bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Wolbachia manipulates host pre-imaginal learning in a parasitoid wasp

Authors: Pouria Abroon[1], Ahmad Ashori[1], Anne Duplouy[2,3], Hossein Kishani Farahani[4]

Affiliations:

1 Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

2 Department of Biology, Lund University, Lund, Sweden

3 Organismal and Evolutionary Biology Research Program, The University of Helsinki, Helsinki, Finland

4 Equipe Recherches Agronomiques, Agronutrition, Carbonne, France

Corresponding author: Hossein Kishani Farahani

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Abstract:

The Hopkin's host-selection principle (HHSP) suggests that organisms at higher trophic levels demonstrate a preference for the host species on which they developed during larval stage. Although investigated in many herbivorous and predatory insects, the HHSP has, to our knowledge, never been tested in the context of insects hosting selfish endosymbiotic passengers such as the maternally inherited bacterium *Wolbachia pipientis*. Here, we investigate the effect of *Wolbachia* infection on host pre-imaginal learning in the parasitoid wasp *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae). We compare host-choice in *Wolbachia*-infected and uninfected adult female parasitoids after rearing them on two different Lepidopteran hosts, namely the flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) or the grain moth *Sitotroga cerealella* (Lep.: Gelechiidae). We show that in *T. brassicae*, *Wolbachia* affect the pre-imaginal learning ability of female wasps. *Wolbachia* infected wasps do not show any host preference and easily switch hosts in the laboratory, while uninfected wasps significantly prefer to lay eggs on the host species they developed on. We discuss how the facilitation of a generalist strategy by *Wolbachia* may allow *T. brassicae* to escape intraspecific competition with their uninfected counterparts, and may have important evolutionary consequences for the host and its symbionts.

Keywords:

Pre-imaginal learning, host preference manipulation, parasitoid,

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Introduction:

Learning from experience is a property embedded into the survival strategies of most animals, including insects [1-3]. For example, learning from olfactory cues enables many insects to optimize foraging, mating, and the completion of other behaviors in their complex local environments [4-6]. Parasitoid wasps especially rely on such innate mechanisms to directly identify, detect and parasitize their hosts [7, 8], or indirectly detect the host plants or any other characteristics of the environment in which the parasitoid has previously encountered its host [9]. Consequently, olfactory learning in parasitoids may contribute to behavioral optimization through increasing the speed and the efficiency of detecting suitable hosts, which should also positively affect the fitness of the parasitoid [10-12]. In the Ichneumonoid parasitoid wasps, *Hyposoter horticola* and *Venturia canescens*, females use olfactory cues from conspecific deterrent markings, to avoid costly interference and competition for resources (hosts) between individuals [13, 14]. Similarly, it has been shown that *Trichogramma brassicae* wasps reared from larvae feeding on tomato, later parasitized significantly more hosts on tomato plants than on lettuce [15].

The learning process of olfactory cues in insects was previously suggested to either occur during adult emergence, or at the young adult stage [9, 16-19]. Learning at adult emergence relies on traces of chemical cues from hosts, inside or outside the host body, which influence adult behavior potentially during a ‘sensitive period’ associated with adult emergence [20- 24] This phenomenon, learning at emergence, has been proposed to occur in two different ways. First, as a result of larval experiences, that implies that the larva can learn from its environment and that this memory can be transferred from pre-imaginal stages to the adult—the so-called Hopkins’ host selection principle [25]; second, at eclosion of the imago, in which the larval environment is

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

carried over to the adult stages and olfactory learning occurs during the contact of the young wasp with olfactory cues at emergence—the so-called "chemical legacy" hypothesis [19, 26]. Two ectoparasitoid wasps, *Hyssopus pallidus* and *Aphidius ervi*, of the codling moth, *Cydia pomonella*, rely on chemical cues learned during their respective pre-imaginal stages to find hosts [17, 19]. Early experience of olfactory stimuli associated with their host is an important driver of parasitoid foraging choices, notably leading to host selection [27].

Successful host detection and choice may depend on a parasitoid's ability to learn different cues during its contact period with its host [2, 28, 29], and is most likely to affect the fitness of the offspring that will develop within the chosen host. Host specificity in parasitoid wasps varies from highly specific, with many species only parasitizing one unique host species, to generalist, with species using a wide range of hosts [30-32]. Furthermore, studies have shown that in certain conditions, specialist parasitoids may successfully lay eggs in new host species [33, 34] , which may lead to evolutionary changes in host specificity. Such change in host-specificity may occur through two different strategies. A 'host switch' is a sudden or accidental colonization of a new host species by a few individuals capable to establish a new and viable population in the new host, while a 'host-shift' is a gradual change of the relative role of a particular host species as primary versus secondary host. Host preference is also by no means static, but is characterized by behavioral plasticity that allows parasitoids to switch hosts when their preferred host is unavailable and by learning host cues associated with positive or negative experiences[35- 37].

Wolbachia is a single bacterial lineage in the alpha-group of the Proteobacteria. The bacterium benefits from strategies, such as thelytoky that increases the number of *Wolbachia*-transmitting females in the host population [38-42]. Similarly, *Wolbachia* can also successfully spread in their

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

host populations by positively affecting various of their host's life history traits, including fecundity, and survival to pathogens or environmental stresses [43-48].

The parasitoid wasp *T. brassicae* (Westwood) (Hym.: Trichogrammatidae) is an egg parasitoid widely used as a biological control agent of various Lepidoptera pest species [49-53]. As it is often the case in Trichogramma wasps, *T. brassicae* can reproduce by arrhenotokous parthenogenesis, with unfertilized eggs producing viable haploid male offspring [54-56]. However, in certain lineages of *T. brassicae*, only females can emerge from unfertilized eggs [54, 56, 57]. In these particular cases, studies have shown that the wasps are naturally infected by the endosymbiotic bacterium *Wolbachia*, which modifies the reproductive system of the wasps, such that *Wolbachia*-infected individuals reproduce through the tlythokous parthenogenesis instead [43, 57]. As the embryo grows up in host eggs, their pre-imaginal learning abilities are likely to be affected by direct cues from their hosts, but less by chemical cues linked to host food or environmental chemical cues. We hypothesized that the manipulated pre-imaginal learning and host-preference in *T. brassicae* by *Wolbachia* may support host shift and adaptation to new intracellular environments in parasitoid wasps.

Material and methods:

Parasitoids

We compared two lineages of *Trichogramma brassicae*: one *Wolbachia* infected (*Wolbachia wBaT.bra* registered as FJ441291 in Genbank), and one uninfected. Previous studies have shown that the *Wolbachia*-infected and *Wolbachia*-free lineages carry the same genetic background [44]. This characteristic allowed us to avoid the use of antibiotics against *Wolbachia*, and their potential confounding effects on the physiology and behavior of antibiotic treated insects [58-60].

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Both lineages came from colonies maintained by the Ecology and Behavior Laboratory of the University of Tehran, Iran, and were originally collected in 2016 from a Cornfield in north of Iran (Baboulsar Region, South of Caspian Sea, Iran). Both original lineages have been reared on *Ostrinia nubilalis* (Lep.: Crambidae) in the laboratory for two generations before experimental design. Parasitoids were reared on egg cards (2×5 cm), loaded with one-day old eggs of *O. nubilalis*. The egg cards were placed into emergence canisters and held in incubators at 25±1ºC, 16L: 8 D and 50±5% RH. Emergence canisters were closed cardboard cylinders (500 ml, 63×161 mm) with a glass vial (50 ml, 26×93 mm).

For all experiments described below, we used two different Lepidoptera species as host to the parasitoids: the flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and the grain moth *Sitotroga cerealella* (Lep.: Gelechiidae). Lepidoptera eggs were obtained from a culture maintained at the Insectary and Quarantine Facility, University of Tehran. The cultures were reared at 25±1ºC on wheat flour and yeast (5%). Newly emerged and mated female moths were kept in glass containers (500 ml) to provide eggs. Eggs were collected daily to ensure that the eggs used in the experiments were no more than 24 h old. The parasitoids were reared at 25±1ºC, 50±5% RH, and 16:8 L: D on eggs of either Lepidoptera hosts for more than ten generations.

Wolbachia screening

We determined *Wolbachia* presence in female wasps, which produced only female offspring [43], by PCR screening for the *Wolbachia surface protein* (*wsp*) gene using the *Wolbachia* specific primers 81F/691R [43,61]. The *Wolbachia* infected line was monitored for the infection throughout the study by PCR. Also uninfected strain was screened randomly to check on any contamination or horizontal transfer between the lines throughout the experiment.

Experimental design

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

All treatments were carried out in an insectary room under controlled conditions with a temperature of 25±1ºC and 55±5% RH. Individual *Trichogramma* females were presented with patches of host eggs fixed with water onto small pieces of white cardboard. The experiments ended once the female wasps left the patches of eggs that were offered to them. All parasitized eggs were incubated at 25 ± 1 °C, L16:D8, and 50 +/-5% R.H. for 4–5 days until the eggs blackened, suggesting that the parasitoid had pupated within its host. Parasitized host eggs were then cut out of the cardboard patch and placed individually in gelatin capsules to await the emergence of the parasitoid progeny. We then measured the total number of parasitoids that emerged. Parasitism rate was calculated as the ratio of blacked eggs compared to the total number of provided host eggs for each wasp.

Immediately after wasp emergence, mated female wasps were removed from their gelatin capsules and maintained for an hour in new tubes individually. During this period, the wasps were never in contact with hosts, and were kept in a separate growth chamber than their future hosts. The emerging parasitoids sex was determined by morphological differentiation of the antennae [52]. We calculated the sex ratio of the emerging progeny for each female wasp.

A) Innate host preference

We first tested the innate preference of newly collected female wasps, infected or not by *Wolbachia*, toward both experimental hosts (*E. kuehniella* and *S. cerealella)*, after emerging from the European corn borer, *O. nubilalis*. Thirty newly emerged wasps infected by *Wolbachia*, and 30 newly emerged uninfected wasps were exposed to eggs from each experimental host in a choice and non-choice test.

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

In the choice experiment: wasps were exposed to 50 one-day old eggs of *E. kuehniella* and 50 on day old eggs of *S. cerealella* (Ntotal=100 eggs), simultaneously. Eggs of each host species were attached to small cards (1× 5 cm) separately and were placed in a glass tubes (2×10cm).

In the non-choice experiment: newly collected wasps were exposed to patch containing 100 one-day old eggs of each experimental host separately. Each egg card contained 100 eggs fixed on labeled, white cardboard cards (2.5× 5 cm) of either host later exposed to a wasp in a glass tubes (2×10cm). For each test, a naive (no previous oviposition experience) one-day old female wasp was released on each egg patch.

B) Host preference after early imaginal experience

To study host preference after early imaginal experience, wasps were reared on either of the two experimental hosts for 10 consecutive generations prior to the experiment. We studied the effect of *Wolbachia* presence and experimental host species during immature developmental time on pre-imaginal learning ability using a full factorial design: each of the two parasitoid lineages were offered to parasitize each of the two Lepidoptera host species, with 30 replicates for each treatment. The four treatments scheme includes Treatment T1: uninfected wasps reared on *E. kuehniella*, T2: uninfected wasps reared on *S. cerealella*, T3: *Wolbachia*-infected wasps reared on *E. kuehniella*, and T4: *Wolbachia*-infected wasps reared on *S. cerealella* (Figure 1)**.**

Data analysis

All statistical analyzes were done by SAS software [62]. To compare egg-laying preference of the female wasps, we implemented a Generalized Linear model in the procedure GENMOD of the SAS software (ver. 9.1), with the binomial family error and logit link. After this global test, the least square estimates of the proportions in each level were compared by the Chi-square approximation (an option offered by GENMOD).

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

The rate of emergence was calculated based on the total number of emerged wasps and the total number of oviposited eggs, and was analyzed for each experiment using generalized linear models based on a binomial logit distribution [63]. The numbers of female and male offspring produced by uninfected wasps were compared by Student's t-test.

In all cases, the explanatory variables were the host species and the *Wolbachia* infection, and when a significant effect of the treatment was found, the tests were followed by Bonferroni's *posthoc* multiple comparison tests. The two-by-two comparisons were evaluated at the Bonferroni-corrected significance level of $P = 0.05/k$, where k is the number of comparisons. Data are presented as means ±SE (through the results and Table 1).

Results:

Host preference experiments

A) Innate host preference

Effects of *Wolbachia* infection status, host species, and their interaction on the preference of the female parasitoid wasps are shown in Table 1. As showed in Figure 1, both lineages displayed no significant preference for either experimental host *E. kuehniella* or *S. cerealella*, and showed the same response toward the two hosts in both the choice and the no choice tests.

B) Host preference after early imaginal experience

In the choice experiment: A significant interaction between wasp *Wolbachia* infection status, and host species was observed on the wasps' host preference (Table 1). The uninfected wasps reared on *E. keueneilla* significantly preferred the hosts that were reared on, *E. keuene*, (χ^2=9.77, P=0.0018, N=100), while *Wolbachia*-infected wasps did not show any preference for either host (χ^2=0.26, P=0.611, N=100) (Figure 3). Similarly, *w*hen given the choice, uninfected wasps reared

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

on *S. cerealella* showed a significant preference toward the host they were reared on (χ^2=14.55, P=0.0001, N=100) (Figure 3), while *Wolbachia* infected wasps reared on *S. cerealella* showed no preference toward either hosts (χ^2=0.11, P=0.74, N=100).

In the no-choice experiment: Uninfected wasps reared on *E. keueneilla* laid more eggs on *E. keueneilla* (χ^2=6.79, P=0.009, N=100). However, no significant difference was observed in number of eggs laid in both hosts by infected wasps (χ^2=0.2, P=0.653, N=100) (Fig 3). Uninfected wasps reared on *S. cerealella*, again significantly oviposited more on the host they were on (χ^2=11.09, P=0.0009, N=100), while *Wolbachia* infected wasps reared on *S. cerealella* oviposited similarly on both hosts in no choice tests (χ^2=0.57, P=0.45, N=100) (Figure 3).

Emergence rate

The parasitoid infection status (χ^2=8.44, P=0.003), the host species (χ^2=4.86, P=0.0275), and their interaction (χ^2=42.41, P<0.0001) had a significant effect on the emergence rate of the parasitoid. Uninfected wasps reared on *S. cerealella* showed significant lower emergence rate when they were reared for one generation on *E. kuehniella* (Figure 4). Similarly, emergence rate of uninfected wasps reared on *E. kuehniella* was significantly lower when wasps were reared on *S. cerealella* for one generation (Figure 4). All other treatments showed similar emergence rates.

Sex ratio

Reared uninfected wasps on *E. kuehniella* laid more female eggs in *E. kuehniella* eggs (t = 7.668, df = 1, P<0.0001) compared to uninfected wasps exposed to *S. cerealella*; their observed offspring sex ratio was 1:3 (male: female). Also uninfected wasps reared on *S. cerealella* produced more female offspring in patches containing *S. cerealella* eggs (t = 2.677, df = 1, P = 0.015) compared to uninfected wasps exposed to *E.kuehniella*, with a ratio of 1:3. All produced

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

215 offspring by infected wasps in both hosts were females. Due to *Wolbachia*-induced thelytoky, all
216 offspring from *Wolbachia*-infected wasps were females.
217
218 **Discussion:**
219 According to our results, *Wolbachia*-infected specimens of the parasitoid wasp *Trichogramma*
220 *brassicae* do not follow the Hopkin's host-preference principle, while their uninfected
221 counterparts do. *Wolbachia* manipulates the host preference of the parasitoid wasp, such that
222 female wasps do no discriminate between eggs from *E. kuehniella* and *S. cerealella*. In contrast,
223 uninfected wasps of the same species show a significant preference towards parasitizing the
224 Lepidopteran host species from which they emerged. We also show that when choice is not given,
225 *Wolbachia*-infected wasps lay the same amount of eggs in both Lepidopteran hosts offered, with
226 similar emergence rates from both hosts, contrasting with uninfected wasps, which significantly
227 lay more eggs and consequently show higher emergence rates in the host species they emerged
228 from. The family Trichogrammatidae is one of the earliest branching families of the superfamily
229 Chalcidoidea [64, 65]. Although many members of this family are generalists, host specificity
230 has also been reported [66-68]. The two *T. brassicae* lineages used for this study were collected
231 from a same unique location in Iran, from a same host species, *Ostrinia nubilalis*, and were later
232 shown to share the same genetic background, we believe they originally share similar host
233 preference. Thus, the differences observed in our study are more likely only due to the insects'
234 *Wolbachia* infection status.
235 By relaxing strict host preference through modifying pre-imaginal learning ability in its host,
236 generalist *Wolbachia* infected individuals could oviposit in and thus benefit from a wider range
237 of hosts resource [69, 70]. They may as well benefit from a more nutritionally balanced diet, and

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

238 show higher capacity to confront variable environments [71-73]. In contrast, the information-
239 processing hypothesis suggests that highly efficient decision-making should evolve faster in
240 specialist foragers compared to generalists [74-77]. Additionally, specialists may also evolve as
241 better competitors on their unique hosts, and thus show higher survival, but could also be more
242 susceptible to population fluctuation of the host than generalist species [78, 79].
243 According to our results, *Wolbachia* infected wasps show similar host preference behavior even
244 after emerging from different hosts. Previously, [80] shown that there are no differences in
245 olfactory responses between conditioned and unconditioned *Wolbachia* infected *T. brassicae*
246 toward peppermint odor. The fixed behaviors were suggested to be a consequence of *Wolbachia*
247 affecting organs structure and function [81, 82], and thus potentially leading to poor information
248 integration, and no differentiation between different host species and host qualities [44]. Studies
249 have also shown that *Wolbachia* can directly affect a range of neurotransmitters with potential
250 impacts on subsequent fitness-related behaviors in various insects [48, 83, 84]. Learning capacity
251 may allow the host to tune behavior to an adaptive solution, especially when the environment is
252 not informative enough for the specific foraging behavior to be optimized [85]. Through
253 manipulating the time dedicated to cues learning and/or information processing, *Wolbachia* may
254 thus appear beneficial for the host that can utilize and develop on different host species.
255 Specialists have long been seen as potential evolutionary dead-ends [86], but more recent
256 research indicates that transition from specialist to generalist phenotype often occur [87]. Switch
257 to a new host however will most likely require genetic, physiologic, and ecological adaptations
258 from the parasitoid wasps [33, 37, 88, 89]. Our study suggests that symbionts like *Wolbachia* can
259 be powerful evolutionary motors in the transition from specialization to generalization [90-92],
260 as their presence may decrease adaptations costs to the new hosts [93,94]. To switch and survive

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

in/on new hosts, the parasitoids must overcome many new ecological and physiological barriers imposed by the new hosts such as overcoming host immune system [95]. *Wolbachia* has been shown to be involved in nutritional provisioning for its hosts, such as providing some elements missing from their host diet or environment [96-99]. In *T. brassicae*, [100] showed that *Wolbachia* may provide for the larval development of their host, as individuals previously reared on hosts of different qualities showed no fitness differences at the adult stage. The details of this potential provision however remain unclear.

Conclusion

We show here that *Wolbachia* can indeed manipulate its host preference for certain resource, and maintain a generalist parasitism strategy in *T. brassicae*. Although the details of the genetic and physiological background of the behavioral manipulation still remain to be investigated, we believe this phenotype is conserved in nature as it may support the spread of the symbiotic strain in unstable environments, in which specialists may be at higher risk of extinction. *Wolbachia* has been considered as “a generalist in host use” as it is thought to be present in at least 40% of all insects [101]. Although a strict maternal transmittion of the symbiont would lead to co-evolution of the symbiont and its hosts, phylogenetic studies have shown that the phylogenetic trees of *Wolbachia* strains is rarely congruent with those of their insect hosts [102]. These results suggest that *Wolbachia* transfer horizontally between host species more often than previously thought. By restraining its host ability to preferentially parasitize the Lepidoptera host species it emerge from, the *Wolbachia* strain present in T. brassicae may thus increase its chance to transfer horizontal and potentially establish in a wider range of naïve species populations.

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Acknowledgements:

We appreciate Prof. Jean-Sébastien Pierre for his help and comments in improving statistical analyses. Also we are grateful to Seyedeh Samira Qazaei for the technical support provided.

This study was financially supported by the University of Tehran, and Academy of Finland (Grant #321543 to AD), and the Marie-Curie Sklodowska Individual fellowship (#120586, Host Sweet Home to AD), but the sponsor had no involvement in the study design, the collection, analysis, and interpretation of data, the writing, or where to submit the paper for publication.

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

References:

1. Papaj DR, Prokopy RJ. 1989. Ecological and evolutionary aspects of learning in phytophagous insects. In T. E. Mittler (Ed.). *Ann. Revi. Of entomo.* **34**,315-350.
2. Dukas R. 2008 Evolutionary biology of insect learning. *Annu. Rev. Entomol.* **53**, 145–160. (doi: 10.1146/annurev.ento.53.103106.093343)
3. Chittka L, Niven J. 2009. Are bigger brains better?. *Curr. Biol.* **19(21)**, 995–1008. (doi: 10.1016/j.cub.2009.08.023)
4. Ishii Y, Shimada M. 2009 The effect of learning and search images on predator–prey interactions. *Popul. Ecol.* **52(1)**,27-35.
5. Deisig N, Dupuy F, Anton S, Renou M. 2014 Responses to Pheromones in a Complex Odor World, Sensory Processing and Behavior. *Insects* **5(2)**,399–422. (doi: 10.3390/insects. 5020399)
6. Conchou L, Lucas P, Meslin C, Proffit M, Staudt M, Renou M. 2019 insect Odorscapes, From Plant Volatiles to Natural Olfactory Scenes .*Front. in Physiol.* **10**,972
7. Prevost G, Lewis WJ. 1990 Heritable differences in the response of the braconid wasp*Microplitis croceipes* to volatile allelochemicals. *J. Insect Behav.* **3**,277–287.
8. Gu H, Dorn S. 2000 Genetic variation in behavioural response to herbivore infested plants in the parasitic wasp *Cotesia glomerata*. *J. Insect Behav*. **13**,141–157.
9. Turlings TCJ, Wäckers F, Vet LEM, Lewis WJ, Tumlinson JH. 1993 Learning of host-finding cues by hymenopterous parasitoids, pp. 51–78, in D. R. Papaj and A. C. Lewis (Eds.). Insect Learning, Ecological and Evolutionary Perspectives. Chapman and Hall, New York.
10. Bouchard Y, Cloutier C. 1985 Role of olfaction in host finding by aphid parasitoidAphidius nigripes (Hymenoptera, Aphidiidae). *J. Chem. Ecol.* **11**, 801. (doi: 10.1007/ BF00988307)

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

11. Wäckers FL, LewisWJ. 1994 Olfactory and visual learning and their combined influence on host site location by the parasitoid Microplitis croceipes (Cresson). *Bio. Cont.* **4**, 105-112.
12. Meiners T, Wäckers FL, Lewis WJ. 2003 Associative learning of complex odours in parasitoid host location. *Chemi. Sen.* **28(3)** ,231-6.
13. Couchoux C, van Nouhuys S. 2015 Effects of Intraspecific Competition and Host-Parasitoid Developmental Timing on Foraging Behaviour of a Parasitoid Wasp. *J. Insect Behav.* **27(3)**,283–301. (doi:10.1007/s10905-013-9420-6)
14. Kishani Farahani H, Moghadassi Y, Alford L, van Baaren J. 2019 Effect of interference and exploitative competition on associative learning by a parasitoid wasp, a mechanism for ideal free distribution?. *Ani. Behav.***151**,157-163.
15. Bjorksten TA, Hoffmann AA. 2004 Plant cues influence searching behaviour and parasitism in the egg parasitoid Trichogramma nr. Brassicae. *Eco. Entom.* **23(4)**, 355-36.
16. Fujiwara C, Takabayashi J, Yano S. 2000 Oviposition experience on a host-infested plant affects flight and antennal searching behaviour of Cotesia kariyai toward the host-plant complex. *Entomol. Exp. Applic.* **97**, 251–256.
17. Gutiérrez-Ibáñez C, Villagra CA, Niemeyer HM. 2007 Pre-pupation behaviour of the aphid parasitoid Aphidius ervi (Haliday) and its consequences for pre-imaginal learning. *Naturwissenschaften* **94**, 595–600.
18. de Queiroz AP, de Freitas Bueno A, Pomari-Fernandes A, Bortolotto OC, Mikami AY, Olive L. 201. Influence of host preference, mating, and release density on the parasitism of Telenomus remus (Nixon) (Hymenoptera, Platygastridae). *Revi. Brasil. De. Entomo.* **61** , 86–90.

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

19. Gandolfi M, Mattiacci L, Dorn S. 2003 Mechanisms of behavioral alterations of parasitoids reared in artificial systems. *J. Chem. Ecol.* **29**, 1871–1887.

20. Arenas A, Farina WM. 2008 Age and rearing environment interact in the retention of early olfactory memories in honeybees. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* **194**, 629–640. (doi: 10.1007/s00359008-0337-z)

21. Arenas A, Giurfa M, Farina WM, Sandoz JC. 2009 Early olfactory experience modifies neural activity in the antennal lobe of a social insect at the adult stage. *Eur. J. Neurosci.* **30**, 1498–1508.

22. Behrends A, Scheiner R .2009 Evidence for associative learning in newly emerged honey bees (Apis mellifera). *Anim. Cogn.* **12**,249–255. (doi: 10.1007/s10071-008-0187-7)

23. 23. Giunti G, Canale A, Messing RH, Donati E, Stefanini C, Michaud JP, Benelli G. 2015 Parasitoid learning, Current knowledge and implications For biological control. *Bio. Cont.* **90**, 208–219. (doi:10.1016/ j.bioco.2015.06.007)

24. Grosso JP, Barneto JA, Velarde RA, Pagano EA, Zavala JA , Farina WM .2018 An Early Sensitive Period Induces Long-Lasting Plasticity in the Honeybee Nervous System. *Front. Behav. Neurosci.* **12**,11. (doi: 10.3389/fnbeh.2018.00011)

25. Barron AB. 2001 The life and death of Hopkins' host-selection principle. *J. Insect Behav.* **14**, 725–737.

26. Corbet SA.1985 Insect chemosensory responses, a chemical legacy hypothesis. *Ecol. Entomol.* **10**,143–153. (doi:10.1111/j.1365-2311. 1985.tb00543.x)

27. Luquet M, Tritto O, Cortesero AM, Jaloux B, Anton S. 2019 Early Olfactory Environment Influences Antennal Sensitivity and Choice of the Host-Plant Complex in a Parasitoid Wasp. *Insects* **10**, 127.

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

28. Rehman A, Powell W. 2010 Host selection behaviour of aphid parasitoids (Aphidiidae, Hymenoptera) . *J. Pla. Breed. And. Cro. Scien.* **10**,299-311.

29. Fors L, Mozuraitis R, Blazyte-Cereskiene L, Verschut TA, Hamback PA. 2018 Selection by parasitoid females among closely related hosts based on volatiles, Identifying relevant chemical cues. *Ecol. And. Evol.* **8**,3219-3228.

30. Strand MR, Obrycki JJ. 1996 Host Specificity of Insect Parasitoids and Predators, Many factors influence the host ranges of insect natural enemies, *BioSci*. **46(6)**, 422–429. (doi:10.2307/1312876)

31. Korenko S, Michalková V, Zwakhals K, Pekár S. 2011 Host specificity and temporal and seasonal shifts in host preference of a web-spider parasitoid Zatypota percontatoria. *J. Of Insect Sci.* **11**,101.

32. van Oudenhove L, Mailleret L, Fauvergue X. 2017 Infochemical use and dietary specialization in parasitoids, a meta-analysis. *Ecol. Evol.* **7(13)**,4804–4811. (doi:10.1002/ece3.2888)

33. Jones TS, Bilton AR, Mak L, Sait SM. 2015 Host switching in a generalist parasitoid, contrasting transient and transgenerational costs associated with novel and original host species. *Ecol. Evol.* **5(2)**,459–465. (doi:10.1002/ece.3.1333)

34. Jones AL, Jennings DE, Hooks CRR, Shrewsbury PM. 2017 Field surveys of egg mortality and indigenous egg parasitoids of the brown marmorated stink bug, Halyomorpha halys, in ornamental nurseries in the mid-Atlantic region of the USA. *J. Pest. Sci.* **90**, 1159. (doi: 10.1007/s10340-017-0890-8)

35. Wolf GH, Riffell Ja. 2018 Olfaction, experience and neural mechanisms underlying mosquito host preference. *J. Exp. Bio.* **221**,157131. (doi:10.1242/ jeb.15713)

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

384 36. Agosta SJ, Klemens JA. 2008 Ecological fitting by phenotypically flexible genotypes,
385 implications for species associations, community assembly and evolution. *Ecol. Lett.* **11**,
386 1123–1134.
387 37. Araujo SB, Braga MP, Brooks DR, Agosta SJ, Hoberg EP, von Hartenthal FW, Boeger WA.
388 2015 Understanding Host-Switching by Ecological Fitting. *PloS One* **10(10)**,e0139225.
389 (doi:10.1371/ journal. 0139225)
390 38. van Nouhuys S, Kohonen M, Duplouy A. 2016 *Wolbachia* increases the susceptibility of a
391 parasitoid wasp to hyperparasitism. *J. Expe. Bio.* **219**, 2984-2990. (doi: 10.1242/jeb. 140699)
392 39. Ahmed MZ, Li SJ, Xue X, Yin XJ, Ren SX, Jiggins FM, Greeff JM, Qiu BL. 2015 The
393 Intracellular Bacterium Wolbachia Uses Parasitoid Wasps as Phoretic Vectors for Efficient
394 Horizontal Transmission. *PLOS Pathog.* **11(2)**, e1004672.
395 (doi:10.1371/journal.ppat.1004672.)
396 40. Furihata S, Hirata M, Matsumoto H, Hayakawa Y. 2015 Bacteria Endosymbiont, Wolbachia,
397 Promotes Parasitism of Parasitoid Wasp Asobara japonica. *PloS One* **10(10)**, e0140914.
398 (doi:10.1371/journal.pone.0140914)
399 41. Werren JH, Baldo L, Clark ME. 2008 *Wolbachia*, master manipulators of invertebrate
400 biology. *Nat. Rev. Microbiol.* **6**, 741–751.
401 42. Duplouy A, Couchoux C, Hanski I, van Nouhuys S. 2015 Wolbachia Infection in a Natural
402 Parasitoid Wasp Population. *PloS One* **10(8)**, 0134843. (doi:10.1371/ journal. pone.
403 0134843)
404 43. Farrokhi S, Ashouri A, Shirazi J, Allahyari H, Huigens ME. 2010 A comparative study on
405 the functional response of Wolbachia-infected and uninfected forms of the parasitoid wasp
406 Trichogramma brassicae. *J. Insect. Sci.* **10**,167.

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

44. Kishani Farahani H, Ashouri A, Goldansaz SH, Ainouche A, van Baaren J. 2015 Does Wolbachia infestation affect decision making in a parasitic wasp?. *Entomol. Exp. App.* **155(2)**,102-116.

45. Caragata EP, Real KM, Zalucki MP, McGraw EA. 2011 Wolbachia infection increases recapture rate of fieldreleased Drosophila melanogaster. *Symbiosis* **54**,55–60.

46. Peng Y, Nielsen JE, Cunningham JP, McGraw EA. 2008 Wolbachia infection alters olfactory-cued locomotion in Drosophila spp. *Appl. Environ. Microbiol.* **74**,3943–3948.

47. Peng Y, Wang Y. 2009 Infection of Wolbachia may improve the olfactory response of Drosophila. *Chin. Sci. Bull.* **54**,1369–1375.

48. Rohrscheib CE, Bondy E, Josh P, Riegler M, Eyles D, van Swinderen B, Weible MW, Brownlie JC.2015 Wolbachia Influences the Production of Octopamine and Affects Drosophila Male Aggression. *Appli. And Enviro. Microbio.* **81 (14)**, 4573-4580. (dio: 10.1128/AEM.00573-15)

49. Babendreier D, Kuske S, Bigler F. 2003 Non-target host acceptance and parasitism by Trichogramma brassicae Bezdenko (Hymenoptera, Trichogrammatidae) in the laboratory. *Bio. Cont.* **26**, 128–138.

50. Kuske S, Widmer F, Edwards PJ, Turlings TCJ, Babendreier D, Bigler F. 2003 Dispersal and persistence of mass released Trichogramma brassicae (Hymenoptera, Trichogrammatidae) in non-target habitats. *Bio. Cont.* **27**,181–193.

51. Hoffmann MP, Walker DL, Shelton AM. 1995 Biology of Trichogramma ostriniae (Hym., Trichogrammatidae) reared on Ostrinia nubilalis (Lep., Pyralidae) and survey for additional hosts. *Entomophaga* **40**, 387– 402.

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

429 52. Pinto JD. 1998 Systematics of the North American species of Trichogramma Westwood
430 (Hymenoptera, Trichogrammatidae). *Mem. Entomol. Soc. Wash.* **22**,1–287.
431 53. Mansfield S, Mills NJ. 2002 Host egg characteristics, physiological host range, and
432 parasitism following inundative releases of Trichogramma platneri (Hymenoptera,
433 Trichogrammatidae) in walnut orchards. *Environ. Entomol.* **31**, 723–731.
434 54. Hohmann CL, Luck RF, Stouthamer R. 2001 Effect of Wolbachia on the survival and
435 reproduction of Trichogramma kaykai Pinto and Stouthamer (Hymenoptera,
436 Trichogrammatidae). *Neotrop. Entomol.* **30(4)**, 607-612.
437 55. Pannebakker BA, Beukeboom LW, van Alphen JJ, Mrakefield PM, Zwaan.BJ. 2004 The
438 Genetic Basis of Male Fertility in Relation to Haplodiploid Reproduction in Leptopilina
439 clavipes (Hymenoptera, Figitidae). *Genetics* **168**, 341–349 .
440 56. Ma W-J, Schwander T. 2017 Patterns and mechanisms in instances of endosymbiont-induced
441 parthenogenesis. *J. Evol. Bio.* **30(5)**,868–888. (doi: 10.1111/jeb.13069)
442 57. Poorjavad N, Goldansaz SH, Machtelinckx T, Tirry L, Stouthamer R, van Leeuwen T. 2012
443 Iranian Trichogramma, ITS2 DNA characterization and natural Wolbachia infection. *BioCon.*
444 **57**,361–374.
445 58. Timmermans M, Ellers J. 2009 Wolbachia endosymbiont is essential for egg hatching in a
446 parthenogenetic arthropod. *Evol. Ecol.* **23**,931–942.
447 59. Dedeine F, Vavre F, Fleury F, Loppin B, Hochberg ME, Boulétreau M. 2001 Removing
448 symbiotic Wolbachia bacteria specifically inhibits oogenesis in a parasitic wasp. *Proc. Natl.*
449 *Acad. Sci. USA* **98**,6247–6252.
450 60. Hoffmann A, Turrelli M, Harshman L. 1990 Factors affecting the distribution of cytoplasmic
451 incompatibility in Drosophila simulans. *Genetics* **126**, 933–948.

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

61. Braig HR, Zhou W, Dobson S, O'Neill SL. 1998 Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont Wolbachia. *J. Bacter*. **180**, 2373-2378.

62. SAS Institute. 2003 SAS User's Guide: Statistics version 9.1. SAS Institute, Cary, NC, USA.

63. Crawly MJ.1993 GLIM for ecologists. Oxford. Blackwell scientific publications.

64. Heraty JM, Burks RA, Cruaud A, Gibson GAP, Liljeblad J, Munro J, Rasplus JY, Delvare G, Jansta P, Gumovsky A, et al. 2013 A phylogenetic analysis of the megadiverse Chalcidoidea (Hymenoptera). *Cladistics* **29(5)**,466–542.

65. Munro JB, Heraty JM, Burks RA, Hawks D, Mottern J, Cruaud A, Rasplus JY, Jansta P. 2011 A molecular phylogeny of the Chalcidoidea (Hymenoptera). *PloS One* **6(11)**, e27023.

66. Pluke R, Leibee GL. 2006 Host Preferences of Trichogramma pretiosum and the Influence of Prior Ovipositional Experience on the Parasitism of Plutella xylostella and Pseudoplusia includens Eggs. BioCont. **51(5)**:569-583. (dio:10.1007/s10526-005-1033-3).

67. Paraiso O, Hight SD, Kairo MT, Bloem S.2013 Host specificity and risk assessment of Trichogramma fuentesi (Hymenoptera: Trichogrammatidae), a potential biological agent of Cactoblastis cactorum (Lepidoptera: Pyralidae). *Flo. Entomol.* **96(4)**:1305-1310. (doi:10.1653/024.096.0409)

68. Iranipour S, Vaez N, Nouri Ghanbalani G, Asghari Zakaria R, Mashhadi Jafarloo M. 2010 Effect of host change on demographic fitness of the parasitoid, Trichogramma brassicae. *J. of Insect Sci.* **10(1)**, 78.

69. Campan E, Benrey B. 2004 Behavior and performance of a specialist and a generalist parasitoid of bruchids on wild and cultivated beans. *Bio. Cont.* **30**,220-228.

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

70. Reudler JH, Biere A, Harvey JA, van Nouhuys S. 2011 Differential performance of a specialist and two generalist herbivores and their parasitoids on Plantago lanceolata. *J. Chem. Ecol.* **37(7)**,765–778. (doi:10.1007/s10886-011-9983-7)

71. Bernays EA, Bright KL, Gonzalez N, Angel J. 1994 Dietary mixing in a generalist herbivore, tests of two hypotheses. *Ecology* 75, 1997–2006.

72. Bernays EA, Minkenberg O. 1997 Insect herbivores, different reasons for being a generalist. *Ecol.* **78**,1157–1169.

73. Funk D J, Bernays EA. 2001 Geographic variation in host specificity reveals host range evolution in Uroleucon ambrosiae aphids. *Ecology* **82**,726–739.

74. Egan SP, Funk DJ. 2006 Individual advantages to ecological specialization, insights on cognitive constraints from three conspecific taxa. *Proc. R. Soc.* **273**, 843–848. (doi:10.1098/rspb.2005.3382)

75. Bernays EA, Wcislo WT. 1994 Sensory capabilities, information-processing, and resource specialization. *Q. Rev. Biol.* **69**,187–204. (doi:10.1086/418539).

76. Bernays EA. 2001 Neural limitations in phytophagous insects, implications for diet breadth and evolution of host affiliations. *Annu. Rev. Entomol.* **46**, 703–727. (doi:10. 1146/annurev. ento.46.1.703)

77. Jacob S, Laurent E, Haegeman B, Bertrand R, Prunier JG, Legrand D, Cote J, Chaine AS, Loreau M, Clobert J, Schtickzelle N. 2018 Habitat choice meets thermal specialization, Competition with specialists may drive suboptimal habitat preferences in generalists. *Nati. Acad. Of Sci.* **115 (47)**, 11988-11993. (dio: 10.1073/ pnas. 1805574115)

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

78. Habermannová J, Bogusch P, Straka J. 2013 Flexible Host Choice and Common Host Switches in the Evolution of Generalist and Specialist Cuckoo Bees (Anthophila, *Sphecodes*). *PloS One* **8(5)**, e64537. (doi:10.1371/ journal.pone.0064537)

79. Peers MJ, Thornton DH, Murray DL. 2012 Reconsidering the specialist-generalist paradigm in niche breadth dynamics, resource gradient selection by Canada lynx and bobcat. *PloS One* **7(12)**,e51488. (doi:10.1371/journal.pone.0051488)

80. Kishani Farahani H, Ashouri A, Goldansaz SH, Shapiro MS, Piere JS, van Baaren J. 2016 Decrease of memory retention in a parasitic wasp, an effect of host manipulation by Wolbachia?. *Insect. sci.* **24(4)**,569-583. (dio: 10.1111/1744-7917.12348)

81. Albertson R, Tan V, Leads RR, Reyes M, Sullivan W, Casper-Lindley C. 2013 Mapping Wolbachia distributions in the adult D rosophila brain. Cel. Microbio. **15(9)**:1527-44.

82. Strunov AA, Ilinskii YY, Zakharov IK, Kiseleva EV. 2013 Effect of high temperature on survival of Drosophila melanogaster infected with pathogenic strain of Wolbachia bacteria. *Rus. J. of Gen.: App. Res.* **3(6)**:435-43.

83. Gruntenko NE, Ilinsky YY, Adonyeva NV, Burdina EV, Bykov RA, Menshanov PN, Rauschenbach IY. 2017 Various Wolbachia genotypes differently influence host Drosophila dopamine metabolism and survival under heat stress conditions. *BMC evol. biol.* **17(2)**, 252. (doi:10.1186/s.12862-017-1104-y)

84. Morioka E, Oida M, Tsuchida T, Ikeda M. 2018 Nighttime activities and peripheral clock oscillations depend on Wolbachia endosymbionts in flies. *Sci. Rep.* **8**,15432 (doi:10.1038/s41598-018-33522-8)

85. Trimmer PC, Houston AI. 2014 An Evolutionary Perspective on Information Processing. *Top. In Cogni. Sci.* **6** , 312–330.

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

518 86. Moran NA. 1988 The evolution of host-plant alternation in aphids, evidence for
519 specialization as a dead end. *Am. Nat.* **132**, 681–706. (doi:10.1086/j.284882)
520 87. Vamosi JC, Armbruster WS, Renner SS. 2014 Evolutionary ecology of specialization,
521 insights from phylogenetic analysis. *Proc. Biol. Sci.* **281(1795)**, 20142004.
522 (doi:10.1098/rspb.2014.2004)
523 88. Schluter D. 2000 The Ecology of Adaptive Radiation. Oxford, UK, Oxford University Press.
524 89. Hamerlinck G, Hulbert D, Hood GR, Smith JJ, Forbes AA. 2016 Histories of host shifts and
525 cospeciation among free-living parasitoids of Rhagoletis flies. *J. Evol. Biol.* **29**, 1766-1779.
526 (doi:10.1111/jeb.12909)
527 90. Cavalier-Smith T. 1992 The number of symbiotic origins of organelles. Biosyst. 28,91–106.
528 91. Corsaro D, Venditti D, Padula M, Valassina M.1999 Intracellular life. *Crit. Rev. Microbio.*
529 25,39–79.
530 92. Searcy DG. 2003 Metabolic integration during the evolutionary origin of mitochondria. *Cell.*
531 *Res.***13**,229–38.
532 93. Fleury F, Vavre F, Ris N, Fouillet P, Boulétreau M. 2000 Physiological cost induced by the
533 maternally-transmitted endosymbiont Wolbachia in the Drosophila parasitoid Leptopilina
534 heterotoma. *Parasitology* **121(5)**,493–500.
535 94. Sachs JL, Wilcox TP. 2006 A shift to parasitism in the jellyfish symbiont Symbiodinium
536 microadriaticum. *Proc. Bio. Sci.* **273**,425–9.
537 95. Strand MR, Pech LL. 1995 Immunological basis for compatibility in parasitoid-host
538 relationships. *Annu. Rev. Entomol.* **40**,31–56.

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

96. Brownlie JC, Cass BN, Riegler M, Witsenburg JJ, Iturbe-Ormaetxe I, McGraw EA, O'Neill SL. 2009 Evidence for metabolic provisioning by a common invertebrate endosymbiont, Wolbachia pipientis, during periods of nutritional stress. *PLoS patho.* **5(4)**:e1000368.

97. Hosokawa T, Koga R, Kikuchi Y, Meng XY, Fukatsu T. 2010 Wolbachia as a bacteriocyte-associated nutritional mutualist. *Proc. of the Nati. Acad. of Sci.* **107(2)**, 769-74.

98. Nikoh N, Hosokawa T, Moriyama M, Oshima K, Hattori M, Fukatsu T. 2014 Evolutionary origin of insect–Wolbachia nutritional mutualism. *Proc. of the Nati. Acad. of Sci.* **111(28)**,10257-62.

99. Moriyama M, Nikoh N, Hosokawa T, Fukatsu T. 2015 Riboflavin provisioning underlies Wolbachia's fitness contribution to its insect host. *MBio.* **6(6)**, e01732-15.

100. Kishani Farahani H, Ashouri A, Zibaee A, Abroon P, Alford L, Piere JS, van Baaren J. 2017 Early life nutritional quality effects on adult memory retention in a parasitic wasp. *Behav. Ecol.***10**,1093. (doi,10.1093/beheco.042)

101. Baldo L, Dunning Hotopp JC, Jolley KA, Bordenstein SR, Biber SA, Choudhury RR, Hayashi C, Maiden MC, Tettelin H, Werren JH.2006 Multilocus sequence typing system for the endosymbiont *Wolbachia pipientis*. *Appl. Environ. Microbiol.* **72**, 7098–7110. (doi: 10.1128/AEM.00731-06)

102. Kajitoch L, Kolasa M, Kubisz D, Gutowski JM, Ścibior R, Mazur MA, Holecová M. 2019 Using host species traits to understand the Wolbachia infection distribution across terrestrial beetles. *Scienti. Repo.* **9**, 847.

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Table 1. Effects of lineage, host species and the interaction of these two factors on host preference of uninfected and infected wasps. Significant results are shown in bold.

		lineage		**Host**		**Interaction**	
		χ^2	**P value**	χ^2	**P value**	χ^2	**P value**
Innate preference	**Choice**	0.43	0.511	0.02	0.889	0.02	0.889
	No choice	0.02	0.889	0.02	0.889	0.79	0.373
reared on *E. keueneilla*	**choice**	4.36	**0.036**	1.63	0.20	**7.7**	**0.005**
	No choice	4.95	**0.026**	1.02	0.313	2.57	0.109
Reared on *S. cerealella*	**Choice**	14.48	**0.0001**	0.24	0.622	11.57	**0.0007**
	No choice	8.93	**0.002**	0.61	0.433	3.76	0.052

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

571

572 Figure legends

573 Figure 1. Schematic view of experimental design of host preferences after pre-imaginal learning.

574 Figure 2. Innate preference of *Trichogramma brassiacae* wasps, *Wolbachia* infected and
575 uninfected, toward *Ephestia kuehniella* and *Sitotroga cerealella* in choice and no choice tests.
576 Wasps were originally all reared on *Ostrinia nubilalis*. (N.s.) stands for Not significant.

577 Figure 3. Host preference of *Trichogramma brassiacae* wasps, *Wolbachia* infected and
578 uninfected, after pre-imaginal experience on *Ephestia kuehniella* and *Sitotroga cerealella* in
579 choice and no choice tests. Different letters indicate significant differences.

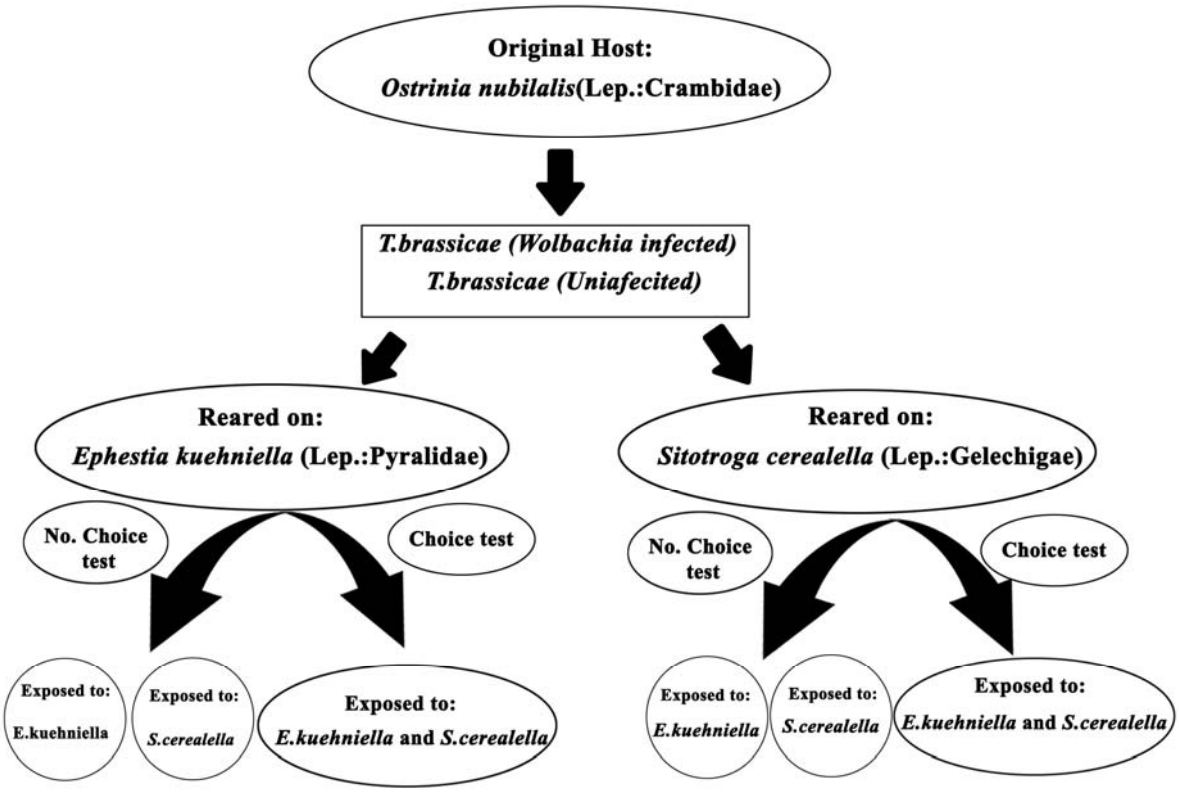
580 Figure 4. Emergence rate from *Ephestia kuehniella and Sitotroga cerealella* hosts of
581 *Trichogramma brassiacae* parasitoid wasps, either *Wolbachia* infected and uninfected. Different
582 letters indicate significant differences.

583

584

585

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



586

587

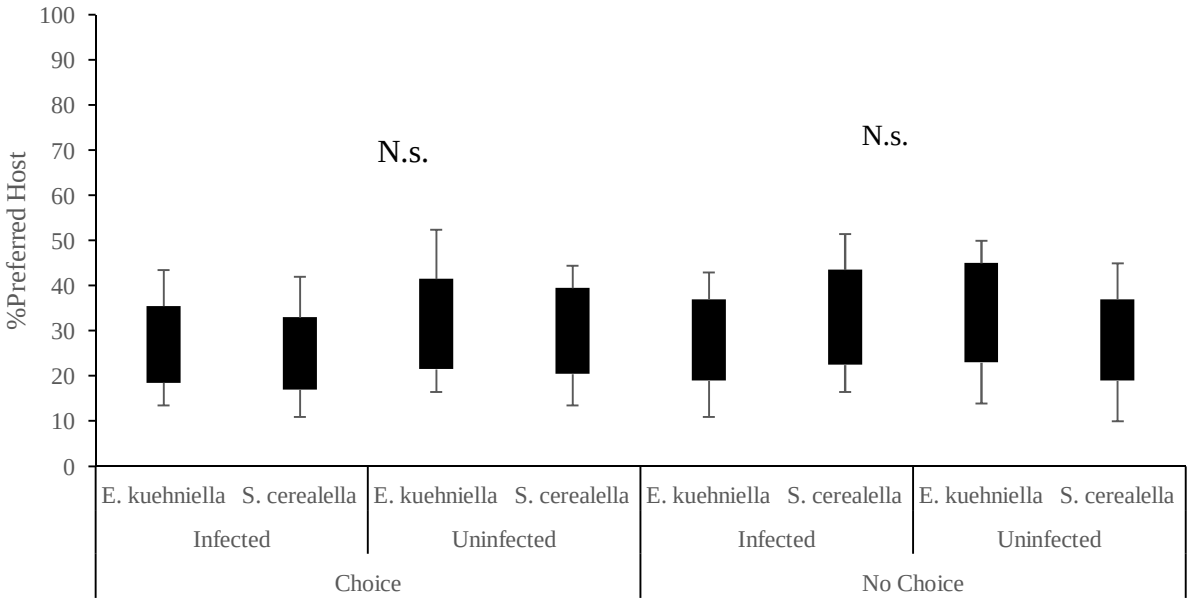
588 Figure 1.

589

590

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

591

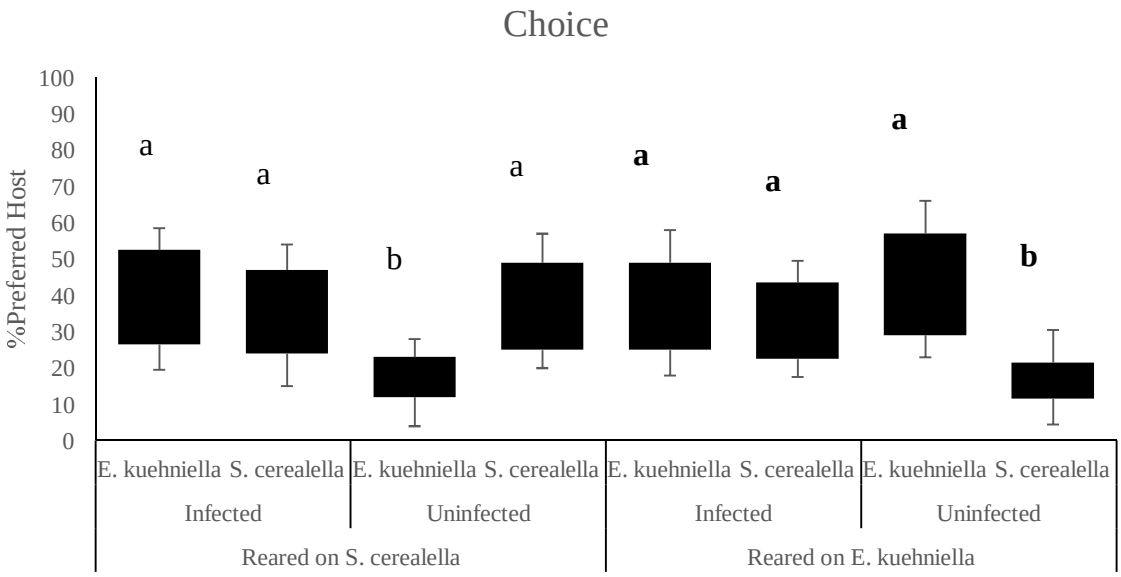


592

593 Figure 2.

594

bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.



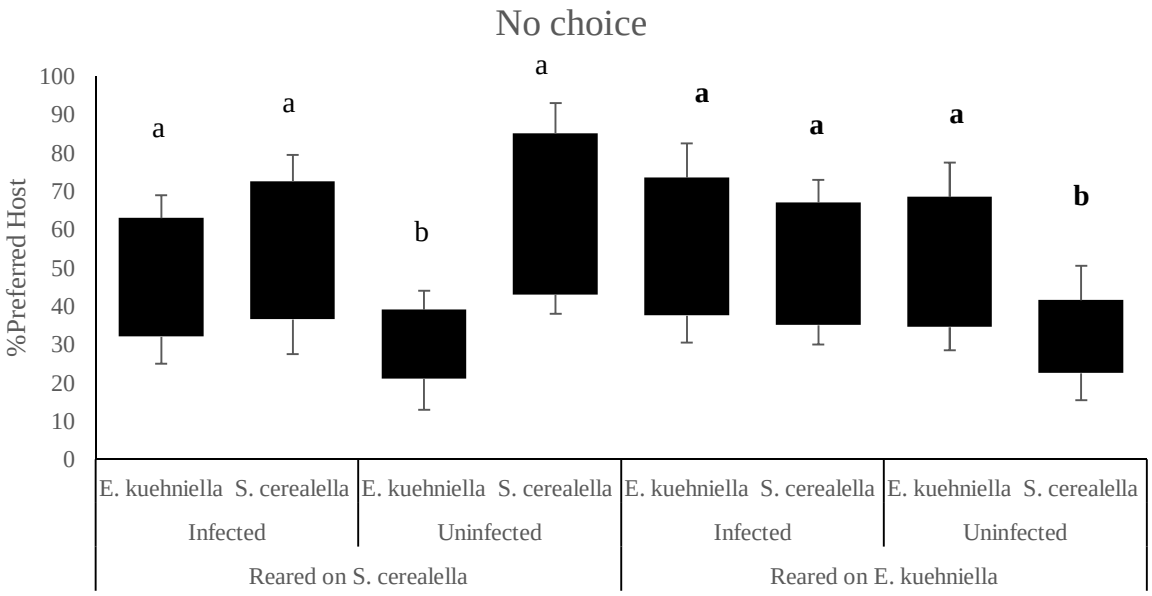


Figure 3.

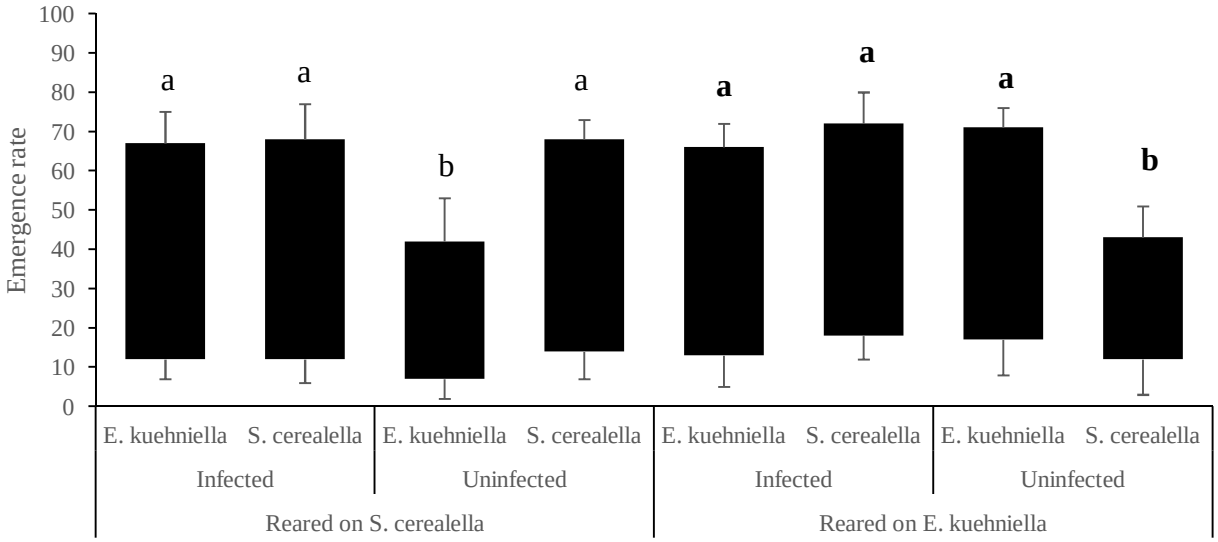
bioRxiv preprint doi: https://doi.org/10.1101/825455; this version posted October 31, 2019. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

602

603

604

605



606

607 Figure 4.

608

609

610