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1 **Plant choice for oviposition in the phytophagous insect *Bemisia***
2 ***tabaci*: cytotype, including symbionts, knows best!**

3

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17

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20 **Abstract**

21 Most phytophagous insects harbour symbiotic microorganisms that may facilitate, at
22 multiple levels, the exploitation of plants. Actually, very little is known about the role of these
23 symbionts in insects' host plant choice. The whitefly *Bemisia tabaci* is a good model to
24 explore these topics. It harbours a nutritional 'primary' endosymbiont allowing it to thrive on
25 plant sap, and up to seven 'secondary' endosymbionts (S-symbionts) whose roles in plant
26 utilization are currently under study. Here, we first investigated the influence of cytotype on
27 females' oviposition rate on hibiscus and lantana, respectively a favourable and an
28 unfavourable host plant for *B. tabaci*. We then addressed whether cytotype affects whiteflies'
29 plant choice for oviposition. Using genetically homogeneous whiteflies harbouring different
30 cytotypes, we showed that oviposition rate was significantly lower on lantana than on
31 hibiscus, but that this reduction varied between cytotypes. Moreover, according to their
32 cytotype, whiteflies preferred hibiscus over lantana, or showed no preference. Thus, cytotype
33 influenced plant choice for oviposition. Altogether, our results indicate that, without a
34 cytotype that facilitates the utilization of lantana, whiteflies may avoid this host plant. Since
35 cytotypes harbour different S-symbionts' combinations, we argue that the influence of these
36 symbionts on whitefly oviposition behaviour is very likely.

37 **Keywords:** *Bemisia tabaci*, cytotype, oviposition behaviour, plant choice, symbionts

38 **Highlights**

- 39 • Cytotype (including symbionts) affects whiteflies oviposition rate
- 40 • Cytotype determines plant choice for oviposition
- 41 • Different symbionts' combinations affect whitefly oviposition behaviour

42 Introduction

43 Microbial symbiosis is widespread in eukaryotes. Some symbionts are parasites with
44 negative effects on their host. Other symbionts are mutualists with beneficial effects, or
45 commensals with no apparent effects (Buchner, 1965; Ferrari & Vavre, 2011; Tipton et al.,
46 2019). Infection by parasites often induces host behavioural changes. Some of these changes
47 improve transmission and/or survival of the parasite at the expense of host fitness and are
48 considered as ‘parasite’s manipulations’ (Heil, 2016; Lewis & Lize, 2015; Poulin, 2010; Vale
49 et al., 2018). On the contrary, other behavioural changes benefit the host and participate to its
50 defence strategies against parasites (Hart, 2011; Vale et al., 2018). While symbiont-driven
51 behavioural alterations have been frequently observed in conflicting interactions, host
52 behavioural modifications in mutualistic or commensal interactions have received less
53 attention. However, recent discoveries indicate that these types of symbiotic interactions may
54 have various effects on insect behaviours related to locomotion (Schretter et al., 2018),
55 reproduction (Najarro et al., 2015; Sharon et al., 2010), foraging (Akami et al., 2019;
56 Angelella et al., 2018; Leitão-Gonçalves et al., 2017; Leybourne et al., 2020; Qiao et al.,
57 2019; Wong et al., 2017) or interactions with other organisms (Dion et al., 2011; Polin et al.,
58 2014; Sochard, Bellec, et al., 2021) (for a review, see also Hosokawa & Fukatsu, 2020).

59 Most insect species are associated with microbial symbionts, which colonize their gut,
60 body cavities or cells (Hansen & Moran, 2014). In phytophagous insects, mutualistic
61 symbionts can facilitate the utilization of food sources that are unbalanced (*e.g.* phloem sap)
62 or difficult to digest (*e.g.* wood) through the provisioning of essential and otherwise limiting
63 nutrients, the detoxification of plant defence compounds or the breakdown of plant polymers,
64 respectively (Frago et al., 2012; Hansen & Moran, 2014). Much less is known about the
65 influence of symbionts in host plant selection. Recent works have shown that the gut

66 symbiotic bacteria can affect food choice and foraging decisions under laboratory conditions
67 in the fruit fly *Drosophila melanogaster* (Leitão-Gonçalves et al., 2017; Qiao et al., 2019;
68 Wong et al., 2017) and the oriental fruit fly *Bactrocera dorsalis* (Akami et al., 2019). Other
69 studies on phloem sap-feeding hemipterans have demonstrated that infection by intracellular
70 bacteria can affect their interaction with the plants by modifying plant probing behaviour, like
71 in the cowpea aphid *Aphis craccivora* (Angelella et al., 2018) and the bird cherry oat aphid
72 *Rhopalosiphum padi* (Leybourne et al., 2020). On the other hand, intracellular symbionts have
73 no or only moderate influence on host plant selection in the pea aphid *Acyrtosiphon pisum*
74 (Sochard, Dupont, et al., 2021; Sochard, le Floch, et al., 2021). Studies on a broader spectrum
75 of host-symbiont associations are needed to better assess the role of symbionts in insect plant
76 choice. This may also shed light on the potential impact of symbiosis on hosts' ecological
77 diversification and speciation (Brucker & Bordenstein, 2012; Vavre & Kremer, 2014). Indeed,
78 for phytophagous insects, being restricted to specific plants is likely to limit gene flow
79 between populations (Bird et al., 2012; Schluter, 2001).

80 The tobacco whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) constitutes an
81 interesting model to address this question. It represents a highly diversified cryptic species
82 complex and, to date, 42 putative species have been proposed in phylogenetic analyses based
83 on mitochondrial cytochrome oxidase 1 (*mtCOI*) sequences (de Barro et al., 2011; Dinsdale et
84 al., 2010; Firdaus et al., 2013; Roopa et al., 2015). Previous studies reported significant
85 variation in plant preference and/or utilization capabilities between *B. tabaci* species. Some
86 are 'generalist' species with a broad host plant range, while others can be considered as
87 'specialist' species, with a restricted host plant range (Malka et al., 2018). Differences can
88 also be seen within species: indeed, in some species, genetic mitochondrial groups can be
89 identified, as in the case of the Mediterranean (MED) species, which can be divided into three
90 groups, Q1, Q2 and Q3 (Mouton et al., 2015; Vyskočilová et al., 2018). While the Q1 and Q2

91 groups are relatively polyphagous (Vyskočilová et al., 2019), field observations and
92 laboratory experiments indicate that the Q3 group, only identified in Burkina Faso to date, has
93 a more restricted host range and seems specialized on lantana (*Lantana camara*) (Romba et
94 al., 2018; Romba & Gnankiné, 2018), a host plant that is otherwise unfavourable to the MED
95 species (Benhamou et al., 2021). Other comparative experiments revealed host range
96 differences between Q1 and Q2, and among Q1 populations from different geographical areas
97 (Vyskočilová et al., 2019).

98 As other phloem sap-feeding insects, *B. tabaci* harbours an obligate ‘primary’
99 intracellular symbiont (P-symbiont) that provide essential amino acids which are not present
100 in sufficient quantities in the phloem sap for whiteflies’ development, survival and
101 reproduction (Baumann, 2005; Douglas, 2009). This γ -proteobacterium, *Portiera*
102 *aleyrodidarum* (hereafter *Portiera*), is common to all members of the *B. tabaci* species
103 complex (Thao & Baumann, 2004). *Portiera* resides within the cytoplasm of specialized host
104 cells, the bacteriocytes, associated to form an organ, the bacteriome (Gottlieb et al., 2008).
105 *Portiera* is vertically transmitted through a mechanism which is unique to whiteflies, with an
106 entire bacteriocyte being transferred to the next generation (Buchner, 1965; Luan et al.,
107 2016,2018). In addition to this P-symbiont, *B. tabaci* harbour facultative ‘secondary’
108 symbionts (S-symbionts). Seven bacterial genera of S-symbionts have been reported to date:
109 *Arsenophonus*, *Cardinium*, *Fritschea*, *Hamiltonella*, *Hemipteriphilus*, *Rickettsia* and
110 *Wolbachia* (Bing et al., 2013; Everett et al., 2005; Gottlieb et al., 2006; H. Wang et al., 2020;
111 Zchori-fein & Brown, 2002), with up to four present in the same insect body (Zchori-Fein et
112 al., 2014). They all co-occur within the same bacteriocytes as *Portiera*, but some of them can
113 also colonize other tissues of the host (Gottlieb et al., 2008). *B. tabaci* S-symbionts are
114 predominantly vertically transmitted, but some of them can also be horizontally transmitted,
115 for instance through the phloem when whiteflies feed upon the same plant (Caspi-Fluger et

116 al., 2012; S. Li et al., 2016; Y. Li et al., 2017). Analyses of *B. tabaci* S-symbionts suggest that
117 some of them could contribute to their hosts' nutrition. Indeed, *Portiera*'s genome has
118 undergone drastic reduction (Rao et al., 2015) as a consequence of its prolonged intracellular
119 lifestyle (Mccutcheon & Moran, 2012; Moran et al., 2008). It has lost almost all genes
120 involved in the synthesis of vitamins and cofactors, and several of its essential amino acid
121 pathways are incomplete (Rao et al., 2015), functions that S-symbionts may replace or
122 complement (Opatovsky et al., 2018; Rao et al., 2015; Ren et al., 2020; Y.-B. Wang et al.,
123 2020). As an example, *Hamiltonella* can provide vitamins and cofactors (Rao et al., 2015;
124 Ren et al., 2020; Y.-B. Wang et al., 2020), and could also complete the missing steps of the
125 essential amino acid lysine biosynthesis pathway of *Portiera* (Rao et al., 2015). Interestingly,
126 by producing metabolites able to interact with their hosts' neurons or sensory organs or that
127 are precursors of neurotransmitters, S-symbionts may directly influence their hosts' behaviour
128 (Engl & Kaltenpoth, 2018; Shropshire & Bordenstein, 2016). Importantly, S-symbiont
129 composition differs between mitochondrial groups, both across (Zchori-Fein et al., 2014) and
130 within (Gnankiné et al., 2013; Gueguen et al., 2010) species, raising the possibility that these
131 symbionts may contribute to *B. tabaci* species complex ecological diversification.

132 Recently, we demonstrated that S-symbionts might affect the range of the host plants
133 suitable for *B. tabaci*. Indeed, we have shown that cytotype (referring to the cytoplasmic
134 characteristics of the insect line, including the mitochondria and intracellular symbionts)
135 affects whitefly capability to feed and oviposit on lantana (*Lantana camara*), an unfavourable
136 host plant to *B. tabaci* (Benhamou et al., 2021). The aims of the present study were to
137 investigate whether whiteflies exhibit plant choice for oviposition and whether the S-symbiont
138 community may influence their oviposition behaviour. To fulfil these objectives, we
139 performed choice experiments between a plant known to be favourable for *B. tabaci*, hibiscus

140 (*Hibiscus moscheutos*), and the unfavourable lantana, using genetically homogeneous
141 whiteflies harbouring different cytotypes (and, hence, different S-symbionts).

142 **Material and Methods**

143 *Insects*

144 Three *B. tabaci* parental lines (F0) from the Mediterranean (MED) species were used
145 in this study: AA(Q1-HW), BB(Q1-HR) and CC(Q2-ARW) that belong to the mitochondrial
146 groups Q1 or Q2, and that are associated with different S-symbionts (**Table 1**). The first
147 uppercase double letters indicate females' diploid nuclear genotype (AA, BB or CC) (males
148 are haploid as *B. tabaci* reproduces by arrhenotoky). Characters within **parenthesis** refer to
149 their cytotype, including their mitochondrial group (Q1 or Q2) and S-symbionts (HW, HR or
150 ARW; A: *Arsenophonus*, H: *Hamiltonella*, R: *Rickettsia*, W: *Wolbachia*). Insect lines were
151 reared on hibiscus plants in mesh cages in climate-controlled rooms at 26°C ± 1°C and 70%
152 relative humidity with a 14L:10D photoperiod. The same climate-controlled conditions have
153 been used for the whole study.

154 **Table 1. *Bemisia tabaci* laboratory lines used in this study.**

| Line | Nuclear Genotype | Cytotype | | Collection information | | |
|------------|------------------|---------------------|--------------------------|------------------------|--------------------------|-------------------------------------|
| | | Mitochondrial group | S-Symbionts ^a | Year | Location (Country) | Plant |
| AA(Q1-HW) | AA | Q1 | HW | 2012 | Tympaki (Greece) | Eggplant <i>Solanum</i> sp. |
| BB(Q1-HR) | BB | Q1 | HR | 2012 | Les Ponts-de-Cé (France) | Mandevilla <i>Mandevilla</i> sp. |
| CC(Q2-ARW) | CC | Q2 | ARW | 2018 | Lyon (France) | Lantana <i>Lantana camara</i> |

^a A: *Arsenophonus*, H: *Hamiltonella*, R: *Rickettsia*, W: *Wolbachia*.

156 *Collection of F1 females*

157 To separate cytotype-related and genotype-related effects, experiments were
158 performed on F1 females obtained from the 9 possible crosses between the F0 parental lines
159 (**Table 2**, experimental design shown in **Fig. S1**), based on the procedure described in
160 Benhamou *et al.* (Benhamou et al., 2021). These crosses produced **either** F1 females with
161 identical nuclear genotypes **(the two letters refer to the maternally (first) and paternally**
162 **(second) inherited nuclear genotype)** but different cytotypes [*e.g.* AB(Q1-HW) and BA(Q1-
163 HR)], or the opposite, *i.e.* females with the same cytotype but different genotypes [*e.g.*
164 AA(Q1-HW), AB(Q1-HW) and AC(Q1-HW)]. The three lines were synchronized as follows:
165 adults from the hibiscus stock cages were allowed to mate and oviposit for 7 days on cotton
166 leaves. To keep them fresh, leaves were fixed on their adaxial side on a 1cm layer of 2% Agar
167 (Sigma-Aldrich®) in plastic Petri dishes (90mm in diameter). Petri dishes were placed upside-
168 down to simulate the situation of leaves attached to a plant with the abaxial surface down.
169 Cotton was used as it is not involved in the subsequent experiments, thus avoiding possible
170 effect of the F0 individuals' plant on the traits assessed on the following F1 generation (**Fig.**
171 **S1**). We chose cotton because it is a known favourable plant for the *B. tabaci* MED species
172 groups Q1 and Q2 (Romba & Gnankiné, 2018; Vyskočilová et al., 2018). F0 females were
173 collected within 5 hours after emergence. Individuals' sex was determined based on their
174 genitalia, by observation under a stereomicroscope (Leica EZ4, Leica Microsystems GmbH,
175 Wetzlar, Germany). F0 females (<5-h-old) were considered virgin as newly emerged adults
176 do not immediately mate (Li et al., 1989). As *B. tabaci* reproduces by arrhenotoky, which
177 means that unmated females produce only haploid eggs that develop into males, this was
178 confirmed by the absence of females in their offspring. Each cross was made on cotton
179 (90mm in diameter Petri dishes) using groups of 15 F0 males and 15 F0 females (3 replicates
180 for each). These 30 individuals were left for 1 week on the same cotton leaf to mate and

181 oviposit. Upon emergence, F1 virgin females (<5-h-old) were collected for further
 182 experiments as described hereafter. The whole procedure (crosses and experiments) was
 183 independently performed three times, treated as “experimental blocks” in the statistical
 184 analyses.

| | | Mother (F0) ^a | | |
|-----------------------------|-----------|--------------------------|-----------|------------|
| | | AA(Q1-HW) | BB(Q1-HR) | CC(Q2-ARW) |
| Father (F0) ^b | A(Q1-HW) | AA(Q1-HW) | BA(Q1-HR) | CA(Q2-ARW) |
| | B(Q1-HR) | AB(Q1-HW) | BB(Q1-HR) | CB(Q2-ARW) |
| | C(Q2-ARW) | AC(Q1-HW) | BC(Q1-HR) | CC(Q2-ARW) |

^a F0 and F1 Females identification: Nuclear genotype(Cytotype); Nuclear genotype: letters indicate the maternally (first) and the paternally (second) inherited nuclear genotype; Cytotype: Mitochondrial group (Q1 or Q2) + S-symbionts; S-symbionts: A: *Arsenophonus*; H: *Hamiltonella*; R: *Rickettsia*; W: *Wolbachia*.

^bF0 Fathers' genotypes (A, B or C) are indicated by only one letter as males are haploid.

185 **Table 2. Nuclear genotypes and cytotypes of *B. tabaci* F1 females.**

186 *Cytotype determination*

187 For each parental line (**Table 1**) and F1 cross (**Table 2**), mitochondrial group (Q1 or
 188 Q2) and symbiont infection status (*Portiera*, *Arsenophonus*, *Hamiltonella*, *Rickettsia*,
 189 *Wolbachia*) were checked on 8 females to ensure that the expected cytotype was correct. Total
 190 DNA was extracted from individual whiteflies using the NucleoSpin 95 Tissue kit (Macherey
 191 Nagel GmbH, Düren, Germany) following the manufacturer's instructions. Samples were
 192 crushed in a mix of 60µL of lysis buffer and 8µL of protein K (22mg/mL) with 1.4mm sterile
 193 stainless-steel beads using a TissueLyser (Qiagen, Hilden, Germany). Elution was done in
 194 100µL of buffer and DNA was then stored at -20°C. The mitochondrial haplotype was
 195 checked by PCR-RFLP according to the method described in Henri *et al.* (Henri *et al.*, 2013),
 196 allowing to differentiate between the Q1 and Q2 groups. The infection status was determined
 197 using quantitative PCR (qPCR) using the following genes: *16S* rRNA for *Arsenophonus*,

198 *dnaK* for *Hamiltonella*, *16S* rRNA for *Portiera*, *gltA* for *Rickettsia* and *ftsZ* for *Wolbachia*
 199 (Table 3). The β -actin gene from *B. tabaci* (Table 3) was used as a DNA extraction control.
 200 The PCR amplifications were performed on a CFX-96 Real Time PCR machine (Bio-Rad,
 201 Hercules, USA). Each reaction consisted of 5 μ L of SYBR® Green 2x Supermix (Bio-rad) in
 202 addition to 2 μ L of DNA sample, 2 μ L of nuclease-free water, and 0.5 μ L of forward and
 203 reverse primers (500nM).

204 **Table 3. qPCR primers used in this study.**

| Organism | Gene | Primers | Primer sequence | Fragment size (bp) | Hybridization T° (°C) | Reference |
|-----------------------|---------------------------------|-------------|------------------------------------|--------------------|-----------------------|-----------------------------|
| <i>Bemisia tabaci</i> | <i>β-actin</i> | wf-Bactin-F | 5'-TCTTCCAGCCATCCTTCTTG-3' | 130 | 63 | (Sinisterra et al., 2005) |
| | | wf-Bactin-R | 5'-CGGTGATTTCCTTCTGCATT-3' | | | |
| <i>Portiera</i> | <i>16S</i> | Port73-F | 5'-GTGGGGAATAACGTACGG-3' | 193 | 60 | (Caspi-Fluger et al., 2011) |
| | | Port266-R | 5'-CTCAGTCCCAGTGTGGCTG-3' | | | |
| <i>Arsenophonus</i> | <i>16S</i> | ArsF3 | 5'-GTCGTGAGGAARGTGTARGGTT-3' | 765 | 63 | (Duron et al., 2008) |
| | | ArsR3 | 5'- CCTYTATCTCTAAAGGMTCGCTGGATG-3' | | | |
| <i>Hamiltonella</i> | <i>dnaK</i> | dnaK-F | 5'-GGTTCAGAAAAAAGTGGCAG-3' | 155 | 60 | (Moran et al., 2005) |
| | | dnaK-R | 5'-CGAGCGAAAGAGGAGTGAC-3' | | | |
| <i>Rickettsia</i> | <i>gltA</i> | glt375-F | 5'-TGGTATTGCATCGCTTTGGG-3' | 199 | 60 | (Caspi-Fluger et al., 2011) |
| | | glt574-R | 5'-TTTCTTTAAGCACTGCAGCAGC-3' | | | |
| <i>Wolbachia</i> | <i>ftsZ</i> | F2 | 5'-TTGCAGAGCTTGGACTTGAA-3' | 400 | 55 | (Vavre et al., 1999) |
| | | R2 | 5'-CATATCTCCGCCACCAGTAA-3' | | | |

205 *Plants*

206 Plants were grown in the same climate-controlled conditions as the ones used for
 207 rearing lines, in insect-free rooms. Cotton, *Gossypium hirsutum* L. (Malvaceae) cv. 'Elpida',
 208 and hibiscus, *Hibiscus moscheutos* L. (Malvaceae) cv. 'Honeymoon', were grown from seeds
 209 while lantana, *Lantana camara* L. (Verbenaceae) was vegetatively propagated from cuttings
 210 in pots using a 75:25 (v/v) mix of potting soil and vermiculite. For all experiments performed
 211 in this study, fully developed leaves, randomly collected from 2-month-old plants, have been
 212 used.

213 *No-choice oviposition and egg hatching rate*

214 The influence of whitefly cytotype on females' oviposition and egg hatching rate on
215 hibiscus and lantana was investigated using no-choice oviposition tests. F1 virgin females
216 (<5-h-old), which developed on cotton, were put individually on hibiscus or lantana leaf disks
217 (20mm in diameter, to standardize the leaf area) fixed on an agar layer in Petri dishes (55mm
218 in diameter), and placed upside down. After 7 days, females were removed and the number of
219 eggs laid (oviposition) was recorded under a stereomicroscope. Ten days later, the number of
220 eggs that hatched was estimated by counting the number of larvae (including both live and
221 dead larvae). This method was used because the larvae, even dead, are easier to identify than
222 the empty eggs. We also calculated the number of eggs that did not hatch (number of eggs
223 laid – number of eggs hatched). Thirteen to 17 individual measures were obtained per
224 cytotype/genotype combination and per plant.

225 *Plant choice for oviposition*

226 Plant choice for oviposition was investigated in binary choice experiments between
227 hibiscus and lantana. The experimental setup used was inspired from the one developed by
228 Shah and Liu (Shah & Liu, 2013). Choice arenas (produced 1 hour before use) consisted of
229 one lantana and one hibiscus leaf disk (20mm in diameter), fixed on their adaxial side on an
230 agar layer in a Petri dish (90mm in diameter). Disks were arranged opposite and at equal
231 distance (20mm) from the center of the arena (**Fig. S1**). For this experiment, we used 2-d-old
232 females to ensure they were old enough to oviposit from the very beginning of the tests, at it
233 lasted less time than the previous no choice experiments. Newly emerged virgin females (<5-
234 h-old), which developed on cotton, were transferred on fresh cotton leaves (55mm in diameter
235 Petri dishes) for 2 days. Tests were initiated every day in the morning at the same hour with
236 the introduction of one F1 female (2-d-old) into the center of each arena, which were then

237 placed upside down. Thirty-two hours later, the female's location was recorded and the
238 number of eggs laid on each leaf disk was counted under a stereomicroscope. As far as
239 possible, experiments were run every day for all nine cytotype/genotype combination
240 simultaneously. Measures were considered to be valid when females were found alive on a
241 leaf disk and if they laid at least one egg at the end of the tests. Thereby, 31 to 37 valid
242 measures were obtained per cytotype/genotype combinations.

243 *Statistical analyses*

244 For no-choice experiments, oviposition and egg average hatching rate were analysed
245 by a generalized linear model (GLM) (with a log link and a negative binomial or a quasi-
246 binomial error structure, respectively) with the plant species, the insect cytotype, the insect
247 nuclear genotype (as a nested effect within the cytotype) and the experimental block set as
248 explanatory variables. The average hatching rate was calculated according to the formula (a),
249 where i is the replicate number and N is the total number of replicates. Eggs that hatched were
250 considered as 'successes' ('No.successes'), and eggs that did not hatch as 'failures'
251 ('No.failures').

$$252 \quad (a) \text{ average proportion of successes} = \frac{\sum_{i=1}^N (\text{No.successes})}{\sum_{i=1}^N (\text{No.successes} + \text{No.failures})}$$

253 For the plant choice experiment, oviposition was analysed by a GLM (with a quasi-
254 binomial error structure) with the insect cytotype, the insect nuclear genotype (integrated into
255 the model as a nested effect within the cytotype) and the experimental block set as
256 explanatory variables. The response variable is expressed as the average proportion of eggs
257 laid on lantana compared to the total number of eggs laid on lantana (accounted as
258 'successes') plus hibiscus (accounted as 'failures'), given by the formula (a). The number of
259 eggs laid on hibiscus or lantana according to females cytotype and genotype in the plant

260 choice experiment was analysed using a GLM (with a negative binomial error structure).
261 From the complete models, we performed a model simplification through a backward
262 procedure (Crawley, 2013). Multiple comparison analyses were carried out by Tukey's test
263 (Tukey, 1949) to investigate statistical relationships between explanatory variables levels
264 using the *emmeans* R package (Russell, 2020). The *emmeans* R package was also used to
265 extract means, average proportions and 95% confidence intervals (95%CI) from the models.
266 All data analyses were performed using the R software (R Development Core Team 2011;
267 <http://www.R-project.org>).

268 ***Data accessibility***

269 The datasets generated and analysed in this study can be obtained from the Zenodo
270 online repository at: <https://zenodo.org/record/6414033>.

271 ***Ethical note***

272 This research adheres to the ASAB/ABS Guidelines for the Use of Animals in
273 Research, the legal requirements of France and the institutional guidelines of the University of
274 Lyon.

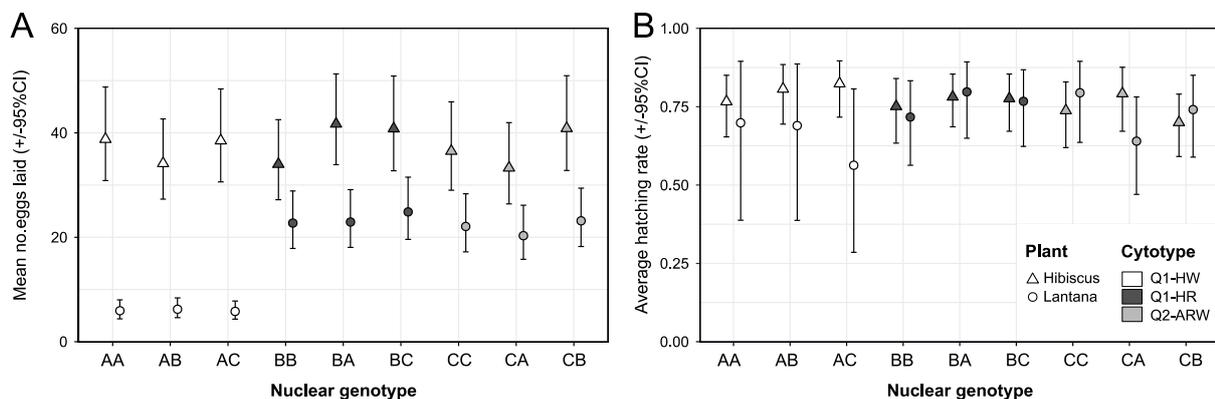
275 **Results**

276 ***No-choice oviposition and hatching rate***

277 The influence of whitefly cytotype on females' ability to feed and produce offspring
278 on favourable and non-favourable plants were investigated by measuring females' oviposition
279 and egg hatching rate on hibiscus or lantana in no-choice experiments (**Fig. 1**). For the
280 number of eggs laid, there was no difference between the three experimental blocks [$\chi^2(2) =$

281 1.60, $P = 0.45$]. For a given cytotpe, there was no difference between genotypes [$\chi^2(6) =$
 282 3.57, $P = 0.74$]. There was also no tripartite interaction between plant, cytotpe and genotype
 283 [$\chi^2(6) = 1.43$, $P = 0.96$]. However, there was a significant interaction between the plant
 284 species and the insect cytotpe [$\chi^2(2) = 103.20$, $P < 0.001$]. This interaction results from the
 285 fact that, while the number of eggs laid was homogeneous among cytotpes on hibiscus
 286 (mean, 37.71; standard error [SE]: 1.31) ($P > 0.99$), it was significantly reduced on lantana for
 287 all cytotpe/genotype combinations ($P < 0.001$), but to different extents between cytotpes.
 288 Indeed, females with the cytotpe Q1-HW consistently laid a lower number of eggs on
 289 lantana (mean, 5.98; SE: 0.74) than females with cytotpes Q1-HR (mean, 23.50; SE: 1.40) (P
 290 < 0.001) or Q2-ARW (mean: 21.88; SE: 1.41) ($P < 0.001$) that laid a similar number of eggs
 291 on this plant ($P = 0.98$) (**Fig. 1A**). These results confirm our previous data (Benhamou et al.,
 292 2021) showing that lantana is globally a less favourable host plant than hibiscus, but also
 293 highlight that some cytotpes mitigate this effect.

294 Regarding the average egg hatching rate (**Fig. 1B**), there was no difference between
 295 experimental blocks [$F(2,202) = 1.91$, $P = 0.15$]. We also did not detect any influence of the
 296 plant species [$F(1,238) = 1.35$, $P = 0.25$] or insect cytotpe [$F(2,238) = 0.65$, $P = 0.52$], and
 297 no interaction between these two factors [$F(2,238) = 1.26$, $P = 0.29$]. There was no difference
 298 between genotypes for each cytotpe [$F(6,238) = 0.27$, $P = 0.95$], and no interaction between
 299 plant, cytotpe and genotype [$F(6,238) = 0.72$, $P = 0.63$].

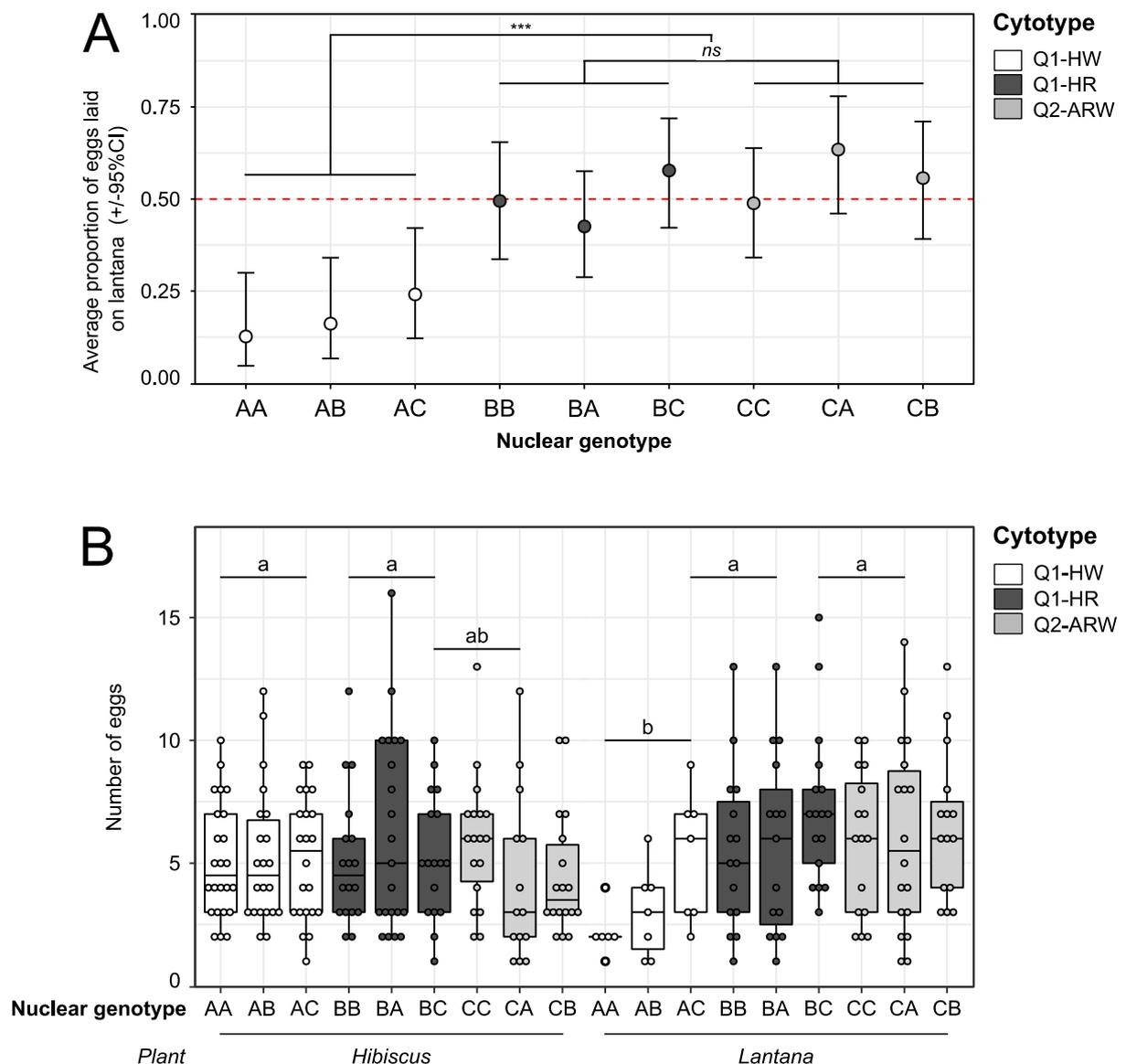


300 **Figure 1. Effect of the insects' genotype and cytotype on *B. tabaci* females' oviposition**
301 **and egg hatching rate on hibiscus and lantana (no choice experiment).** (A) Mean number
302 of eggs laid per female [\pm 95% confidence interval (CI)] over 7 days. (B) Average egg
303 hatching rate (\pm 95% CI) after 10 days. All individuals are F1 females obtained from crosses
304 of laboratory lines: AA(Q1-HW), BB(Q1-HR), or CC(Q2-ARW). F1 female legend key:
305 Nuclear genotype: letters indicate the maternally (first) and the paternally (second) inherited
306 nuclear genotype; Cytotype: mitochondrial group+S-symbionts (A: *Arsenophonus*, H:
307 *Hamiltonella*, R: *Rickettsia*, W: *Wolbachia*). Thirteen to 17 individual measures were
308 obtained per type of F1 female and per plant.

309 *Oviposition choice according to the insect cytotype*

310 We then investigated the influence of the whiteflies' cytotype on female plant choice
311 for oviposition between hibiscus and lantana (**Fig. 2**). Oviposition choice is expressed here as
312 the mean proportion of eggs laid on lantana compared to the total number of eggs laid on the
313 two plants (**Fig. 2A**). There was no effect of the experimental block factor [$F(2,278) = 0.13$, P
314 $= 0.88$] and no difference between genotypes for a given cytotype [$F(6,296) = 0.82$, $P = 0.56$].
315 In contrast, there was a significant effect of the insect cytotype on oviposition plant choice
316 [$F(2,296) = 17.46$, $P < 0.001$]: females with the cytotype Q1-HW laid a greater proportion of
317 eggs on hibiscus (around 80%) than on lantana (around 20%), while females with the cytotype
318 Q1-HR or Q2-ARW showed no oviposition preference, *i.e.* the average proportion of eggs
319 laid on hibiscus and lantana was merely 50% (**Fig. 2A**). Most females (95%) laid eggs on a
320 single leaf disk: the one they were on at the end of the experiment. Only a few of them (5%)
321 laid eggs on both leaf disks (**Fig. S2**). During this short-term time period (32h), females that
322 have chosen a single leaf disk laid a similar amount of eggs, about 5, whatever their choice
323 (hibiscus or lantana), and whatever their cytotype or genotype, with one exception: females

324 with the cytotype Q1-HW not only chose lantana less often than hibiscus but, when they did,
 325 they laid a lower number of eggs (mean, 3.55; SE, 0.52) than the ones who chose hibiscus
 326 (mean, 5.16; SE, 0.30) [effect of the interaction between the plant species and the insect
 327 cytotype: $\chi^2(2) = 11.72$, $P = 0.0028$; effect of the insect's nuclear genotype : $\chi^2(6) = 3.62$, $P =$
 328 0.73] (**Fig. 2B**). A similar number of eggs, about 5, was laid by females that oviposited on
 329 both leaf disks (**Fig. S3**).



330 **Figure 2. Effect of the insects' genotype and cytotype on *B. tabaci* females' oviposition**
 331 **plant choice of hibiscus vs lantana. (A) Average proportion of eggs laid on lantana [$\pm 95\%$**
 332 **confidence interval (CI)] compared to the total number of eggs laid on the two plants over the**

333 course of a 32h trial. The oviposition choice was investigated from 31 to 37 individuals per
334 cytotype/genotype combinations. Significance: $p < 0.001$: ***, $p > 0.05$: *ns* (Tukey's test). **(B)**
335 Number of eggs laid by females laying eggs exclusively on hibiscus or lantana (which
336 correspond to 95% of the females). Each dot represents one measure for one female; box
337 length represents the interquartile range; whiskers indicate the lowest and largest data points,
338 excluding outliers. Black horizontal bars correspond to the median. To decipher the plant x
339 cytotype interaction, letters above the boxes indicate statistical groups determined by Tukey's
340 multiple comparison test ($P < 0.05$). Measures were performed on individual F1 females
341 obtained from crosses of laboratory lines: AA(Q1-HW), BB(Q1-HR), or CC(Q2-ARW). F1
342 females legend key: Nuclear genotype: letters indicate the maternally (first) and the paternally
343 (second) inherited nuclear genotype; Cytotype: mitochondrial group + S-symbionts (A:
344 *Arsenophonus*, H: *Hamiltonella*, R: *Rickettsia*, W: *Wolbachia*).

345 **Discussion**

346 In the present study, we demonstrate that whitefly cytotype is a major determinant of
347 both females' oviposition rate in no-choice experiments and oviposition behaviour in choice
348 experiments, while we did not find any influence of the insects' genotype on those traits. We
349 acknowledge the possibility that these observations could result from genetic maternal effects,
350 such as the mother transmitting epigenetic states like DNA methylation or supplying eggs
351 with mRNA or proteins (English et al., 2015). The cytotype is composed of several
352 components but differences due to potential genetic and/or gene expression variability of the
353 mitochondria or the P-symbiont between cytotypes seem unlikely due to (i) the extremely low
354 mitochondrial DNA divergence between Q1 and Q2 populations (Vyskočilová et al., 2018),
355 and (ii) the similar genomic features and metabolic capabilities of *Portiera* across the *B.*
356 *tabaci* species complex (Santos-Garcia et al., 2020; Zhu et al., 2019, 2021). In contrast,

357 cytotypes differ in their S-symbiont composition, harbouring either *Hamiltonella* and
358 *Wolbachia* (Q1-HW), *Hamiltonella* and *Rickettsia* (Q1-HR) or *Arsenophonus*, *Rickettsia* and
359 *Wolbachia* (Q2-ARW). Therefore, it is likely that differences in traits and behaviours between
360 cytotypes result from this composition.

361 In this study, we also show that, in no-choice experiments, oviposition is lower on
362 lantana compared to hibiscus. Similarly to what we observed in a previous study, this could be
363 partially explained by the lower amount of the essential amino acids (EAAs) isoleucine,
364 leucine, phenylalanine and valine available in this plant compared to more favourable plants
365 (Benhamou et al., 2021). We also confirm that some cytotypes attenuate this effect: females
366 with cytotypes Q1-HR or Q2-ARW laid more eggs on lantana than females with cytotype Q1-
367 HW, which could be due to the influence of the S-symbionts on their hosts' free amino acid
368 (AA) metabolism (Benhamou et al., 2021; Opatovsky et al., 2018; Rao et al., 2015). We
369 demonstrate here that this effect is dependent on the host plant species since oviposition is
370 homogeneous on hibiscus among cytotypes.

371 In choice experiments, females with cytotype Q1-HW oviposited more frequently on
372 hibiscus than on lantana, while other females did not show plant preference. Behavioural
373 changes associated to symbionts may be attributable to the host's adaptive response to
374 symbiont infection or to the direct modification by the symbiont of the host behaviour. Here,
375 the choice looks adaptive: the oviposition preference of females with cytotype Q1-HW
376 towards hibiscus could be a response of the whitefly, driven by the lack of a cytotype
377 facilitating the utilization of lantana. Conversely, females with cytotypes Q1-HR and Q2-
378 ARW, with no apparent plant preference, may be less selective considering their relatively
379 advantageous cytotype on lantana. Similar observations have been made in the olive fruit fly
380 *B. oleae*, in which experimental manipulation of the gut bacterial consortium resulted in
381 altered oviposition behaviour (Jose et al., 2019). Uninfected *B. oleae* showed less frequent

382 oviposition on fruits, which may benefit the insect by avoiding oviposition on food sources
383 that are difficult to use in the absence of the symbionts. It cannot be completely excluded that
384 these observations are not an adaptive response of the host to S-symbiont infection. Indeed,
385 the fact that half of the individuals of the cytotypes Q1-HR and Q2-ARW made non-optimal
386 choices by choosing lantana could be due to behavioural manipulation (driven by the
387 symbiont benefit), but the presumed benefit for the symbiont remains unknown.

388 The overall effect of the cytotype on whitefly oviposition behaviour may result as the
389 net effect of the entire symbiotic community or may depend on the infection by a single S-
390 symbiont species. Consistent with this second hypothesis, the avoidance/acceptance of lantana
391 for oviposition can be correlated to the absence/presence of the S-symbiont *Rickettsia*, as
392 whiteflies with *Rickettsia*-associated cytotypes (Q1-HR and Q2-ARW) show the same
393 phenotype. Interestingly, *Rickettsia* has been associated to various phenotypic modifications
394 in *B. tabaci*, such as an increase of the fitness in some environments (Cass et al., 2016; Himler
395 et al., 2011), heat tolerance (Brumin et al., 2011) or resistance to entomopathogenic
396 *Pseudomonas syringae* strains (Hendry et al., 2014) (for a review see also Milenovic et al.,
397 2022). Moreover, *Rickettsia* was recently found to increase *B. tabaci* attraction towards
398 tomato plants infected with the Tomato yellow leaf curl virus (Kliot et al., 2019), supporting
399 the hypothesis that this symbiont affects its host behaviour. Alternatively, it cannot be
400 excluded that whiteflies do not have any preference but that the combination of *Hamiltonella*
401 and *Wolbachia* in females with cytotype Q1-HW decreases the preference for lantana.
402 However, less is known about the impact of this particular S-symbionts combination on their
403 hosts.

404 Mechanisms by which *Rickettsia* or any other S-symbiont may influence *B. tabaci*
405 oviposition behaviour are unknown to date. Studies on a broad spectrum of microorganisms
406 suggest that symbionts may influence their host behaviour directly, through the production of

407 a microbial signal (e.g. some EAAs) able to interact with their hosts' neurons or sensory
408 organs or that are precursors of neurotransmitters (Engl & Kaltenpoth, 2018; Shropshire &
409 Bordenstein, 2016). Symbionts could also influence their host behaviour indirectly, through
410 their effects on host physiological functions (e.g. metabolism, immunity, endocrine system
411 and pheromone production) that ultimately influence the host nervous system (Engl &
412 Kaltenpoth, 2018; Lynch & Hsiao, 2019; Shropshire & Bordenstein, 2016). In *D.*
413 *melanogaster* deprived of their gut bacteria, feeding on a diet depleted in EAAs elicits
414 preference for AA-rich food, while this behaviour can be rescued in flies inoculated with gut
415 bacteria (Leitão-Gonçalves et al., 2017). Therefore, it was proposed that flies are able to
416 detect changes in their inner free EAA concentrations, modulating their preference for AA-
417 rich food. It was also proposed that *D. melanogaster* gut bacteria could provide their host with
418 EAAs, thereby modulating the nutritional status and subsequent food preference for this insect
419 (Leitão-Gonçalves et al., 2017). Similarly, S-symbionts may influence *B. tabaci* oviposition
420 behaviour through their impact on their hosts' amino acid metabolism and nutritional status
421 (Benhamou et al., 2021; Opatovsky et al., 2018; Rao et al., 2015; Ren et al., 2020; Y.-B.
422 Wang et al., 2020). Regarding more specifically *Rickettsia*, genomic and experimental studies
423 in *B. tabaci* MEAM1 (Middle-East Asia Minor 1) species suggest that this S-Symbiont could
424 complement the P-Symbiont and directly contribute to the biosynthesis of several EAAs,
425 including lysine (Bao et al., 2021) and the branched-chain amino acids isoleucine, leucine and
426 valine (Opatovsky et al., 2018). According to this hypothesis, the apparent increase
427 production of EAAs previously reported in *Rickettsia*-associated cytotypes may explain why
428 they show no preference between plants with high (i.e. hibiscus) and low (i.e. lantana)
429 amounts of these specific EAAs (Benhamou et al., 2021). Conversely, the lower level of
430 EAAs production in Q1-HW females may elicit preference for hibiscus. However, the

431 mechanisms by which insects could detect changes in internal free EAAs availability and
432 subsequent changes in behaviour are not known.

433 In conclusion, this work shows that the host plant choice of *B. tabaci* for oviposition is
434 determined by the insect cytotype, most likely by the S-symbiont community. Whether these
435 effects depend on single or multiple S-symbiont species is unknown to date, and further
436 studies should investigate the respective contribution of each S-symbiont, including *Rickettsia*
437 as promising candidate, to host behaviour. This will require separation of S-symbionts from
438 other components of the cytotype, *i.e.*, selective elimination or transfer of symbionts to
439 whiteflies of identical genetic background. In this perspective, recent studies have made
440 encouraging advances in manipulating *B. tabaci* symbiotic communities by selectively
441 eliminating S-symbionts using heat (Shan et al., 2019) or antibiotic treatments (Ren et al.,
442 2020; Shan et al., 2019; Y.-B. Wang et al., 2020). Mechanisms by which S-symbionts mediate
443 the insect behaviour also remain to be determined. Future investigations will help in
444 dissecting the molecular bases of whitefly behaviour to accurately determine the influence of
445 S-symbionts during each host plant selection step (*e.g.* olfaction or probing). Importantly,
446 acting on their hosts' niche choice, S-symbionts could contribute to build-up reproductive
447 isolation between whitefly populations. However, in our framework, reproductive isolation is
448 not complete, as the choice of hibiscus is common to all cytotypes. Future work should
449 explore additional plant combinations to determine whether S-symbionts can lead to complete
450 reproductive isolation through differential host plant choice. Such directions would help to
451 better encompass the influence of symbionts on insects' ecological diversification and
452 speciation.

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454 Declarations of interest: none

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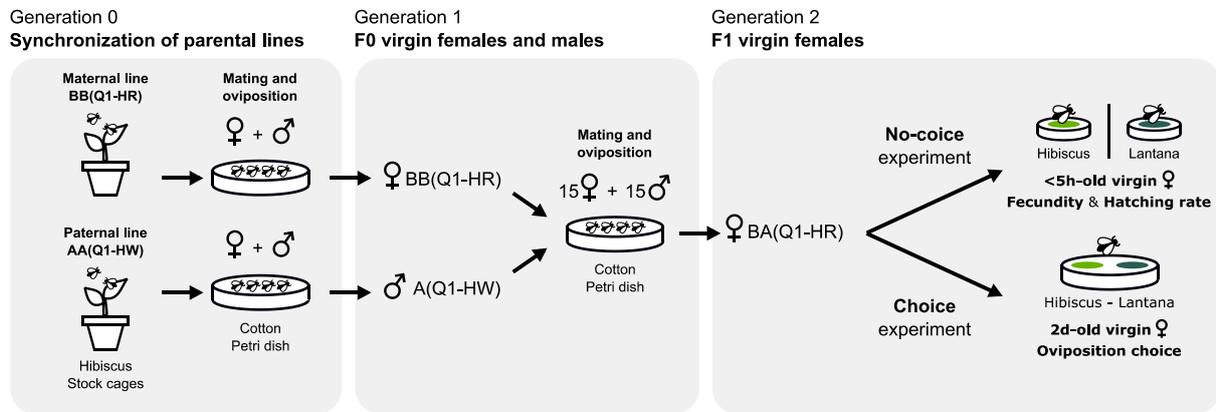
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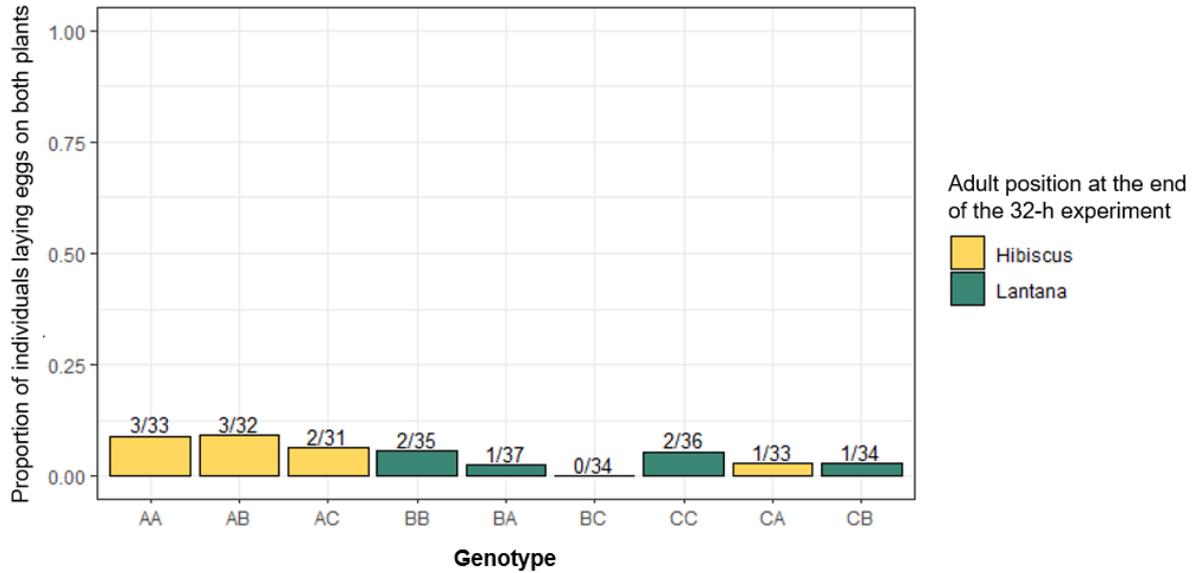
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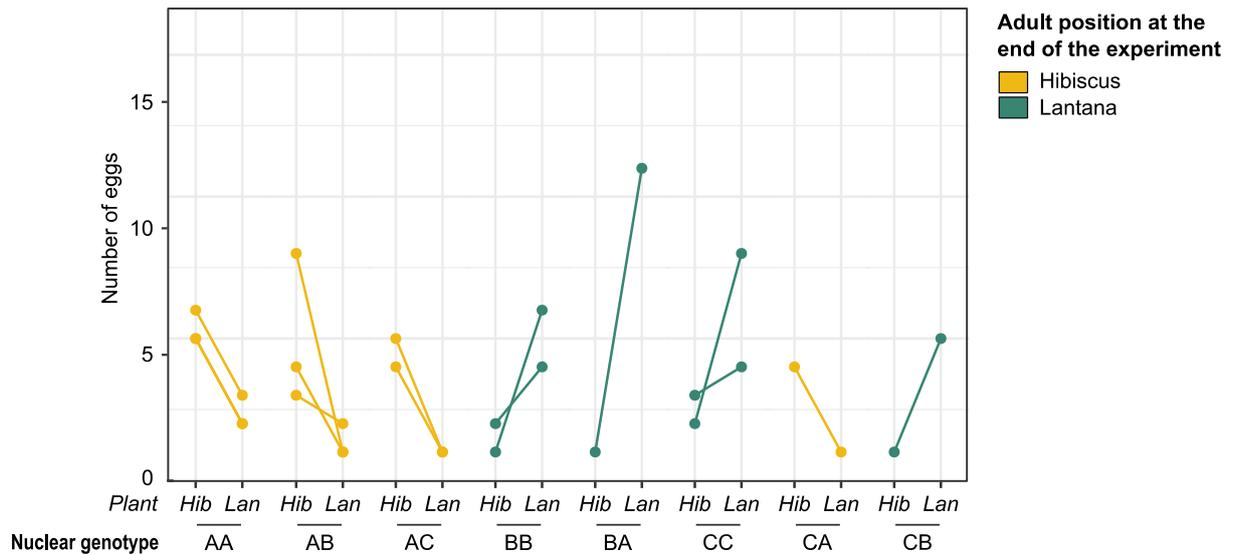
748 **Supplementary material**



749 **Figure S1. Experimental procedure used for no-choice and choice experiments on F1**
 750 **females**, using BA(Q1-HR) F1 females as an example. All experiments were performed on
 751 individual F1 females. Oviposition choice was assessed by counting the number of eggs laid
 752 per female after 32 h on each plant (n=31-27). Fecundity (number of eggs laid) was assessed
 753 over 7 days per F1 female after which the female was removed from the Petri dish. The
 754 hatching rate was calculated as the ratio of larvae (observed 10 days after removing the
 755 female), over the total number of eggs laid per F1 female (see formula (a) from main text)
 756 (n=13-17).



757 **Figure S2. Proportion of *B. tabaci* females laying eggs on both hibiscus and lantana**
 758 **according to their genotype in choice experiments.** Most of females laid eggs on a single
 759 plant species. Colors indicate the female's position at the end of the experiment. Numbers
 760 above bars indicate absolute values (number of females laying eggs on both plants / total
 761 number of females analyzed).



762 **Figure S3. Number of eggs laid by whiteflies ovipositing on both plants in hibiscus vs**
 763 **lantana plant choice for oviposition experiments, according to their nuclear genotype.**
 764 There was no data for genotype BC, as no BC(Q1-HR) female oviposited on both plants. Each
 765 dot pair corresponds to an individual females' oviposition on hibiscus (Hib) and lantana (Lan).
 766 Colors indicate the female's position at the end of the experiment.