

1 **Frequent intertrophic transmission of *Wolbachia* by**
2 **parasitism but not predation**

3 **Zhi-Chao Yan, Lan-Da Qi, Han-Le Ji, Xiao-Xiang Wang, Xiao-Yue Hong*, Yuan-Xi Li***

4 *Department of Entomology, Nanjing Agricultural University, Nanjing 210095, China;*

5 * To whom correspondence should be addressed:

6 Yuan-Xi Li (e-mail: yxli@njau.edu.cn)

7 Xiao-Yue Hong (e-mail: xyhong@njau.edu.cn)

8

9 **Abstract**

10 *Wolbachia* is one of the most pervasive symbionts, estimated to infect ~50% of
11 arthropod species. It is primarily transmitted vertically, inducing a variety of
12 fascinating reproductive manipulations to promote its spread within host populations.
13 However, incongruences between host and *Wolbachia* phylogenies indicate
14 substantial horizontal transmissions, the mechanisms of which are largely unexplored.
15 By systematically analyzing *Wolbachia* surface protein (*wsp*) sequences on NCBI, we
16 found that parasitism, not predation, is the primary route of intertropical *Wolbachia*
17 transmission. This conclusion held after accounting sampling bias. One example of
18 frequent *Wolbachia* transfers is between egg parasitoid wasps, *Trichogramma*, and
19 their lepidopteran hosts. Moreover, both bioinformatics and experimental results
20 showed that *Wolbachia* from the parasitoid wasp *Encarsia formosa* can be transmitted
21 to its whitefly host *Bemisia tabaci*, through unsuccessful parasitism. Once *En.*
22 *formosa* *Wolbachia* is transferred to whiteflies, it can be vertically transmitted within
23 whiteflies and induce fitness costs. To our knowledge, this is the first compelling
24 evidence that *Wolbachia* can be transmitted from parasitoid wasps to their hosts,
25 revealing the bidirectional nature of *Wolbachia* transfers between parasitoids and their
26 hosts. Overall, our findings enrich the current understanding of the horizontal
27 transmission of *Wolbachia* and shed new light on its ecology and evolution.

28 **Keywords:** *Wolbachia*, horizontal transmission, parasitism, parasitoid wasps, *Bemisia*
29 *tabaci*, *Encarsia formosa*

30

31 **Introduction**

32 Symbiosis with microbes, ranging from parasitism to mutualism, is prevalent in both
33 plants and animals (1, 2). The ubiquity of microbial symbionts is likely attributed to
34 their profound impact on host biology, including host survival, development,
35 immunity, reproduction, and even behavior (2, 3). The transmission mode of
36 symbionts is a key factor in shaping the ecology and evolution of both symbionts and
37 their hosts (4-6). In addition to vertical transmission, incongruence between
38 symbionts and host phylogenies indicates a large number of horizontal symbiont
39 transfers across species (1). These events are important, as they allow symbionts to
40 expand their host range and enable hosts to acquire new symbionts and alter their
41 fitness. However, the transmission of symbionts has been relatively little studied
42 compared to their function (1).

43 *Wolbachia* (Rickettsiales, Alphaproteobacteria) is intracellular gram-negative bacteria
44 and one of the most famous endosymbionts that infests ~50% arthropod and several
45 filarial nematode species (7-9). On the one hand, *Wolbachia* can induce a range of
46 fascinating phenotypes, including a variety of reproductive manipulations, provision
47 of nutrients, and alteration of host behavior, thus facilitating its spread among
48 populations (7). On the other hand, although *Wolbachia* is primarily maternally
49 transmitted, there are widespread and frequent horizontal transfers across hosts (10,
50 11). Together, these characteristics make *Wolbachia* the most infectious microbe on
51 Earth in terms of the number of species it infects (7, 8).

52 *Wolbachia* has also received much attention for its applications in controlling pests

53 and vector-borne diseases (8). Specific strains of *Wolbachia* are artificially transfected
54 into target pests and subsequently released into the field to either suppress pest
55 populations or replace populations to depress the spread of vector-borne diseases (12,
56 13). Understanding how *Wolbachia* spreads horizontally is critical in assessing its
57 successful application and potential risks (14). This is because the released *Wolbachia*
58 may leak into natural pest populations, frustrating population suppression strategies
59 based on the cytoplasmic incompatibility (CI) of *Wolbachia*. It may also spread to
60 nontarget organisms, potentially disrupting their population dynamics, reducing
61 genetic diversity, and even leading to extinction. Therefore, the lack of a thorough
62 understanding of *Wolbachia* transmission and its consequences could hinder its
63 broader application (14).

64 Despite the extensive interest in the horizontal transmission of *Wolbachia*, our
65 understanding of this subject remains incomplete (15). Similar to other symbionts,
66 *Wolbachia* host shifts may occur through three main routes: parasitism, predation, and
67 shared plant or other food sources (15). The relative contributions of these three
68 routes to the transmission process remain unclear. Multiple surveys report a
69 significant similarity in *Wolbachia* sequences between parasitoid wasps and their
70 respective hosts, suggesting that parasitism may serve as a primary route for
71 *Wolbachia*'s host shift (16-21). However, without quantitative tests, this observation
72 could simply reflect a bias in research focus. For the intertrophic transmission of
73 *Wolbachia* between parasitoid wasps and their hosts, experimental evidence has
74 shown that parasitoid wasps can acquire hosts' *Wolbachia* and vertically transmit

75 them for several generations (22). However, it remains uncertain whether *Wolbachia*
76 can be transferred from parasitoid wasps to their hosts. Some have argued that the
77 transfer of *Wolbachia* between parasitoid wasps and their hosts is unidirectional, from
78 host to wasp, as all parasitoid wasps emerge from their hosts, but parasitized hosts are
79 eventually killed or castrated if not killed immediately (17, 23, 24).

80 In this study, our objective was to elucidate the role of parasitism and other potential
81 routes in *Wolbachia* horizontal transmission and to investigate whether *Wolbachia* can
82 be transferred from parasitoids to their hosts. We conducted a systematic survey of
83 *Wolbachia* surface protein (*wsp*) sequences from the NCBI database and executed
84 experiments using the whitefly *Bemisia tabaci* and its parasitoid wasp *Encarsia*
85 *formosa*. Our study illuminates the crucial role of parasitism in *Wolbachia* intertrophic
86 transmission, demonstrates the bidirectional nature of *Wolbachia* transfer between
87 parasitoids and their hosts, and thus expands the current understanding of *Wolbachia*
88 horizontal transmission.

89 **Results**

90 ***Wolbachia* is frequently transmitted between parasitoid wasps and their hosts**

91 To investigate potential horizontal transmission of *Wolbachia*, we retrieved 4685 *wsp*
92 sequences from the NCBI database. Out of these 4685 sequences, 4253 could be
93 assigned to 1377 species. We constructed a phylogenetic tree of *wsp* sequences (Fig.
94 1a) and extracted the minimum genetic distances of *wsp* between every species pair.
95 Based on the relationships between species, we defined the species pairs into
96 categories “Parasitism”, “Plant-sharing”, “Predation” and “Others” (for details, see

97 Methods and Materials). Among these species pairs, 16.5% (53 out of 321) in
98 “Parasitism” pairs had the minimum interspecific *wsp* distances less than 0.01 (i.e., >
99 99% identity). This proportion is significantly greater than the 6.3% (145 out of 2294)
100 in “Plant-sharing” pairs, the 1.1% (13 out of 1146) in “Predation” pairs, and the 1.5%
101 (14120 out of 943315) in “Others” pairs (χ^2 test; all comparisons: $p < 1e^{-5}$).
102 Consistently, the minimum interspecific *wsp* distances in “Parasitism” relationships
103 were significantly shorter than those in “Plant-sharing”, “Predation”, and “Other”
104 relationships (Fig. 1b; Mann–Whitney U test (MWUT); all comparisons: $p < 1e^{-12}$).
105 The minimum *wsp* distances in “Plant-sharing” were also significantly smaller than
106 those in “Others” (Fig. 1b; MWUT; $W = 730215564$, $p < 2.2e^{-16}$). However, the
107 minimum *wsp* distances showed no significant difference between “Predation” and
108 “Others” (Fig. 1b; MWUT; $W = 699285646$, $p = 0.096$).
109 To test whether these effects were merely due to sampling bias, we further obtained
110 divergent times from TimeTree for 95.2% (901,615 out of 947,376) of the species
111 pairs. As expected, the minimum interspecific *wsp* distance increased as the divergent
112 time increased (Fig. 1c; Spearman's correlation; $\rho = 0.14$, $p < 2.2e^{-16}$). Considering the
113 impact of divergent time, linear regression analyses were conducted with the *wsp*
114 distance as the dependent variable and divergent time as the independent variable. We
115 found that both “Parasitism” and “Plant-sharing” had significant effects (both tests: p
116 $< 2e^{-16}$). The estimated effect of “Parasitism” (-0.28) was more profound than that of
117 “Plant-sharing” (-0.13) ($p = 3.9e^{-16}$). Given that we cannot obtain divergence times for
118 all species pairs, especially for those closely related, we also classified the species

119 pairs according to their last common ancestor. We divided all species pairs into six
120 categories based on whether the two species belonged to the same genus, family, order,
121 class, phylum, or kingdom. Similar results were observed (Supplementary Note 1). To
122 further rule out potential influences of sampling bias, subsampling analyses were
123 conducted using three methods: 1) shuffling species relationships, 2) subsampling by
124 controlling divergent time, and 3) subsampling by controlling the last common
125 ancestor (for details, see Methods and Materials). For all three methods, both
126 “Parasitism” and “Plant-sharing”, but not “Predation”, exhibited significantly shorter
127 minimum interspecific distances of *wsp* than randomly generated controls (Fig. S1;
128 both “Parasitism” and “Plant-sharing”: $p < 0.001$; “Predation”: $p > 0.05$). These
129 results confirmed that parasitism and plant-sharing promote interspecific transfers of
130 *Wolbachia*, rather than being due to sampling bias.

131 An example of frequent *Wolbachia* transfers between parasitoids and their hosts is
132 observed in *Trichogramma* wasps and their lepidopteran hosts (Fig. 1d).
133 *Trichogramma* is a genus of generalist egg parasitoids, targeting mainly lepidopterans
134 such as moths and butterflies (25). Fig. 1d displays representative *wsp* sequences from
135 *Trichogramma* and lepidopterans, illustrating potential interspecific transitions of
136 *Wolbachia*. In the analyzed dataset, 16 out of 23 (69.6%) *Trichogramma* species
137 exhibited the minimum interspecific *wsp* distance of less than 0.01 with at least one
138 lepidopteran species. Similarly, 79 out of 254 (31.1%) surveyed lepidopteran species
139 displayed a minimum interspecific *wsp* distance of less than 0.01 with at least one
140 *Trichogramma* species. These results suggest frequent *Wolbachia* transitions between

141 *Trichogramma* wasps and lepidopterans.

142 Collectively, these findings support that *Wolbachia* are frequently transmitted between
143 parasitoid wasps and their hosts.

144 ***Wsp* phylogeny suggests transfer directions of *Wolbachia* between the whitefly *B.*
145 *tabaci* and its parasitoid wasps**

146 However, the interspecific identities of *wsp* between parasitoids and their hosts
147 typically offer no clues about the direction of *Wolbachia* transmission. We noticed
148 frequent *Wolbachia* transfers between the whitefly *B. tabaci* and its parasitoid wasps
149 (Fig. 2a). Notably, one of the juvenile parasitoids of *B. tabaci* is *En. formosa*.
150 *Wolbachia* induces parthenogenesis and has evolved into an obligate symbiont in *En.*
151 *formosa*, exhibiting a 100% infection rate (26, 27). This system presents a unique
152 opportunity to infer the directions of *Wolbachia* transmission between parasitoids and
153 their hosts.

154 One clade of the *wsp* phylogeny contained 99 sequences from *B. tabaci*, 14 sequences
155 from *Encarsia* and *Eretmocerus* parasitoid wasps of *B. tabaci*, and 8 other sequences
156 (Fig. 2a). The prevalence of *B. tabaci* sequences interspersed with those of the
157 parasitoids suggests that the transmission direction of *Wolbachia* in this clade may be
158 primarily from *B. tabaci* to its parasitoid wasps. Notably, in another clade of *wsp*, nine
159 *En. formosa* and five *B. tabaci* *wsp* sequences are clustered together, along with one
160 *wsp* sequence detected in cotton and 12 sequences from other parasitoid wasps of *B.*
161 *tabaci*, such as *En. inaron*, *En. lutea*, *En. bimaculata*, *Er. mundus*, and *Aimtus*
162 *hesperidum* (Fig. 2a). Given that *Wolbachia* is obligate with a 100% infection rate in

163 *En. formosa*, it is reasonable to infer that the transmission direction of *Wolbachia* in
164 this clade was from *En. formosa* to *B. tabaci*.

165 ***Wolbachia* can be transmitted from the parasitoid wasp *En. formosa* to its**
166 **whitefly host in cage experiments**

167 *Bemisia tabaci* is a species complex of at least 40 cryptic species (28). Infection rates
168 of *Wolbachia* reported across multiple populations within these cryptic species exhibit
169 dramatic fluctuations, ranging from 0% to 100% (28-31). To investigate whether
170 *Wolbachia* can be transmitted from parasitoid wasps to their whitefly hosts, we first
171 established a *Wolbachia*-free iso-female line of *B. tabaci* Q biotype. Subsequently, we
172 conducted outdoor cage experiments using the *Wolbachia*-free *B. tabaci* and its
173 parasitoid *En. formosa*, which was 100% infected with *Wolbachia* (Fig. 2b). After
174 introducing *En. formosa*, the *Wolbachia* infection rate in whiteflies increased from
175 zero to 4.17% after 20 days and reached 10.83% after 100 days (Fig. 2c; ANOVA;
176 $F_{5,17}=11.44$, $p < 0.001$). Correspondingly, the female ratio of whiteflies decreased
177 from 69.17% to 60.08% (Fig. 2d; ANOVA; $F_{5,17}=3.14$, $p = 0.048$). In contrast, the
178 whitefly population with no exposure to wasps maintained a zero infection rate of
179 *Wolbachia* (Fig. 2c), and the female ratio showed no significant changes (Fig. 2d;
180 ANOVA; $F_{5,17} = 0.076$, $p = 0.99$). Additionally, the Sanger sequencing results showed
181 that *wsp* sequences from both whitefly and *En. formosa* were identical (Fig. S2).
182 These results indicate that *Wolbachia* from *En. formosa* can be rapidly transmitted to
183 its whitefly host.

184 **Parasitism failure transmits *Wolbachia* from the parasitoid wasp *En. formosa* to**

185 **its whitefly host**

186 We hypothesized that *Wolbachia* was transmitted from parasitoid wasps to their hosts
187 through unsuccessful parasitism. To test this hypothesis, we performed parasitism
188 experiments on wasp individuals and applied irradiation treatment to wasps to reduce
189 their parasitism success rate. After 60 Gy irradiation, the fecundity of *En. formosa* in
190 12 h significantly decreased (Fig. 3a; Student's t test; $t = 12.91$, $p < 0.001$), and the
191 parasitism success rate on whiteflies drastically declined from 78.8% to 9.9% (Fig. 3b;
192 Student's t test; $t = 13.09$, $p < 0.001$). Correspondingly, the *Wolbachia* infection rate
193 increased from 7.5% to 78.3% in whiteflies survived from parasitism (Fig. 3c;
194 Student's t test; $t = 6.61$, $p < 0.001$), despite a decrease in the *Wolbachia* titer in *En.*
195 *formosa* post irradiation (Fig. S3). More details can be found for irradiation treatments
196 at different dosages (Supplementary Note 2). Fluorescence in situ hybridization (FISH)
197 assays further showed that *Wolbachia* was injected into the host nymphs along with
198 *En. formosa* eggs (Fig. 3 d-f and S4). These results indicate that parasitism failure can
199 transfer *Wolbachia* from the parasitoid wasps *En. formosa* to their whitefly hosts.

200 **Vertical transmission and fitness cost of *Wolbachia* in whiteflies after horizontal**
201 **transfer from *En. formosa***

202 Next, we investigated the vertical transmission and fitness cost of *Wolbachia* in its
203 new host, *B. tabaci*, following its horizontal transfer from *En. formosa*. The vertical
204 transmission rate varied from 22.2% to 33.3% across generations in whiteflies, with a
205 slight but statistically insignificant increase from G₁ to G₅ (Fig. 4b; ANOVA; $F_{4,40} =$
206 2.09, $p = 0.10$). Moreover, *Wolbachia* was also detected in the G₃ whiteflies,

207 specifically in the nymph's bacteriocytes, the abdomen of male adults, and the ovaries
208 of female adults (Fig. 4 and S5). MLST typing confirmed that the *Wolbachia* strain
209 introduced into the whiteflies was identical to the strain from *En. formosa* (Fig. S6).
210 We then examined the impact of the introduced *Wolbachia* on the fitness of whiteflies.
211 Compared to uninfected females, *Wolbachia*-infected females showed decreased
212 fecundity (Fig. 5a; Student's t test; $t = 8.51$, $p < 0.001$). Moreover, offspring from
213 infected mothers exhibited a diminished egg hatching rate (Fig. 5b; Student's t test; $t =$
214 8.33 , $p < 0.001$), a lower survival rate among nymphs (Fig. 5c; Student's t test; $t =$
215 13.54 , $p < 0.001$), and a decreased ratio of females in adults (Fig. 5d; Student's t test; t
216 $= 12.29$, $p < 0.001$), although there was no significant difference in the development
217 time from egg to adult (Fig. 5e; Student's t test; $t = 1.51$, $p < 0.001$). We also
218 investigated the effects of paternal infection with *Wolbachia*. Regardless of whether
219 the female was infected with *Wolbachia*, the infection status in males showed no
220 significant influence on their fecundity, the hatching rate of their offspring's eggs, the
221 survival rate of their nymph offspring, or the female ratio of their adult offspring
222 (Table 1; Student's t test; all comparisons: $p > 0.05$).

223 Collectively, these results indicate that *Wolbachia* from *En. formosa*, when shifted
224 into the new host *B. tabaci*, exhibits a low rate of vertical transmission and a
225 substantial fitness cost, without apparent reproductive manipulation phenotypes.

226 **Discussion**

227 **The effect of parasitism on *Wolbachia* horizontal transmission**

228 As with previous studies, we utilized the sequence similarities of *wsp* to infer

229 potential horizontal transfers of *Wolbachia*. Here, we systematically investigated all
230 *wsp* sequences in the NCBI database, enabling us to examine the effect of potential
231 factors such as parasitism, plant-sharing, and predation. Our findings clearly indicate
232 that parasitism exhibits shorter interspecific *wsp* distances than plant-sharing and
233 predation.

234 Moreover, the fact that herbivores sharing plants have identical *wsp* sequences does
235 not necessarily imply plant-mediated horizontal transfer of *Wolbachia*. This is because
236 species that share the same plants often have recent divergent times (Fig. 1b), which
237 increases the potential for hybridization and also sharing common parasitoid wasps.
238 Therefore, the plant-sharing category overestimates the extent of plant-mediated
239 *Wolbachia* horizontal transmission, further supporting the notion that parasitism is the
240 primary route of *Wolbachia* horizontal transmission.

241 **Directions of *Wolbachia* transmission between parasitoids and their hosts**

242 However, investigations based on *Wolbachia* sequence similarity have significant
243 limitations. First, determining the direction of transfer is challenging based solely on
244 the identity of *Wolbachia* strains. In certain exceptional cases, inferences of transfer
245 direction might be drawn from the prevalence of *Wolbachia* infection in the two
246 species (15). For example, *Wolbachia* is obligate in *En. formosa* and exhibits a 100%
247 infection rate across all populations (27). This unique instance of horizontal
248 *Wolbachia* transfer between *En. formosa* and its whitefly hosts provides compelling
249 evidence, suggesting that *Wolbachia* is likely transmitted from the parasitoid wasp to
250 its host, rather than the reverse. Second, the PCR detection of *wsp* does not

251 necessarily indicate horizontal transfer of *Wolbachia* (24). Instead, it could merely
252 represent contamination that arises during predation or parasitism. The contamination
253 can be *Wolbachia*-infected tissues or even just fragmented *Wolbachia* DNA, which
254 could be found on the surface, within the gut, or inside the body cavity (as in the case
255 of parasitized hosts).

256 To verify *Wolbachia* transmission from parasitoid wasps to their hosts, we conducted
257 outdoor cage experiments and indoor tests using *Wolbachia*-free whitefly *B. tabaci*
258 and its parasitoid *En. formosa*. *Wolbachia* was detected by nested PCR in whitefly
259 adults (G₀) that survived parasitism. *Encarsia formosa* parasitizes *B. tabaci* nymphs,
260 which undergo a pseudo-pupal stage to reach adulthood (26, 32). In our experiments,
261 PCR detection of *Wolbachia* typically occurred 3~7 days post-parasitism, minimizing
262 risks of contamination from parasitism. *Wolbachia* was also detected by PCR in
263 subsequent generations (G₁–G₅) of whiteflies and induced notable fitness costs. PCR
264 sequencing confirmed that the *Wolbachia* strain in the *B. tabaci* matched that of *En.*
265 *formosa*. Furthermore, FISH assays revealed a tissue-specific distribution of
266 *Wolbachia* in both nymphs and adults of the whiteflies, matching previously reported
267 patterns (33). Collectively, these findings provide compelling evidence that *Wolbachia*
268 from *En. formosa* can be horizontally transmitted to *B. tabaci*, beyond mere DNA
269 contamination.

270 Although previous research has demonstrated that parasitoids can acquire *Wolbachia*
271 from their hosts, the reverse direction of transmission, from host to parasitoid, has
272 been largely overlooked and lacks supportive experimental evidence. One possible

273 reason for this oversight is that all parasitoids emerge from their hosts, but hosts are
274 eventually killed by the parasitism of parasitoid wasps (17, 23, 24). However,
275 parasitoid wasps' success in parasitizing their hosts does not always reach 100% (34).
276 Some hosts can manage to survive after parasitism. Various factors can influence the
277 outcome of parasitoid-host interactions, including environmental conditions, the
278 species and genotype of both wasps and hosts, the host's age, and the presence of
279 symbiotic bacteria within the host (35-37). Moreover, we used radiation to reduce the
280 parasitism success rate, which notably enhanced the transfer of *Wolbachia* from *En.*
281 *formosa* to its whitefly host.

282 **Potential intertrophic transmission network of *Wolbachia***

283 A previous study reported that parasitoid wasps can act as vectors to transmit
284 *Wolbachia*, without the necessity of being infected themselves (38). Through the
285 probing actions of the *Eretmocerus* parasitoid, *Wolbachia* can be transmitted among
286 whitefly hosts (38). This is often referred to as the 'dirty needle' model. Conversely,
287 hosts can also serve as vectors for *Wolbachia* transmission among parasitoid wasps.
288 *Wolbachia* can be transferred from infected to noninfected *Trichogramma* wasps
289 through superparasitism (39, 40). However, the *Wolbachia* transmission of these two
290 modes is restricted within the same trophic level. In contrast, the transmission of
291 *Wolbachia* between parasitoid wasps and hosts can cross trophic levels. Our findings,
292 when combined with existing knowledge, suggest that the intertrophic transmission of
293 *Wolbachia* is bidirectional between parasitoid wasps and their hosts. This greatly
294 enhances our understanding of the horizontal transmission of *Wolbachia*.

295 Interestingly, we found on NCBI that a strain of *Wolbachia* detected in the cotton
296 plant was identical to the *Wolbachia* from *En. formosa*, based on the *wsp* sequence
297 and MSLT typing (Fig. 2 and S6). This *Wolbachia* strain was probably transmitted to
298 the cotton plant from the feeding of whiteflies (41). Given that *En. formosa* is 100%
299 infected with its obligate *Wolbachia* strain, a possible transmission route could be
300 from *En. formosa* to whiteflies via parasitism and then to the cotton plant through the
301 feeding of whiteflies. This finding indirectly supports our conclusion that *Wolbachia*
302 can be transmitted from parasitoid wasps to their hosts. This suggests that once the
303 *Wolbachia* of parasitoids is transmitted to herbivorous hosts, it may further spread to
304 host plants. The reverse transmission route can also be possible. It is likely that
305 *Wolbachia*'s widespread and complex horizontal transmission network is established
306 through such bidirectional transmissions across multiple trophic levels, e.g.,
307 "plant-herbivore-parasitoid-hyperparasitoid". Further investigations are needed to test
308 these hypotheses.

309 ***Wolbachia* establishment after host transfers**

310 Moreover, the physical transfer of *Wolbachia* often represents merely the first step of
311 its establishment in a new host (15). Several subsequent steps are required for
312 *Wolbachia* to establish itself within new species, e.g., entry into germ cells, vertical
313 transmission, and mechanisms that promote its spread within the population (15).
314 First, we found that *Wolbachia* from *En. formosa* was enriched in the ovaries of
315 whiteflies and vertically transmitted after entering *B. tabaci*. However, the vertical
316 transmission efficiency is low, ranging from 22.2% to 33.3%. We also noted an

317 absence of reproductive manipulations by the newly introduced *Wolbachia* in
318 whiteflies. The reduced female ratio after infection does not support the induction of
319 parthenogenesis, feminization or male-killing by *Wolbachia*. We neither observed
320 cytoplasmic incompatibility, where the mating of infected males and uninfected
321 females resulted in reduced offspring hatching.

322 In contrast, the introduced *Wolbachia* from *En. formosa* reduced whiteflies' egg
323 laying and hatching, larval survival, and female proportion, demonstrating significant
324 fitness costs in the new host. This is likely due to *Wolbachia*'s coevolution with its
325 host in *En. formosa*, which may have led to the loss of its ability to colonize new host
326 species. Given the low vertical transmission rate, high fitness cost, and lack of clear
327 reproductive manipulations, it is reasonable to predict that the spread of *Wolbachia* in
328 its new host population will be limited. Finally, these factors, together with the
329 frequency of *Wolbachia* introductions by parasitoids and its spread via parasitoid or
330 plant vectors, shape the dynamics and equilibrium of *Wolbachia*. These dynamics
331 could shift with the emergence of reproductive manipulation or other beneficial
332 phenotypes that promote *Wolbachia* spread, probably through gene mutation,
333 recombination or horizontal gene transfer within *Wolbachia* (2). There are still many
334 questions waiting to be further studied in these steps of *Wolbachia* host shifts.

335 **Conclusions**

336 By investigating *wsp* sequences from the NCBI database, we found frequent
337 intertrophic transmission of *Wolbachia* by parasitism but not predation. Combining
338 bioinformatics and experimental approaches, we demonstrated that *Wolbachia* can be

339 transmitted from the parasitoid wasp *En. formosa* to the host *B. tabaci*. To our
340 knowledge, this is the first compelling evidence that *Wolbachia* can be transmitted
341 from parasitoid wasps to their hosts, thus revealing the bidirectional nature of
342 *Wolbachia* transfers between parasitoids and their hosts. These findings enrich our
343 knowledge of the *Wolbachia* transmission network and have significant implications
344 for understanding the ecology of *Wolbachia*, as well as for evaluating the release of
345 *Wolbachia* in pest control.

346 **Materials and Methods**

347 ***Wsp* sequence retrieval and phylogenetic analyses**

348 tblastn was conducted against the NCBI “nr/nt” database using the *wsp* protein
349 AAS14719.1 from *Wolbachia* of *Drosophila melanogaster* (July 2023). Default
350 settings were used with a maximum target sequence of 5000 and the organism
351 limitation of *Wolbachia* (taxid:953). The sequences were filtered to remove those
352 shorter than 300 bp or containing premature stop codons. *Wsp* sequences were
353 translated into proteins, aligned using MAFFT v7.475 (42), and reverse translated into
354 codons using PAL2NAL v14 (43). The phylogenetic trees were constructed using
355 IQ-Tree v2.2.0 (44), with the best model selected by the built-in ModelFinder (45).
356 The phylogenetic trees were visualized using iTOL v6 (46).

357 **Statistics for genetic distances of *wsp***

358 Genetic distances of *wsp* sequences were extracted from the *wsp* phylogeny using a
359 custom Python script. Species interaction relationships were extracted from the GloBI
360 database (August 2023) (47). Parasitic associations were extracted using interaction

361 types of “parasiteOf” or “parasitoidOf”, excluding social parasitism to focus on direct
362 biological parasitism. Predation associations were extracted using interaction types of
363 “preysOn” or “eats”. Given the relatively broad dietary range of predators, a
364 genus-to-genus expansion was adopted for the predation relationships. Herbivorous
365 interactions were extracted using the interaction types “hasHost” or “eats”,
366 specifically targeting taxa within the kingdom Plantae. Extracted relationships were
367 manually curated to verify the accuracy. Divergent times between species were
368 extracted from TimeTree v5 via its application programming interface (API
369 (September 2023) (48). To test the effects of special features of sampled species pairs,
370 three subsampling methods were employed using a custom Python script. For each
371 method, 1000 replicates were randomly generated. For species pair shuffling, in every
372 replication, the pairs of species were randomly rearranged to create new combinations.
373 To control divergent time, pairs of species were randomly sampled from the
374 background to match the divergent times in the tested category (i.e., parasitism,
375 plant-sharing or predation). The sampling background pools were all species pairs
376 excluding the specific tested category. A similar process was applied to control the last
377 common ancestor. Linear model analyses, statistical tests, and data visualization were
378 executed using R v4.3.1.

379 **Insect rearing**

380 Both whiteflies *B. tabaci* and the parasitoid wasps *En. formosa* were maintained in
381 nylon cages at $25\pm 1^\circ\text{C}$, $70\pm 5\%$ RH, and a L:D photoperiod of 16:8 h. *Bemisia tabaci*
382 was originally collected from the campus of Nanjing Agricultural University in 2012

383 and determined to be the Q biotype. A *Wolbachia*-free iso-female line of *B. tabaci* was
384 then established. *En. formosa* was initially acquired from Beijing Ecoman
385 Biotechnology Co., Ltd. in 2013 and was maintained on *B. tabaci* on tomato plants.

386 **PCR and Sanger sequencing**

387 *Bemisia tabaci* or *En. formosa* were initially washed using ethanol and ultrapure
388 water three times each. DNA was extracted from individuals using the STE method
389 (49). To avoid false-negative results, a nest-PCR targeting the *wsp* gene was employed
390 for detecting *Wolbachia* in whiteflies (Fig. S7) as previously described (49). PCRs
391 were performed using DreamTaq Green PCR Master Mix (Thermo Scientific)
392 according to the manufacturer's protocol. To confirm whether the *Wolbachia* present
393 in whiteflies and their parasitoid wasps belonged to the same strain, PCR and Sanger
394 sequencing were performed on *Wolbachia* genes, namely, *wsp*, *gatB*, *coxA*, *hcpA*, *ftsZ*,
395 and *fbpA*, for MSLT typing (50). The primers used for *wsp* nest-PCR and MSLT
396 typing are listed in Table S1.

397 **FISH detection of *Wolbachia***

398 The location of *Wolbachia* in whiteflies was determined using fluorescence in situ
399 hybridization as previously reported (27). To enhance the detection signal, two 5'
400 rhodamine-labeled probes, W1: 5'-AATCCGGCCGARCCG ACCC-3' and W2:
401 5'-CTTCTGTGAGTACCGTCATTATC-3', were used to target *Wolbachia* 16S rRNA
402 (51). Stained samples were examined under a Zeiss LSM 700 confocal microscope
403 (Carl Zeiss, Germany). The specificity of the method was confirmed using
404 *Wolbachia*-free whiteflies as negative controls.

405 **Outdoor cage experiments for *Wolbachia* transmission**

406 To assess the potential transmission of *Wolbachia* from *En. formosa* to *B. tabaci*, six
407 cages (80 cm x 80 cm x 80 cm, enclosed with a 120 mesh nylon screen) were
408 established on the Nanjing Agricultural University campus. Initially, six 20-cm tall
409 tomato plants were placed in each cage, with two additional plants introduced at
410 30-day intervals. Each cage was populated with 100 female and 100 male
411 *Wolbachia*-free whiteflies. After a 20-day period, ten *En. formosa* wasps were
412 introduced into each of three randomly selected cages, following the random sampling
413 of 40 adult whiteflies from each cage. Subsequently, an additional 40 adult whiteflies
414 were sampled from each cage at 20-day intervals. These sampled whiteflies were
415 sexed based on their morphologies and screened for *Wolbachia* infection using *wsp*
416 nest PCR.

417 ***Wolbachia* transmission from irradiated *En. formosa* to *B. tabaci***

418 To explore the possibility of *Wolbachia* transmission from *En. formosa* to *B. tabaci*
419 due to unsuccessful parasitism, we subjected *En. formosa* wasps to radiation at the
420 Nanjing Aerospace Irradiation Center. Wasps that had emerged within 24 h were
421 exposed to Co⁶⁰ radiation at doses of 60, 80 and 100 Gy (dose rate of 2 Gy/min). A
422 single irradiated wasp was subsequently introduced into a Petri dish, which contained
423 a tomato leaf infested with *Wolbachia*-free 3rd or 4th instar whitefly nymphs. The
424 parasitic behaviors of *En. formosa* were monitored under a Nikon SMZ800
425 stereomicroscope (Nikon Instrument Inc., Tokyo, Japan). Wasps were removed after
426 12 h of parasitism. Only whitefly nymphs that had been punctured by wasps were

427 kept until the emergence of either parasitoid wasps or whiteflies. The newly emerged
428 whiteflies were then collected for *Wolbachia* detection using *wsp* nested PCR. In each
429 replication, five irradiated *En. formosa* were randomly selected for parasitization,
430 with four replications performed in total. Nonirradiated wasps were used as controls.

431 ***Wolbachia* transmission across generations in whiteflies**

432 The parasitized and subsequently emerged whiteflies were denoted as the initial
433 generation (G_0). To investigate the transmission rate of *Wolbachia* across whitefly
434 generations, a pair of female and male newly emerged whitefly adults (G_0) were
435 randomly chosen and introduced into a Petri dish containing a tomato leaf. After 5 d
436 of oviposition, the whitefly adults (G_0) were removed and sampled for *Wolbachia*
437 detection using *wsp* nested PCR. Only the eggs laid by *Wolbachia*-infected females
438 were allowed to develop into adults. In a similar manner, upon the emergence of G_1
439 adults, whiteflies were paired and placed into individual Petri dishes to produce the G_2
440 generation and then sampled for *Wolbachia* detection. This procedure was repeated
441 from the G_0 to G_5 generations. A parallel control experiment was conducted using
442 *Wolbachia*-free whiteflies. Nine replications were conducted for each generation.

443 **Effects of *Wolbachia* on whitefly fitness**

444 Given the inability to obtain a whitefly strain with 100% *Wolbachia* infection, we
445 selected individuals from the infected whitefly population, which initially acquired
446 *Wolbachia* through parasitism of irradiated *En. formosa* and subsequently maintained
447 for over five generations. Whitefly nymphs were individually isolated into PCR tubes
448 at their 4th instar stage. After emergence, female and male whitefly adults were paired

449 and allowed to oviposit for five days within a Petri dish containing a tomato leaf.
450 These adults were then removed for *Wolbachia* detection via PCR. For the
451 *Wolbachia*-infected female adults, we recorded several fitness parameters. These
452 included the total number of eggs laid, the number of first instar nymphs, the
453 developmental time from egg to eclosion, and the number of eclosed male and female
454 offspring. This procedure was replicated with 60 pairs, and whiteflies from the
455 original *Wolbachia*-free population were used as controls. Additionally, we examined
456 the effects of various paternal and maternal infection status combinations on whitefly
457 fitness. A similar procedure was employed, with the exception that all whitefly adults
458 were sourced from the infected population, and their infection status was determined
459 through *wsp* nested PCR.

460 **Statistics of the experimental data**

461 Statistical analysis of experimental data was conducted using SPSS version 18.0
462 (SPSS Inc., Chicago, USA). The proportions were arcsine-square root transformed.
463 Two independent samples were compared using Student's T test. One-way ANOVA
464 was performed for analysis of multiple samples. Means were then compared using
465 Honest Student's Tukey test. Data were visualized using GraphPad Prism 6 software
466 (GraphPad Software, San Diego, USA).

467 **Data and code availability**

468 The alignment files, phylogenetic trees, and custom scripts can be accessed on
469 FigShare (<https://doi.org/10.6084/m9.figshare.24718119>).

470 **References**

- 471 1. M. Bright, S. Bulgheresi, A complex journey: transmission of microbial symbionts. *Nat.*
472 *Rev. Microbiol.* **8**, 218-230 (2010).
- 473 2. J. Perreau, N. A. Moran, Genetic innovations in animal-microbe symbioses. *Nat. Rev.*
474 *Genet.* **23**, 23-39 (2022).
- 475 3. A. E. Douglas, Multiorganismal insects: diversity and function of resident
476 microorganisms. *Annu. Rev. Entomol.* **60**, 17-34 (2015).
- 477 4. A. Mestre, R. Poulin, J. Hortal, A niche perspective on the range expansion of
478 symbionts. *Biol. Rev. Camb. Philos. Soc.* **95**, 491-516 (2020).
- 479 5. J. L. Sachs, R. G. Skophammer, J. U. Regus, Evolutionary transitions in bacterial
480 symbiosis. *Proc. Natl. Acad. Sci. USA* **108 Suppl 2**, 10800-10807 (2011).
- 481 6. R. M. Fisher, L. M. Henry, C. K. Cornwallis, E. T. Kiers, S. A. West, The evolution of
482 host-symbiont dependence. *Nat. Commun.* **8**, 15973 (2017).
- 483 7. J. H. Werren, L. Baldo, M. E. Clark, *Wolbachia*: master manipulators of invertebrate
484 biology. *Nat. Rev. Microbiol.* **6**, 741-751 (2008).
- 485 8. R. Kaur *et al.*, Living in the endosymbiotic world of *Wolbachia*: a centennial review.
486 *Cell Host Microbe* **29**, 879-893 (2021).
- 487 9. J. Porter, W. Sullivan, The cellular lives of *Wolbachia*. *Nat. Rev. Microbiol.* **21**,
488 750-766 (2023).
- 489 10. M. Scholz *et al.*, Large scale genome reconstructions illuminate *Wolbachia* evolution.
490 *Nat. Commun.* **11**, 5235 (2020).
- 491 11. E. Vancaester, M. Blaxter, Phylogenomic analysis of *Wolbachia* genomes from the
492 Darwin Tree of Life biodiversity genomics project. *PLoS Biol.* **21**, e3001972 (2023).

- 493 12. X. Zheng *et al.*, Incompatible and sterile insect techniques combined eliminate
494 mosquitoes. *Nature* **572**, 56-61 (2019).
- 495 13. A. A. Hoffmann *et al.*, Successful establishment of *Wolbachia* in *Aedes* populations to
496 suppress dengue transmission. *Nature* **476**, 454-457 (2011).
- 497 14. E. L. Loreto, G. L. Wallau, Risks of *Wolbachia* mosquito control. *Science* **351**, 1273
498 (2016).
- 499 15. E. Sanaei, S. Charlat, J. Engelstadter, *Wolbachia* host shifts: routes, mechanisms,
500 constraints and evolutionary consequences. *Biol. Rev. Camb. Philos. Soc.* **96**,
501 433-453 (2021).
- 502 16. H. Noda *et al.*, Infection shared among planthoppers (Homoptera: Delphacidae) and
503 their endoparasite (Strepsiptera: Elenchidae): a probable case of interspecies
504 transmission. *Mol. Ecol.* **10**, 2101-2106 (2001).
- 505 17. F. Vavre, F. Fleury, D. Lepetit, P. Fouillet, M. Bouletreau, Phylogenetic evidence for
506 horizontal transmission of *Wolbachia* in host-parasitoid associations. *Mol. Biol. Evol.*
507 **16**, 1711-1723 (1999).
- 508 18. P. Kittayapong, W. Jamnongluk, A. Thipaksorn, J. R. Milne, C. Sindhusake, Infection
509 complexity among insects in the tropical rice-field community. *Mol. Ecol.* **12**,
510 1049-1060 (2003).
- 511 19. H. Hou, G. Zhao, C. Su, D. Zhu, *Wolbachia* prevalence patterns: horizontal
512 transmission, recombination, and multiple infections in chestnut gall wasp-parasitoid
513 communities. *Entomol. Exp. Appl.* **168**, 752-765 (2020).
- 514 20. J. L. Morrow, M. Frommer, D. C. A. Shearman, M. Riegler, Tropical tephritid fruit fly

- 515 community with high incidence of shared strains as platform for horizontal
516 transmission of endosymbionts. *Environ. Microbiol.* **16**, 3622-3637 (2014).
- 517 21. D. Kageyama, S. Narita, T. Imamura, A. Miyanoshita, Detection and identification of
518 *Wolbachia* endosymbionts from laboratory stocks of stored-product insect pests and
519 their parasitoids. *J. Stored. Prod. Res.* **46**, 13-19 (2010).
- 520 22. B. D. Heath, R. D. J. Butcher, W. G. F. Whitfield, S. F. Hubbard, Horizontal transfer of
521 between phylogenetically distant insect species by a naturally occurring mechanism.
522 *Curr. Biol.* **9**, 313-316 (1999).
- 523 23. M. Aboumourad, H. zu Dohna, Unidirectional and heterogenous *Wolbachia* transfer
524 rates among insect host orders. *Preprint at*
525 <https://www.researchsquare.com/article/rs-2698051/v1>, (2023).
- 526 24. D. P. Hughes, P. Pamilo, J. Kathirithamby, Horizontal transmission of *Wolbachia* by
527 strepsipteran endoparasites? A response to Noda et al., 2001. *Mol. Ecol.* **13**, 507-509
528 (2004).
- 529 25. S. M. Smith, Biological control with *Trichogramma*: advances, successes, and
530 potential of their use. *Annu. Rev. Entomol.* **41**, 375-406 (1996).
- 531 26. M. S. Hoddle, R. G. Van Driesche, J. P. Sanderson, Biology and use of the whitefly
532 parasitoid *Encarsia formosa*. *Annu. Rev. Entomol.* **43**, 645-669 (1998).
- 533 27. X. X. Wang, L. D. Qi, R. Jiang, Y. Z. Du, Y. X. Li, Incomplete removal of with
534 tetracycline has two-edged reproductive effects in the thelytokous wasp
535 (Hymenoptera: Aphelinidae). *Sci. Rep.* **7**, 44014 (2017).
- 536 28. S. A. Andreason *et al.*, Whitefly Endosymbionts: Biology, Evolution, and Plant Virus

- 537 Interactions. *Insects* **11**, 775 (2020).
- 538 29. X. L. Bing *et al.*, Diversity and evolution of the *Wolbachia* endosymbionts of Bemisia
539 (Hemiptera: Aleyrodidae) whiteflies. *Ecol. Evol.* **4**, 2714-2737 (2014).
- 540 30. E. Zchori-Fein, T. Lahav, S. Freilich, Variations in the identity and complexity of
541 endosymbiont combinations in whitefly hosts. *BMC Microbiol.* **5**, 310 (2014).
- 542 31. G. Gueguen *et al.*, Endosymbiont metacommunities, mtDNA diversity and the
543 evolution of the *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex. *Mol. Ecol.*
544 **19**, 4365-4376 (2010).
- 545 32. I. D. Weber, C. Czapak, K. C. Albernaz-Godinho, K. M. B. Borges, A. S. G. Coelho,
546 Validation of a sampling method: using one square centimeter for sampling the
547 immature stages of *Bemisia tabaci* in soybean. *Entomol. Exp. Appl.* **170**, 488-494
548 (2022).
- 549 33. P. Shi *et al.*, *Wolbachia* has two different localization patterns in whitefly *Bemisia*
550 *tabaci* Asiall7 Species. *PLoS One* **11**, e0162558 (2016).
- 551 34. a. M P Hassell, J. K. Waage, Host-parasitoid population interactions. *Annu. Rev.*
552 *Entomol.* **29**, 89-114 (1984).
- 553 35. K. M. Oliver, J. A. Russell, N. A. Moran, M. S. Hunter, Facultative bacterial symbionts
554 in aphids confer resistance to parasitic wasps. *Proc. Natl. Acad. Sci. USA* **100**,
555 1803-1807 (2003).
- 556 36. R. Arunkumar *et al.*, Natural selection has driven the recurrent loss of an immunity
557 gene that protects against a major natural parasite. *Proc. Natl. Acad. Sci. USA* **120**,
558 (2023).

- 559 37. J. J. Zhang *et al.*, Effects of host-egg ages on host selection and suitability of four
560 Chinese Trichogramma species, egg parasitoids of the rice striped stem borer, *Chilo*
561 *suppressalis*. *Biocontrol* **59**, 159-166 (2014).
- 562 38. M. Z. Ahmed *et al.*, The intracellular bacterium uses parasitoid wasps as phoretic
563 vectors for efficient horizontal transmission. *PLoS Pathog.* **11**, e1004672 (2015).
- 564 39. M. E. Huigens *et al.*, Infectious parthenogenesis. *Nature* **405**, 178-179 (2000).
- 565 40. M. E. Huigens, R. P. de Almeida, P. A. Boons, R. F. Luck, R. Stouthamer, Natural
566 interspecific and intraspecific horizontal transfer of parthenogenesis-inducing
567 *Wolbachia* in *Trichogramma* wasps. *Proc. Biol. Sci.* **271**, 509-515 (2004).
- 568 41. S. J. Li *et al.*, Plant-mediated horizontal transmission of between whiteflies. *ISME J.*
569 **11**, 1019-1028 (2017).
- 570 42. K. Katoh, D. M. Standley, MAFFT multiple sequence alignment software version 7:
571 improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772-780 (2013).
- 572 43. M. Suyama, D. Torrents, P. Bork, PAL2NAL: robust conversion of protein sequence
573 alignments into the corresponding codon alignments. *Nucleic Acids Res.* **34**,
574 W609-612 (2006).
- 575 44. B. Q. Minh *et al.*, IQ-TREE 2: new models and efficient methods for phylogenetic
576 inference in the genomic era. *Mol. Biol. Evol.* **37**, 1530-1534 (2020).
- 577 45. S. Kalyaanamoorthy, B. Q. Minh, T. K. F. Wong, A. von Haeseler, L. S. Jermini,
578 ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods*
579 **14**, 587-589 (2017).
- 580 46. I. Letunic, P. Bork, Interactive tree of life (iTOL) v3: an online tool for the display and

- 581 annotation of phylogenetic and other trees. *Nucleic Acids Res.* **44**, W242-245 (2016).
- 582 47. J. H. Poelen, J. D. Simons, C. J. Mungall, Global biotic interactions: An open
583 infrastructure to share and analyze species-interaction datasets. *Ecol. Inform.* **24**,
584 148-159 (2014).
- 585 48. S. Kumar *et al.*, TimeTree 5: an expanded resource for species divergence times. *Mol.*
586 *Biol. Evol.* **39**, msac174 (2022).
- 587 49. H. L. Ji, L. D. Qi, X. Y. Hong, H. F. Xie, Y. X. Li, Effects of host sex, plant species, and
588 putative host species on the prevalence of *Wolbachia* in natural populations of
589 *Bemisia tabaci* (Hemiptera: Aleyrodidae): a modified nested PCR study. *J. Econ.*
590 *Entomol.* **108**, 210-218 (2015).
- 591 50. L. Baldo *et al.*, Multilocus sequence typing system for the endosymbiont *Wolbachia*
592 *pipientis*. *Appl. Environ. Microb.* **72**, 7098-7110 (2006).
- 593 51. A. Heddi, A. M. Grenier, C. Khatchadourian, H. Charles, P. Nardon, Four intracellular
594 genomes direct weevil biology: nuclear, mitochondrial, principal endosymbiont, and
595 *Wolbachia*. *Proc. Natl. Acad. Sci. USA* **96**, 6814-6819 (1999).
- 596
- 597

598 **Acknowledgements**

599 This research was funded by “Shuangchuang Doctor” Foundation of Jiangsu Province
600 (202030472), Nanjing Agricultural University startup fund (804018), the Hainan
601 Major Science and Technology Project (ZDKJ2021007), and the Special Fund for
602 Agro-scientific Research in the Public Interest of China (201303019). Bioinformatic
603 analyses were supported by the high-performance computing platform of
604 Bioinformatics Center, Nanjing Agricultural University.

605 **Author contributions**

606 Y.X.L., X.Y.H., and Z.C.Y. conceptualized and designed the research; Z.C.Y. carried
607 out the bioinformatics analysis; L.D.Q., H.L.J., and X.X.W. conducted the
608 experiments; Y.X.L. and Z.C.Y. interpreted the results; Z.C.Y. and L.D.Q. prepared
609 the visualization; Z.C.Y. wrote the initial manuscript; Y.X.L. and X.Y.H revised the
610 manuscript.

611 **Competing interests**

612 The authors declare no competing interests.

613

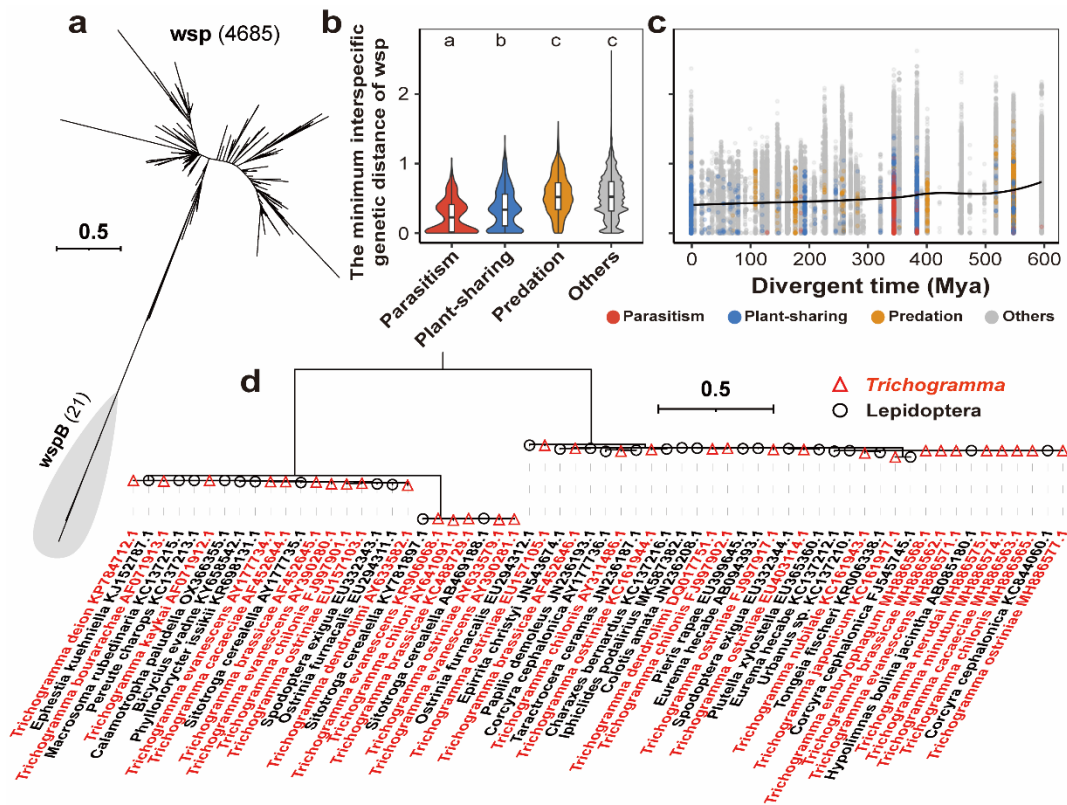
614 **Figures and Tables**

615 **Table 1. Fitness in *Bemisia tabaci* of different cross combinations**

Cross	n	Fecundity (eggs)	Egg hatching rate (%)	Immature survival rate (%)	Female proportion (%)
♀- × ♂-	20	54.0 ± 19.5 a	91.61 ± 2.99 a	73.31 ± 5.29 a	65.81 ± 8.19 a
♀- × ♂+	20	51.7 ± 20.5 a	90.88 ± 4.99 a	72.85 ± 6.33 a	66.43 ± 7.43 a
♀+ × ♂-	20	34.0 ± 12.3 b	82.19 ± 4.54 b	53.66 ± 6.58 b	42.57 ± 9.10 b
♀+ × ♂+	20	28.8 ± 11.1 b	82.16 ± 5.36 b	55.35 ± 7.21 b	48.03 ± 11.99 b

616 Data are presented as the means ± standard errors (SE) (n = 20). ♀: female adult; ♂: male adult; +: *Wolbachia*
617 infected; -: *Wolbachia* uninfected.

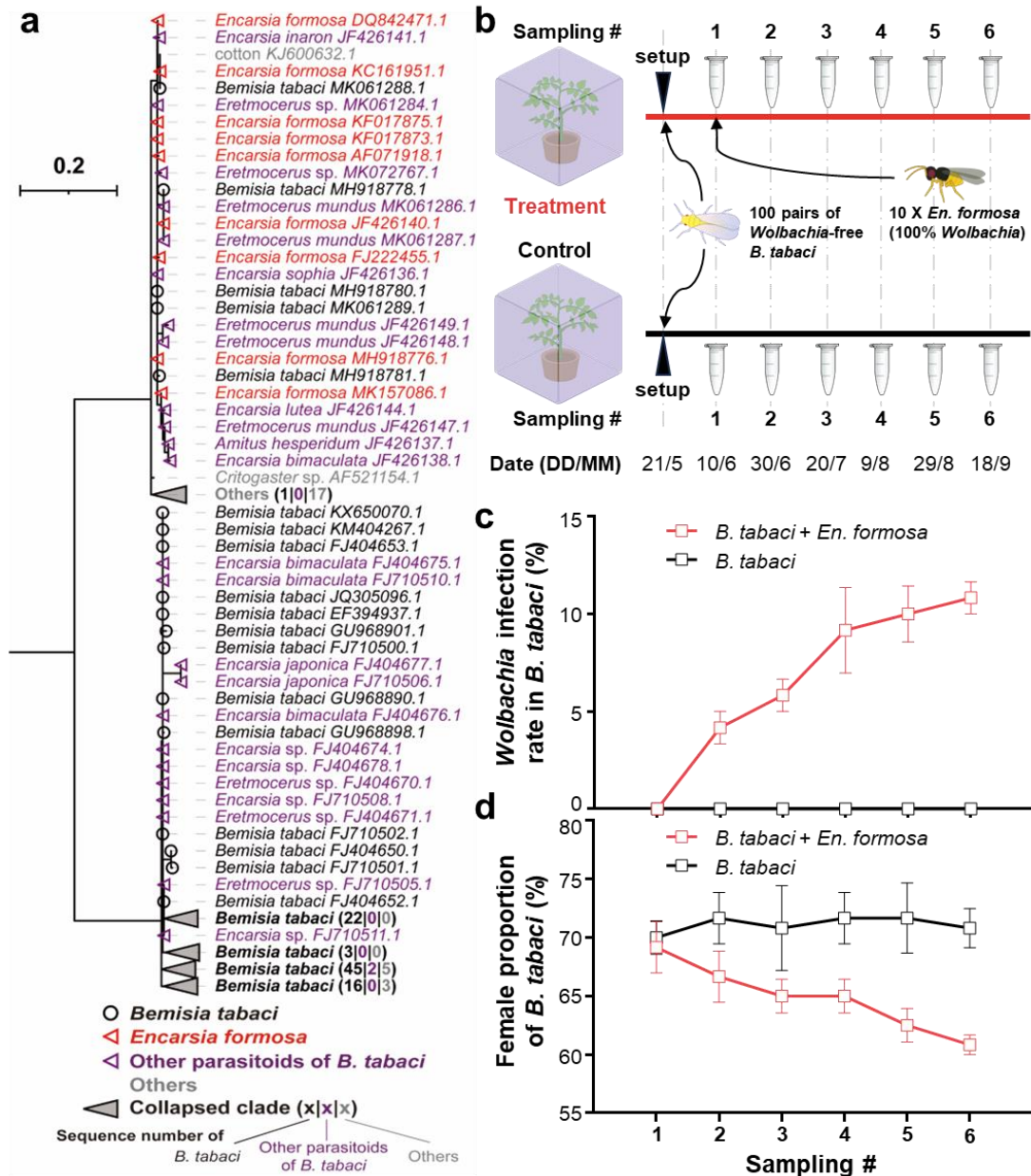
618
619
620



621

622 **Fig. 1. Parasitism facilitates interspecific horizontal transfer of *Wolbachia*.** (a)
623 Phylogeny of *Wolbachia* surface protein (*wsp*) genes retrieved from NCBI. (b) Effect
624 of parasitism, plant-sharing, and predation on the genetic distance of *wsp* between
625 species. (c) Relationship between species divergence time and the genetic distance of
626 *wsp* between species. (d) Phylogeny of representative *wsp* sequences from
627 *Trichogramma* wasps and lepidopterans. Color red and black represent *wsp* sequences
628 from *Trichogramma* wasp and lepidopterans, respectively.

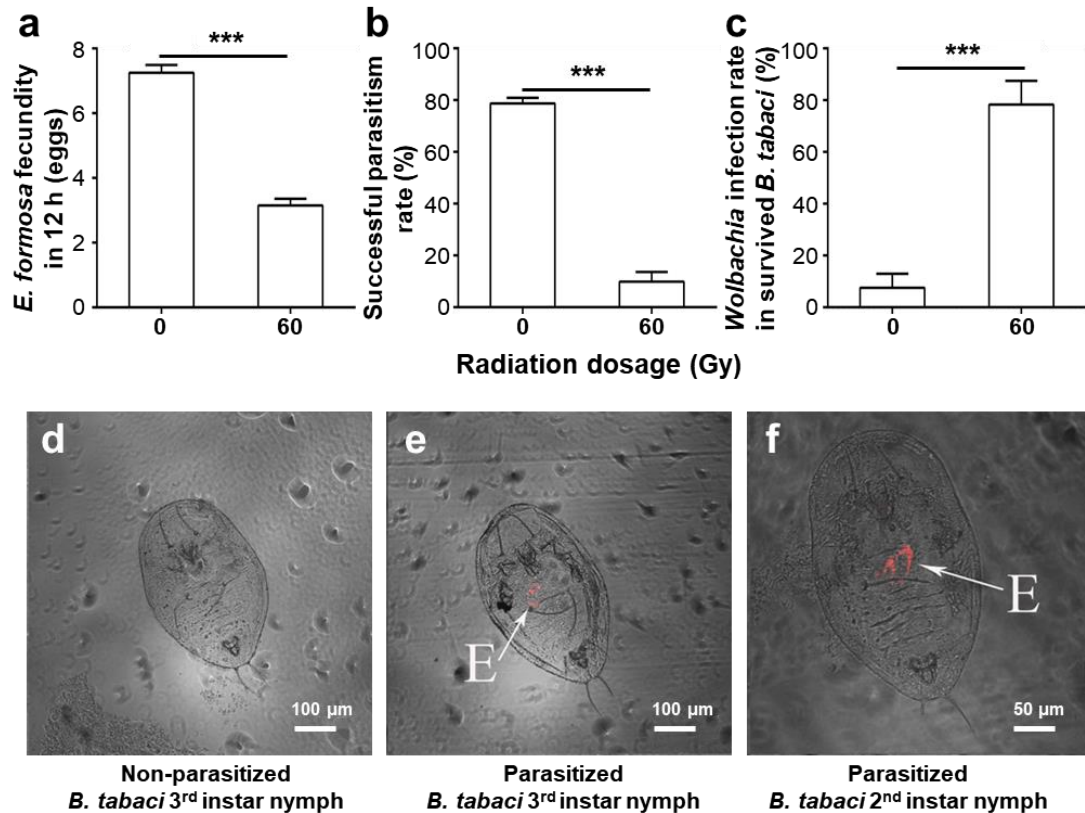
629



630

631 **Fig. 2. Transfer of *Wolbachia* from the parasitoid wasp, *Encarsia formosa*, to its**
632 **host, *Bemisia tabaci*.** (a) Phylogenetic analysis of the *wsp* gene from *B. tabaci* and its
633 parasitoid wasps. The colors black, red, purple, and gray represent *wsp* sequences
634 from *B. tabaci*, *En. formosa*, other parasitoids of *B. tabaci*, and other species,
635 respectively. (b) Scheme of the experimental design for studying the transmission of
636 *Wolbachia* from *En. formosa* to *B. tabaci*. (c–d) Effects of *En. formosa* on (c) the
637 infection rate of *Wolbachia* and (d) the proportion of females in *B. tabaci* populations.
638 Data are presented as the means \pm standard errors (SE) (n = 3).

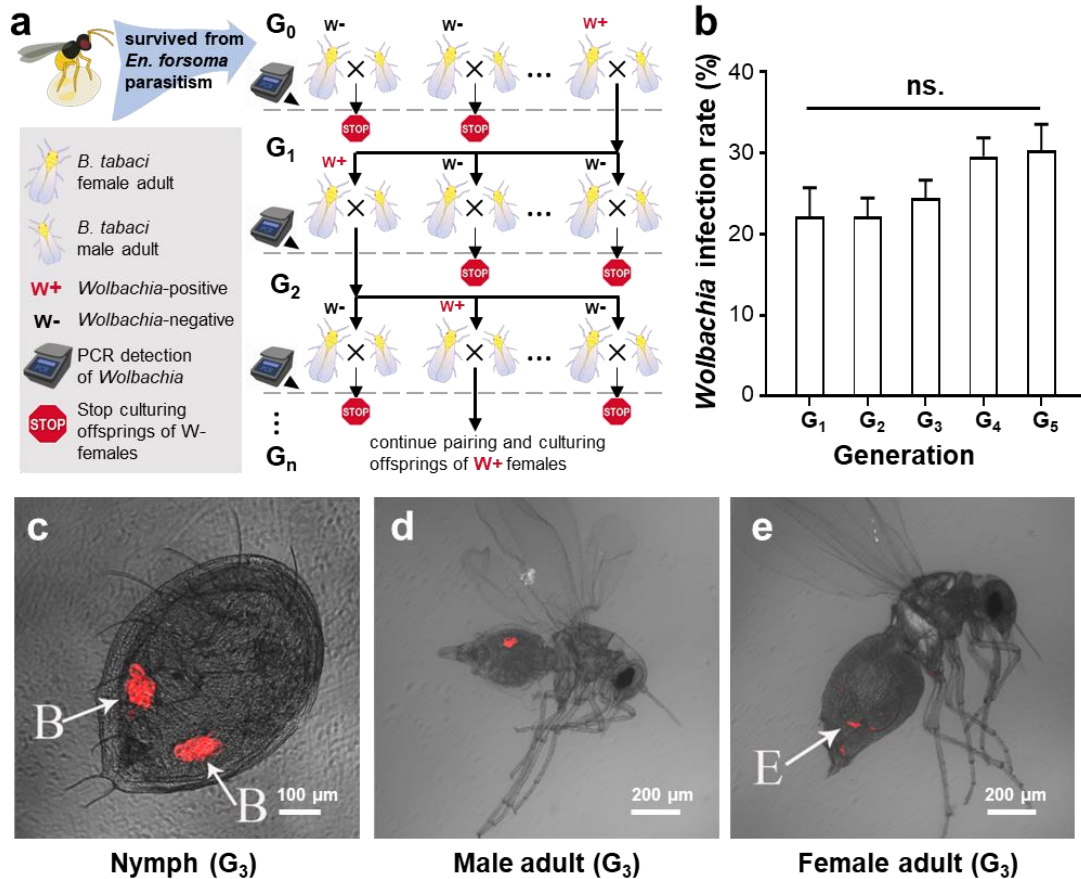
639



640

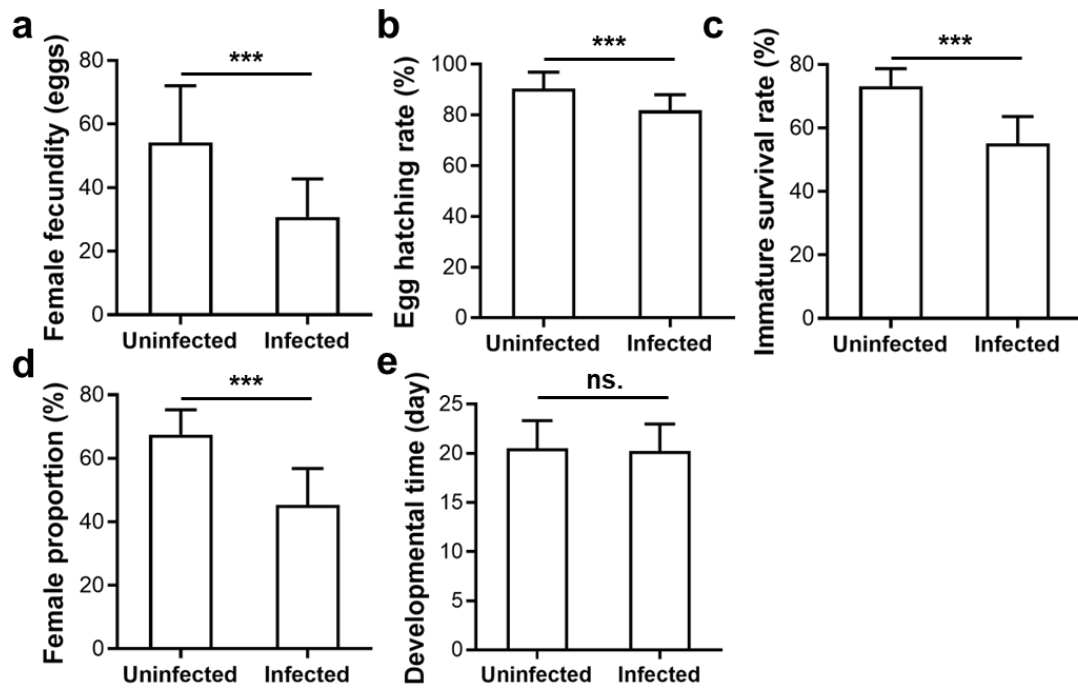
641 **Fig. 3. Parasitism failure mediates *Wolbachia* transfer from *En. formosa* to *B.***
642 ***tabaci*.** (a–c) Effects of 60 Gy radiation on (a) the fecundity of *En. formosa* over a
643 12-hour period, (b) the rate of successful parasitism, and (c) the *Wolbachia* infection
644 rate in surviving whiteflies. Data are presented as the means + SEs (n = 20). ***: $p <$
645 0.001. (d–f) Fluorescence in situ hybridization (FISH) visualization of *Wolbachia* in
646 nonparasitized and parasitized 3rd instar nymphs, as well as parasitized 2nd instar
647 nymphs of *B. tabaci*. The images present a combination of bright field and
648 fluorescence. E: injected eggs from *En. formosa*.

649



650
651
652
653
654
655
656
657
658
659
660
661

Fig. 4. Vertical transmission of *Wolbachia* from *En. formosa* in *B. tabaci*. (a) Scheme of the study design for *Wolbachia* vertical transmission in *B. tabaci*. Adult whiteflies that survived from *En. formosa* parasitism were denoted as G₀. After pairing and oviposition, the infection status of *Wolbachia* in the female parent was examined. Only the offsprings from *Wolbachia*-infected female whiteflies were maintained. (b) The vertical transmission rate of *Wolbachia* across five generations in *B. tabaci*. Data are presented as the means + SEs (n = 9). ns.: no significant differences. (c–e) FISH visualization of *Wolbachia* in G₃ *B. tabaci* (c) nymph, (d) male adult, and (e) female adult. The images present a combination of bright field and fluorescence. B: bacteriocyte; E: eggs in the ovary of a female whitefly.



662

663 **Fig. 5. Fitness costs in *B. tabaci* induced by *Wolbachia* from *En. formosa*.** (a–e)

664 The effects of *En. formosa* *Wolbachia* on *B. tabaci* (a) female fecundity, (b) egg

665 hatching rate, (c) immature survival rate, (d) female proportion, and (e)

666 developmental time. Data are presented as the means + SEs (n = 60). ***: $p < 0.001$;

667 ns.: no significant differences.