2017). Linear functional response by two pupal *Drosophila* parasitoids foraging within single or multiple patch environments. *Plos One*, 12 , e0183525. <https://doi.org/10.1371/journal.pone.0183525>
- Kacsoh, B.Z. & Schlenke, T.A. (2012). High hemocyte load is associated with increased resistance against parasitoids in *Drosophila suzukii* , a relative of *D. melanogaster* . *Plos One*, 7 , e34721. <https://doi.org/10.1371/journal.pone.0034721>
- Kazmer, D.J. & Luck, R.F. (1995). Field test of the size fitness hypothesis in the egg parasitoid *Trichogramma pretiosum* . *Ecology*, 76 , 412-425. <https://doi.org/10.2307/1941200>
- Kenis, M., Tonina, L., Eschen, R., van der Sluis, B., Sancassani, M., Mori, N., Haye, T. & Helsen, H. (2016). Non-crop plants used as hosts by *Drosophila suzukii* in Europe. *Journal of Pest Science*, 89 , 735-748. <https://doi.org/10.1007/s10340-016-0755-6>
- King, B.H. & Charnov, E.L. (1988). Sex-ratio manipulation in response to host size by the parasitoid *Spalangia cameroni* : a laboratory study. *Evolution*, 42 , 1190-1198. <https://doi.org/10.1111/j.1558-5646.1988.tb04179.x>
- King, B.H. & Lee, H.E. (1994). Test of the adaptiveness of sex-ratio manipulation in a parasitoid wasp. *Behavioral Ecology and Sociobiology*, 35 , 437-443. <https://doi.org/10.1007/bf00165847>
- Kingsolver, J.G. & Buckley, L.B. (2018). How do phenology, plasticity, and evolution determine the fitness consequences of climate change for montane butterflies? *Evolutionary Applications*, 11 , 1231-1244. <https://doi.org/10.1111/eva.12618>
- Lafferty, K.D. & Kuris, A.M. (2002). Trophic strategies, animal diversity and body size. *Trends in Ecology & Evolution*, 17 , 507-513. [https://doi.org/10.1016/S0169-5347\(02\)02615-0](https://doi.org/10.1016/S0169-5347(02)02615-0)
- Lee, J.C., Wang, X.G., Daane, K.M., Hoelmer, K.A., Isaacs, R., Sial, A.A. & Walton, V.M. (2019). Biological control of spotted-wing drosophila (Diptera: Drosophilidae): Current and pending tactics. *Journal of Integrated Pest Management*, 10 , 13. <https://doi.org/10.1093/jipm/pmz012>
- Markow, T.A., O'Grady, P.M. (2006). *Drosophila: A Guide to Species Identification and Use* , Academic Press, London.
- Merila, J. & Hendry, A.P. (2014). Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evolutionary Applications*, 7 , 1-14. <https://doi.org/10.1111/eva.12137>
- Morris, R.J. & Fellowes, M.D.E. (2002). Learning and natal host influence host preference, handling time and sex allocation behaviour in a pupal parasitoid. *Behavioral Ecology and Sociobiology*, 51 , 386-393. <https://doi.org/10.1007/s00265-001-0439-x>
- Munkemuller, T., Lavergne, S., Bzeznik, B., Dray, S., Jombart, T., Schiffrers, K. & Thuiller, W. (2012). How to measure and test phylogenetic signal. *Methods in Ecology and Evolution*, 3 , 743-756. <https://doi.org/10.1111/j.2041-210X.2012.00196.x>
- Otto, M. & Mackauer, M. (1998). The developmental strategy of an idiobiont ectoparasitoid, *Dendrocercus carpenteri* : influence of variations in host quality on offspring growth and fitness. *Oecologia*, 117 , 353-364. <https://doi.org/10.1007/s004420050668>
- Petersen, G. & Hardy, I.C.W. (1996). The importance of being larger: Parasitoid intruder-owner contests and their implications for clutch size. *Animal Behaviour*, 51 , 1363-1373. <https://doi.org/10.1006/anbe.1996.0139>

- Poyet, M., Le Roux, V., Gibert, P., Meirland, A., Prevost, G., Eslin, P. & Chabrerie, O. (2015). The wide potential trophic niche of the Asiatic fruit fly *Drosophila suzukii* : The key of its invasion success in temperate Europe? *Plos One*, 10 , e0142785. <https://doi.org/10.1371/journal.pone.0142785>
- Rivero, A. & West, S.A. (2002). The physiological costs of being small in a parasitic wasp. *Evolutionary Ecology Research*, 4 , 407-420.
- Rossi Stacconi, M.V., Buffington, M., Daane, K.M., Dalton, D.T., Grassi, A., Kacar, G., Miller, B., Miller, J.C., Baser, N., Ioriatti, C., Walton, V.M., Wiman, N.G., Wang, X. & Anfora, G. (2015). Host stage preference, efficacy and fecundity of parasitoids attacking *Drosophila suzukii* in newly invaded areas. *Biological Control*, 84 , 28-35. <https://doi.org/10.1016/j.biocontrol.2015.02.003>
- Sagarra, L.A., Vincent, C. & Stewart, R.K. (2001). Body size as an indicator of parasitoid quality in male and female *Anagyrus kamali* (Hymenoptera : Encyrtidae). *Bulletin of Entomological Research*, 91 , 363-367. <https://doi.org/10.1079/ber2001121>
- Santoemma, G., Trivellato, F., Caloi, V., Mori, N. & Marini, L. (2019). Habitat preference of *Drosophila suzukii* across heterogeneous landscapes. *Journal of Pest Science*, 92 , 485-494. <https://doi.org/10.1007/s10340-018-1052-3>
- Segoli, M. & Rosenheim J.A. (2015). The effect of body size on oviposition success of a minute parasitoid in nature. *Ecological Entomology* , 40 , 483-485. <https://doi.org/10.1111/een.12194>
- Sequeira, R. & Mackauer, M. (1992). Covariance of adult size and development time in the parasitoid wasp *Aphidius ervi* in relation to the size of its hosts, *Acyrtosiphon pisum* . *Evolutionary Ecology*, 6 , 34-44. <https://doi.org/10.1007/bf02285332>
- Shaw, M. R. (1994). Parasitoid host ranges. *Parasitoid community ecology* (eds. B. A., Hawkins & W. Sheehan), pp. 111-144, Oxford University Press, Oxford.
- Stephens, D.W. & Charnov, E.L. (1982). Optimal foraging - some simple stochastic models. *Behavioral Ecology and Sociobiology*, 10 , 251-263. <https://doi.org/10.1007/bf00302814>
- Stiling, P. & Cornelissen, T. (2005). What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. *Biological Control*, 34 , 236-246. <https://doi.org/10.1016/j.biocontrol.2005.02.017>
- Team, R.D.C. (2020). R Development Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Teder, T., Tammaru, T. & Pedmanson, R. (1999). Patterns of host use in solitary parasitoids (Hymenoptera, Ichneumonidae): field evidence from a homogeneous habitat. *Ecography*, 22 , 79-86. <https://doi.org/10.1111/j.1600-0587.1999.tb00456.x>
- Tscharntke, T., Bommarco, R., Clough, Y., Crist, T.O., Kleijn, D., Rand, T.A., Tylianakis, J.M., van Nouhuys, S. & Vidal, S. (2007). Conservation biological control and enemy diversity on a landscape scale. *Biological Control*, 43 , 294-309. <https://doi.org/10.1016/j.biocontrol.2007.08.006>
- Ueno, T. (1998). Adaptiveness of sex ratio control by the pupal parasitoid *Itoplectis naranyae* (Hymenoptera : Ichneumonidae) in response to host size. *Evolutionary Ecology*, 12 , 643-654. <https://doi.org/10.1023/A:1006577314205>
- Visser, M.E. (1994). The importance of being large: The relationship between size and fitness in females of the parasitoid *Aphaereta minuta* (Hymenoptera: Braconidae). *Journal of Animal Ecology*, 63 , 963-978. <https://doi.org/10.2307/5273>
- Wang, X.G., Kacar, G., Biondi, A. & Daane, K.M. (2016a). Foraging efficiency and outcomes of interactions of two pupal parasitoids attacking the invasive spotted wing drosophila. *Biological Control*, 96 , 64-71. <https://doi.org/10.1007/s10526-016-9720-9>

- Wang, X.G., Kacar, G., Biondi, A. & Daane, K.M. (2016b). Life-history and host preference of *Trichopria drosophilae*, a pupal parasitoid of spotted wing drosophila. *Biocontrol*, 61, 387-397. <https://doi.org/10.1016/j.biocontrol.2016.02.004>
- Wang, X.G. & Messing, R.H. (2004a). The ectoparasitic pupal parasitoid, *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae), attacks other primary tephritid fruit fly parasitoids: host expansion and potential non-target impact. *Biological Control*, 31, 227-236. <https://doi.org/10.1016/j.biocontrol.2004.04.019>
- Wang, X.G. & Messing, R.H. (2004b). Fitness consequences of body-size-dependent host species selection in a generalist ectoparasitoid. *Behavioral Ecology and Sociobiology*, 56, 513-522. <https://doi.org/10.1007/s00265-004-0829-y>
- Wang, X.G. & Messing, R.H. (2004c). Two different life-history strategies determine the competitive outcome between *Dirhinus giffardii* (Chalcididae) and *Pachycrepoideus vindemmiae* (Pteromalidae), ectoparasitoids of cyclorrhaphous Diptera. *Bulletin of Entomological Research*, 94, 473-480. <https://doi.org/10.1079/ber2004318>
- Wang, X.G., Serrato, M.A., Son, Y., Walton, V.M., Hogg, B.N. & Daane, K.M. (2018). Thermal performance of two indigenous pupal parasitoids attacking the invasive *Drosophila suzukii* (Diptera: Drosophilidae). *Environmental Entomology*, 47, 764-772. <https://doi.org/10.1093/ee/nvy053>
- West, S.A., Flanagan, K.E. & Godfray, H.C.J. (1996). The relationship between parasitoid size and fitness in the field, a study of *Achrysocharoides zwoelferi* (Hymenoptera: Eulophidae). *Journal of Animal Ecology*, 65, 631-639. <https://doi.org/10.2307/5742>
- Wilson, E.E., Mullen, L.M. & Holway, D.A. (2009). Life history plasticity magnifies the ecological effects of a social wasp invasion. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 12809-12813. <https://doi.org/10.1073/pnas.0902979106>
- Wolf, S., Boycheva-Woltering, S., Romeis, J. & Collatz, J. (2020). *Trichopria drosophilae* parasitizes *Drosophila suzukii* in seven common non-crop fruits. *Journal of Pest Science*, 93, 627-638. <https://doi.org/10.1007/s10340-019-01180-y>
- Woltering, S.B., Romeis, J. & Collatz, J. (2019). Influence of the rearing host on biological parameters of *Trichopria drosophilae*, a potential biological control agent of *Drosophila suzukii*. *Insects*, 10, 183. <https://doi.org/10.3390/insects10060183>
- Yi, C.D., Cai, P.M., Lin, J., Liu, X.X., Ao, G.F., Zhang, Q.W., Xia, H.M., Yang, J.Q. & Ji, Q.G. (2020). Life history and host preference of *Trichopria drosophilae* from Southern China, one of the effective pupal parasitoids on the *Drosophila* species. *Insects*, 11, 103. <https://doi.org/10.3390/insects11020103>

Table 1. Phylogenetic signal in fitness traits of the two parasitoid species as measured by Moran's I .

Parameter	<i>T. drosophilae</i>	<i>T. drosophilae</i>	<i>P. vindemmiae</i>	<i>P. vindemmiae</i>
	<i>I</i>	<i>P</i>	<i>I</i>	<i>P</i>
Female offspring development time	-0.044	0.190	-0.171	0.985
Male offspring development time	-0.050	0.270	-0.081	0.085
Offspring survival	0.054	0.014	-0.008	0.106
Offspring per day per female	-0.036	0.287	-0.043	0.296

Parameter	<i>T. drosophilae</i>	<i>T. drosophilae</i>	<i>P. vindemiae</i>	<i>P. vindemiae</i>
Female offspring hind tibia length	-0.050	0.373	-0.076	0.732
Male offspring hind tibia length	-0.053	0.444	-0.050	0.063
Sex ratio	-0.085	0.801	0.290	0.980
Mature eggs per female	-0.056	0.449	0.090	0.310

Table 2. Results of General Linear Models analyzing the effects of host species, host size, parasitoid size and/or their interactions on the fitness of *T.drosophilae* and *P. vindemiae*

Parameter	<i>Trichopria drosophi-lae</i>	<i>Trichopria drosophi-lae</i>	<i>Trichopria drosophi-lae</i>	<i>Trichopria drosophi-lae</i>	<i>Pachycrepoides vin-demiae</i>	<i>Pachycrepoides vin-demiae</i>	<i>Pachycrepoides vin-demiae</i>	<i>Pachycrepoides vin-demiae</i>
	Source	<i>Df</i>	<i>F</i>	<i>P</i>	Source	<i>Df</i>	<i>F</i>	<i>P</i>
Offspring develop-mental time	Host species	17	27.3	0.054	Host species	17	18.4	0.4
	Body size of female wasp	1	0.3	0.595	Body size of female wasp	1	0.8	0.3
Body size of emerged wasp	Sex	1	9.7	0.002	Sex	1	2.0	0.1
	Host species (H)	17	113.4	< 0.001	Host species (H)	17	86.0	<
	Host size (S)	1	65.2	< 0.001	Host size (S)	1	12.3	<
	H × S	17	71.0	< 0.001	H × S	1	9.0	<
	Sex	1	283.7	< 0.001	Sex	17	54.5	<
Mature egg load of female wasps	Host species (H)	1	127.3	< 0.001	Host species (H)	17	50.2	<
	Body size of female wasp (S)	17	68.2	< 0.001	Body size of female wasp (S)	1	25.2	<
	H × S	17	23.8	< 0.001	H × S	17	23.3	0.1

Parameter	<i>Trichopria drosophila</i>	<i>Trichopria drosophila</i>	<i>Trichopria drosophila</i>	<i>Trichopria drosophila</i>	<i>Pachycrepoides vindemiae</i>	<i>Pachycrepoides vindemiae</i>	<i>Pachycrepoides vindemiae</i>	<i>Pachycrepoides vindemiae</i>
Foraging efficiency of female wasps	Natal host (H)	14	49.0	< 0.001	Natal host (H)	16	62.3	< 0.001
	Body size of female wasp (S)	1	0.7	0.412	Body size of female wasp (S)	1	0.4	0.5
	H × S	14	32.8	0.003	H × S	16	25.9	0.0

Figure legends

Figure 1. Number of *Drosophila* pupae parasitized by (A) *T. drosophilae* or (B) *P. vindemiae* in various *Drosophila* species. Phylogenetic tree of tested *Drosophila* species was constructed based on COI gene sequences retrieved from the NCBI database except for *Scaptomyza elmoi* and *Gitona americana* that were collected from this study. Bars refer to mean \pm SE and bars bearing different letters are significantly different (Tukey's HSD, $P < 0.05$).

Figure 2. Offspring survival (%) of (A) *T. drosophilae* or (B) *P. vindemiae* on various *Drosophila* species. Phylogenetic tree of tested *Drosophila* species was constructed based on COI gene sequences retrieved from the NCBI database except for *Scaptomyza elmoi* and *Gitona americana* that were collected from this study. Bars refer to mean \pm SE and bars bearing different letters are significantly different (Tukey's HSD, $P < 0.05$).

Figure 3. Offspring sex ratio (females %) of (A) *T. drosophilae* or (B) *P. vindemiae* developed from various *Drosophila* species. *P. vindemiae* produced only a few female offspring on *D. persimilis* and the data were excluded from the analyses due to small samples. Phylogenetic tree of tested *Drosophila* species was constructed based on COI gene sequences retrieved from the NCBI database except for *Scaptomyza elmoi* and *Gitona americana* that were collected from this study. Bars refer to mean \pm SE and bars bearing different letters are significantly different (Tukey's HSD, $P < 0.05$).

Figure 4. The relationship between host body size (pupal volume) and the body size (hind tibia length) of female (A) *T. drosophilae* or (B) *P. vindemiae*. Data were pooled from all measured individuals developed from various species (see Tables S2, S3).

Figure 5. The relationship between female's body size (hind tibia length) of (A) *T. drosophilae* or (B) *P. vindemiae* and mature egg load. Data were pooled from all measured individuals developed from various species (see Tables S2, S3).

Hosted file

image1.emf available at <https://authorea.com/users/362196/articles/483427-plasticity-of-body-growth-and-development-in-two-cosmopolitan-pupal-parasitoids>

Figure 1

Hosted file

image2.emf available at <https://authorea.com/users/362196/articles/483427-plasticity-of-body-growth-and-development-in-two-cosmopolitan-pupal-parasitoids>

Figure 2

Hosted file

image3.emf available at <https://authorea.com/users/362196/articles/483427-plasticity-of-body-growth-and-development-in-two-cosmopolitan-pupal-parasitoids>

Figure 3

Hosted file

image4.emf available at <https://authorea.com/users/362196/articles/483427-plasticity-of-body-growth-and-development-in-two-cosmopolitan-pupal-parasitoids>

Figure 4

Hosted file

image5.emf available at <https://authorea.com/users/362196/articles/483427-plasticity-of-body-growth-and-development-in-two-cosmopolitan-pupal-parasitoids>

Figure 5

Supplemental materials

Table S1. Information on all tested *Drosophila* species (stock number, origin, diet, potential habitats).

Table S2. Pupal sizes of various *Drosophila* species and offspring fitness of *Trichopria drosophilae* developed from the *Drosophila* species

Table S3. Pupal sizes of various *Drosophila* species and offspring fitness of *Pachycrepoideus vindemiae* developed from the *Drosophila* species

Figure S1. Control mortality of tested *Drosophila* species. Values are mean \pm SE and bars bearing different letters are significantly different (Tukey's HSD, $P < 0.05$).

Table S1. Information on *Drosophila* species tested in this study

Stock Number ¹	Genus	Subgenus	Group	Species	Artificial diet ²	Collection site
14030-0811.02	<i>Drosophila</i>	<i>Sophophora</i>	<i>willistoni</i>	<i>willistoni</i>	Cornmeal	Royal Palm P
14043-0871.18	<i>Drosophila</i>	<i>Sophophora</i>	<i>saltans</i>	<i>sturtevantii</i>	Cornmeal	Wabasso, FL
14021-0251.188	<i>Drosophila</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>simulans</i>	Cornmeal	Santa Cruz Is
14021-0231.131	<i>Drosophila</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>melanogaster</i>	Cornmeal	Catalina Islan
	<i>Drosophila</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>suzukii</i>	Cornmeal	Parlier, CA
14011-0111.00	<i>Drosophila</i>	<i>Sophophora</i>	<i>obscura</i>	<i>persimilis</i>	Cornmeal	Cold Creek, C
14011-0121.262	<i>Drosophila</i>	<i>Sophophora</i>	<i>obscura</i>	<i>pseudoobscura</i>	Cornmeal	Mount St. He
14011-0131.10	<i>Drosophila</i>	<i>Sophophora</i>	<i>obscura</i>	<i>subobscura</i>	Banana	Eugene, OR
13000-0081.32	<i>Drosophila</i>	<i>Drosophila</i>	<i>busckii</i>	<i>busckii</i>	Wheeler-Clayton	Tucson, AZ
15085-1641.69	<i>Drosophila</i>	<i>Drosophila</i>	<i>repleta</i>	<i>hydei</i>	Cornmeal	Berkeley, CA
15010-1021.19	<i>Drosophila</i>	<i>Drosophila</i>	<i>virilis</i>	<i>montana</i>	Cornmeal	Mount Hood,
15030-1161.03	<i>Drosophila</i>	<i>Drosophila</i>	<i>melanica</i>	<i>paramelanica</i>	Cornmeal	Muscataine, IA
15020-1111.10	<i>Drosophila</i>	<i>Drosophila</i>	<i>robusta</i>	<i>robusta</i>	Cornmeal	Rocky Point,
15111-1731.16	<i>Drosophila</i>	<i>Drosophila</i>	<i>immigrans</i>	<i>immigrans</i>	Cornmeal	San Diego, CA
15130-1971.10	<i>Drosophila</i>	<i>Drosophila</i>	<i>guttifera</i>	<i>guttifera</i>	Cornmeal	Gainesville, F
15181-2181.21	<i>Drosophila</i>	<i>Drosophila</i>	<i>cardini</i>	<i>cardini</i>	Banana	Fort Laurerda
15120-1911.00	<i>Drosophila</i>	<i>Drosophila</i>	<i>funnebris</i>	<i>funnebris</i>	Cornmeal	Sturgis, KY
15220-2401.14	<i>Drosophila</i>	<i>Drosophila</i>	<i>tripunctata</i>	<i>tripunctata</i>	Cornmeal	Madison, WI
15150-2101.00	<i>Drosophila</i>	<i>Drosophila</i>	<i>testacea</i>	<i>putrida</i>	Banana	Chadron, NE

Stock Number ¹	Genus	Subgenus	Group	Species	Artificial diet ²	Collection site
20010-2010.01	<i>Chymomyza</i>		<i>fuscimana</i>	<i>amoena</i>	Cornmeal	East Lansing, MI
11010-0011.00	<i>Scaptodrosophila</i>	<i>Scaptodrosophila</i>	<i>victoria</i>	<i>lebanonensis</i>	Cornmeal	Veyo, UT
92000-0075.00	<i>Hirtodrosophila</i>		<i>duncani</i>	<i>duncani</i>	Banana	Jefferson County, KY
80000-2761.03	<i>Samoaia</i>	<i>Samoaia</i>	<i>leonensis</i>	<i>leonensis</i>	Cornmeal	Samoa, USA
31000-2651.04	<i>Scaptomyza</i>			<i>elmoi</i>	Cornmeal	Omogo valley, Kenya
70000-3000.00	<i>Gitona</i>			<i>americana</i>	Cornmeal	San Diego, CA
Field-collected	<i>Drosophila</i>	<i>Sophophora</i>	<i>melanogaster</i>	<i>suzukii</i>	Cornmeal	Parlier, CA

¹ Live specimen were purchased from University of California San Diego Drosophila Stock Center, currently called “ National Drosophila Species Stock Center (NDSSC)” and moved to University of Cornell (drosophilaspecies.com).

² Three different artificial diets were used for different species, including Cornmeal (45g agar, 125g cornmeal, 200g sugar, 70g nutritional yeast, 4.8 L distilled H₂O, 17.7 mL propionic acid 1M (= 74.08 mL/L H₂O), 3.3g Methyl paraben and 33.3 mL 95% ethanol), Banana medium (550 g blended bananas, 300g Karo syrup, 100g nutritional yeast, 100 g powder wheat dry malt, 45 g agar, 4 L distilled H₂O, 10 g Methyl paraben, 100 mL 95% ethanol, 15mL propionic acid 1M, 80 g white sugar) and Wheeler-Clayton (100 g nutritional yeast, 28 gr agar, 30g wheat germ, 30 Hi-Protein baby cereal, 10g Kellogg’s Product 19 cereal, 20 g Special K, 2 jars (2 x 135 g) Gerbers banana baby food, 2 L distilled H₂O, 12 mL propionic acid, and 12 mL 95% ethanol). The cornmeal receipt was adopted from Dalton et al. (2011) while the other two from University of California San Diego Drosophila Stock Center.

Table S2. Pupal sizes of various *Drosophila* species and offspring fitness of *Trichopria drosophilae* developed from the *Drosophila* species

Host species	Host pupal size ¹	Host pupal size ¹	Host pupal size ¹	Developmental time (day) ¹	Developmental time (day) ¹
	Length (mm)	Width (mm)	Volume (mm ³)	Male	Female
<i>D. busckii</i>	2.59 ± 0.03	0.95 ± 0.01	0.12 ± 0.00	0.49 ± 0.01	0.52 ± 0.01
<i>D. cardini</i>	2.84 ± 0.02	1.03 ± 0.01	0.16 ± 0.00	0.54 ± 0.01	0.57 ± 0.01
<i>D. funebris</i>	3.73 ± 0.04	1.27 ± 0.02	0.32 ± 0.01	0.56 ± 0.01	0.58 ± 0.01
<i>D. hydei</i>	4.42 ± 0.06	1.29 ± 0.02	0.40 ± 0.02	0.56 ± 0.01	0.59 ± 0.01
<i>D. immigrans</i>	3.82 ± 0.04	1.36 ± 0.02	0.38 ± 0.01	0.60 ± 0.01	0.63 ± 0.01
<i>D. montana</i>	4.52 ± 0.11	1.26 ± 0.03	0.38 ± 0.03	0.60 ± 0.01	0.60 ± 0.02
<i>D. melanogaster</i>	2.76 ± 0.01	0.97 ± 0.01	0.14 ± 0.00	0.51 ± 0.00	0.54 ± 0.01
<i>D. persimilis</i>	2.94 ± 0.02	1.07 ± 0.01	0.18 ± 0.01	0.51 ± 0.00	0.55 ± 0.01
<i>D. paramelanica</i>	2.91 ± 0.03	1.09 ± 0.01	0.18 ± 0.01	0.53 ± 0.00	0.58 ± 0.01
<i>D. pseudoobscura</i>	2.80 ± 0.03	1.09 ± 0.02	0.18 ± 0.01	0.53 ± 0.00	0.56 ± 0.01
<i>D. putrida</i>	2.27 ± 0.02	0.78 ± 0.01	0.07 ± 0.00	0.47 ± 0.01	0.50 ± 0.00
<i>D. robusta</i>	4.36 ± 0.04	1.38 ± 0.02	0.44 ± 0.02	0.60 ± 0.01	0.63 ± 0.01
<i>D. simulans</i>	2.84 ± 0.03	1.02 ± 0.01	0.16 ± 0.00	0.48 ± 0.00	0.52 ± 0.01
<i>D. sturtevantii</i>	2.99 ± 0.04	1.09 ± 0.02	0.19 ± 0.01	0.52 ± 0.01	0.54 ± 0.01
<i>D. subobscura</i>	2.98 ± 0.04	1.04 ± 0.02	0.17 ± 0.01	0.52 ± 0.00	0.57 ± 0.01
<i>D. willistoni</i>	2.52 ± 0.02	0.86 ± 0.01	0.10 ± 0.00	0.43 ± 0.00	0.47 ± 0.01
<i>H. duncani</i>	2.65 ± 0.05	0.93 ± 0.02	0.13 ± 0.01	0.50 ± 0.01	0.54 ± 0.01
<i>S. lebanonensis</i>	3.19 ± 0.02	0.97 ± 0.01	0.16 ± 0.00	0.51 ± 0.00	0.55 ± 0.01

¹ Values are mean ± SE and subject to analyses of multiple factors’ effects (see statistical results on Table 3).

² Data are were excluded from the analyses due to small samples (only a few individual wasps emerged).

Table 3S. Pupal sizes of various *Drosophila* species and offspring fitness of *Pachycrepoideus vindemiae* developed from the *Drosophila* species

Host species	Host pupal size ¹	Host pupal size ¹	Host pupal size ¹	Developmental time (day) ¹	Developmental time (day) ¹
	Length (mm)	Width (mm)	Volume (mm ³)	Male	Female
<i>D. busckii</i>	2.95 ± 0.05	1.08 ± 0.02	0.19 ± 0.01	0.39 ± 0.00	0.41 ± 0.01
<i>D. cardini</i>	2.67 ± 0.04	1.04 ± 0.02	0.15 ± 0.01	0.39 ± 0.00	0.41 ± 0.01
<i>D. funebris</i>	3.32 ± 0.07	1.17 ± 0.02	0.25 ± 0.01	0.44 ± 0.01	0.45 ± 0.01
<i>D. hydei</i>	3.54 ± 0.12	1.14 ± 0.03	0.25 ± 0.02	0.45 ± 0.02	— ²
<i>D. immigrans</i>	3.82 ± 0.03	1.39 ± 0.02	0.40 ± 0.01	0.46 ± 0.00	0.47 ± 0.01
<i>D. montana</i>	4.57 ± 0.08	1.25 ± 0.02	0.38 ± 0.01	0.44 ± 0.00	0.43 ± 0.02
<i>D. melanogaster</i>	2.86 ± 0.03	1.00 ± 0.01	0.15 ± 0.00	0.41 ± 0.00	0.36 ± 0.05
<i>D. persimilis</i>	2.83 ± 0.05	1.06 ± 0.07	0.17 ± 0.02	0.45 ± 0.02	— ²
<i>D. paramelanica</i>	2.98 ± 0.02	1.08 ± 0.01	0.18 ± 0.00	0.42 ± 0.0	0.43 ± 0.00
<i>D. pseudoobscura</i>	2.84 ± 0.04	1.01 ± 0.01	0.15 ± 0.01	0.40 ± 0.01	— ²
<i>D. putrida</i>	2.54 ± 0.02	0.91 ± 0.01	0.11 ± 0.00	0.36 ± 0.00	0.37 ± 0.01
<i>D. robusta</i>	3.84 ± 0.03	1.22 ± 0.03	0.31 ± 0.01	0.46 ± 0.01	0.48 ± 0.01
<i>D. simulans</i>	2.52 ± 0.03	0.93 ± 0.02	0.12 ± 0.01	0.38 ± 0.00	0.39 ± 0.01
<i>D. sturtevantii</i>	2.71 ± 0.03	1.04 ± 0.02	0.16 ± 0.01	0.42 ± 0.01	0.42 ± 0.01
<i>D. subobscura</i>	2.87 ± 0.03	1.06 ± 0.01	0.17 ± 0.01	0.41 ± 0.006	0.43 ± 0.008
<i>D. willistoni</i>	2.71 ± 0.04	0.92 ± 0.02	0.12 ± 0.01	0.34 ± 0.004	0.37 ± 0.008
<i>H. duncani</i>	2.64 ± 0.03	0.96 ± 0.02	0.13 ± 0.01	0.41 ± 0.004	0.40 ± 0.011
<i>S. lebanonensis</i>	2.69 ± 0.03	0.88 ± 0.01	0.11 ± 0.00	0.39 ± 0.006	0.40 ± 0.007

¹ Values are mean ± SE and subject to analyses of multiple factors' effects (see statistical results on Table 3).

² Data are were excluded from the analyses due to small samples (only a few individual wasps emerged).

Hosted file

image6.emf available at <https://authorea.com/users/362196/articles/483427-plasticity-of-body-growth-and-development-in-two-cosmopolitan-pupal-parasitoids>

Supplemental Figure 1