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5

6 **Response of life history traits to artificial and natural selection for virulence and non-virulence in a**

7 ***Drosophila* parasitoid, *Asobara tabida***

8

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11

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12

13 **Abstract.**

14 Co-evolution of host-parasitoid interactions is determined by the costs of host resistance, which
15 received empirical evidence, and the costs of parasitoid virulence, which have been mostly
16 hypothesized. *Asobara tabida* is a parasitoid which mainly parasitizes *Drosophila melanogaster* and

17 *D. subobscura*, the first species being able to resist to the parasitoid development while the second
18 species is not. To parasitize resistant hosts, including *D. melanogaster*, *A. tabida* develops sticky eggs
19 which prevent encapsulation, but this virulence mechanism may be costly. Inter-individual and inter-
20 population variation in the proportion of sticky eggs respectively allowed us to (i) artificially select
21 and compare life history traits of a virulent and a non-virulent laboratory strain, and (ii) compare a
22 virulent and a non-virulent field strain, to investigate the hypothetical costs of virulence. We
23 observed strong differences between the two laboratory strains. The non-virulent strain invested
24 fewer resources in reproduction and walked less than the virulent one but lived longer. Concerning
25 the field strains, we observed that the non-virulent strain had larger wings while the virulent one
26 walked more and faster. All together, our results suggest that virulence may not always be costly,
27 but rather that different life histories associated with different levels of virulence may coexist at
28 both intra- and inter-population levels.

29

30 **Key words:** encapsulation; geographic variation; host resistance; trade-offs

31

32

33 INTRODUCTION

34

35 Interspecific relationships are major drivers of evolution (van Valen 1973, Janzen 1980), especially in
36 host-parasite interactions where the fitness of each partner is strongly linked to the fitness of the
37 other one (Lambrechts *et al.*, 2006). Many numerical models have investigated how host resistance
38 and parasite virulence may co-evolve (e.g. May & Anderson 1983; van Baalen 1998; Gilchrist &
39 Sasaki, 2002) and empirical studies have brought evidence of such co-evolution events (for review,

40 see Ebert, 2008). In host-parasitoid systems, the interdependency of host and parasite fitness is
41 singular since generally only one of the two partners will survive the interaction (Godfray, 1994).
42 Several factors have been reported to influence the outcome of host attack by a parasitoid, such as
43 host endosymbiont fauna (Gwynn *et al.*, 2005), host nutritional status (Maure *et al.*, 2016), or
44 parasitoid clutch size and sequential oviposition (Kapranas *et al.*, 2012). Sasaki and Godfray (1999)
45 proposed a convincing model predicting issues to the co-evolution of host resistance - i.e. the host's
46 ability to survive the infestation by a parasitoid, and parasitoid virulence, referring in our paper to
47 the parasite's ability to overcome its host's defenses. As for most generic host-parasite systems (e.g.
48 May & Anderson, 1983; Gilchrist & Sasaki, 2002), the outcomes of this model mainly depend on the
49 costs associated with host resistance and parasitoid virulence. For the host, the Sasaki and Godfray
50 (1999) model considers both the costs incurred by the maintenance of the machinery to resist a
51 parasitoid's attack and those incurred by the allocation of resources to the parasite destruction. For
52 the parasitoid, this model assumes a decreased probability of surviving pupation caused by an
53 investment in increased virulence.

54 In order to understand how hosts and parasitoids coevolve, it is important to experimentally
55 investigate possible trade-offs between hosts' resistance, or parasitoids' virulence, and other
56 components of the fitness of each partner (Stearns, 1992). Most known studies are based on
57 artificial selection of resistant hosts, and genomic changes associated with such selection have been
58 identified in *Drosophila melanogaster* (Jalvingh *et al.*, 2014). For instance, Fellowes *et al.* (1999) and
59 Green *et al.* (2000) reported that larvae of *D. melanogaster* lines that had been artificially selected
60 for higher resistance to parasitoid attacks fed more slowly than larvae of control lines, resulting in a
61 decreased competitive ability. Kraaijeveld *et al.* (2001a) later investigated the basis of this trade-off
62 and proposed that *D. melanogaster* larval resources were switched from feeding rate to defensive
63 functions as indicated by an increased haemocytes density in the resistant lines. Conversely to

64 Fellowes *et al.* (1999), Sanders *et al.* (2005) observed an increased resistance in *D. melanogaster*
65 lines selected on the basis of their high feeding competitive ability. More recently, Takigahira *et al.*
66 (2014) selected lines of *D. bipectinata* for their resistance against the parasitoid *Leptopilina victoricae*
67 and observed a reduced longevity in resistant females than in control ones but a longer longevity in
68 males, suggesting different trade-offs depending on gender. To our knowledge, only one study
69 investigated the costs of selection for virulence in parasitoids: Kraaijeveld *et al.* (2001b) compared
70 life history traits of control lines of the solitary koinobiont *Drosophila* endoparasitoid *Asobara tabida*
71 Nees (Hymenoptera: Braconidae) with those of lines artificially selected to be virulent towards *D.*
72 *melanogaster*. No difference was observed between lines except a longer duration of the embryonic
73 development in the selected ones, which may be explained by a reduced nutrient and/or oxygen
74 supply due to the embedment of the egg in the host tissues. The authors hypothesized that this
75 delay of development may reduce larval survival if superparasitism or multiparasitism occurs and
76 could thus be considered as a cost associated with enhanced virulence.

77 In natural systems, parasitoids may avoid hypothetical costs of virulence by attacking preferentially
78 the less resistant host species (Kraaijeveld *et al.*, 1995). Conversely, they may invest resources in
79 increasing virulence when resistant hosts are commonly present (Godfray, 1994). The parasitoid
80 *Asobara tabida* is widespread in Europe and North America (Kraaijeveld & van der Wel, 1994) and
81 may also be present in Asia (Nomano *et al.*, 2015). In this species, Kraaijeveld and van Alphen (1994)
82 reported a cline of low to high virulence from the North to the South of Europe and explained this
83 geographical variation by the composition of the hosts' community. Indeed, *Drosophila subobscura*,
84 which is unable to resist parasitoid development, is the most abundant host species in Northern and
85 Central Europe while *D. melanogaster*, for which an effective immune response against parasitoid
86 eggs is commonly observed, dominates in the Mediterranean Basin (Kraaijeveld & Godfray, 1999).
87 Therefore, it is expected that *A. tabida* parasitoids must invest in high virulence in the South where

88 they mainly attack *D. melanogaster* resistant hosts, while they can select (Kraaijeveld *et al.*, 1995)
89 and successfully parasitize the predominant non-resistant host species, *D. subobscura*, in northern
90 Europe. If virulence is costly as it is often hypothesized, such a difference in need for virulence may
91 lead to selection of different life histories between natural populations, as would do other ecological
92 factors such as climate (Moiroux *et al.* 2010), host distribution (Moiroux *et al.*, 2013) or competition
93 (Vayssade *et al.*, 2012; Vuarin *et al.*, 2012).

94 This study aims at evaluating the possible costs incurred by the selection of virulence on life history
95 traits in the *Drosophila* parasitoid *Asobara tabida*. We compared strains which differed in their
96 ability to overcome hosts' resistance using strains artificially selected in the laboratory and field
97 strains. Encapsulation represents the chief immune response of insect larvae against parasitoids. In
98 *Drosophila*, an endophagous parasitoid egg or larva is recognised as a foreign body by the
99 plasmatocytes, causing aggregation of other haemocytes, the lamellocytes, giving rise to a cellular
100 capsule surrounding the parasite. A cascade of biochemical reactions leads to the melanisation and
101 hardening of the capsule, resulting in the parasitoid death by suffocation or because of the release
102 of cytotoxic radicals (Nappi *et al.*, 1995, for a review see Eslin *et al.*, 2009). This ability to overcome
103 parasitoid development is associated with the ratio of the different haemocyte types circulating in
104 host haemolymph (Gerritsma *et al.*, 2013). In response to this immune defense of insect hosts,
105 parasitoids have evolved several passive and active strategies to avoid encapsulation such as a rapid
106 development, egg deposition into host's tissues where they are protected from the attack by the
107 haemocytes, molecular mimicry, or destruction of the host's immune system using toxins or
108 symbiotic viruses (for reviews, see Vinson, 1990; Strand & Pech, 1995). In *Asobara tabida*, the main
109 mechanism to prevent encapsulation is the concealment of the egg by adhesion to host tissues,
110 which results from the development of a fibrous egg exochorion (Eslin *et al.*, 1996). *A. tabida*
111 females lay sticky eggs that become embedded within the host's tissues therefore being hidden from

112 the circulating haemocytes. Embedded eggs are thus protected from encapsulation and can
113 successfully pursue their development (Prévost *et al.*, 2009).

114 In *A. tabida*, genetic variation exists in the percentage of adhesive (versus non-adhesive) eggs laid by
115 females and thus, in the so designated parasitoid virulence towards *Drosophila* hosts (Kraaijeveld &
116 Godfray, 2009). This inter-individual variation allowed us to artificially select in the laboratory two
117 strains of *A. tabida*, a virulent and a non-virulent one towards the host *D. melanogaster*. The life
118 history traits of the females were compared between the two selected lines and also with the
119 original laboratory strain used for selection. In nature, evolution of life history traits induced by
120 selection for virulence may be influenced by other selection pressures, especially ecological factors.
121 To test if the same differences were observed in the lab and in nature between virulent and non-
122 virulent populations, we also compared life history traits of females originating from naturally
123 virulent and non-virulent populations. A wide range of life history traits associated with various
124 functions, i.e., reproduction, maintenance and locomotion, were measured in these five strains in
125 order to provide a comprehensive overview of the relationship and possible trade-offs between
126 parasitoid virulence and life history traits, considering the hypothesis of a lower performance of
127 these traits in virulent strains compared to non-virulent ones. Implication of ecological factors,
128 especially climate and composition of parasitoid community, in the evolution of traits associated
129 with virulence is discussed.

130

131 **MATERIAL AND METHODS**

132 *STRAINS ORIGIN AND REARING.*

133 **Hosts** The strains of *Drosophila subobscura* Collin and *D. melanogaster* Meigen (Diptera:
134 Drosophilidae) used in our experiments were established from individuals sampled respectively in
135 Amiens (49°53'N, 2°17'E) in 1995 and Lyon (45°44'N, 4°48'E) in 1994, and were reared on fermented
136 diet (yeast 6%, cornflour 39 %, sugar 47%, agar 7.8% and nipagine 0.2%) at 20±1°C, LD 16:8, RH:
137 66%..

138
139 **Parasitoids** The laboratory *Asobara tabida* strain (later called “original lab strain”) used for
140 selection of virulent and non-virulent strains was established from individuals sampled in Saanich
141 (48°29'N, 123°23'W), British Columbia, Canada.

142 The so-called “field strains” used in our study were provided by Roland Allemand and
143 Patricia Gibert from the Université Claude Bernard Lyon 1 (France). Populations were sampled in
144 September 2010 along the Rhône-Saône Valley, South-eastern France, in two localities located 90
145 km apart: Igé in the north (46°22'N, 4°44'E), and Villette in the south (45°35'N, 4°54'E). Samplings
146 were carried out using banana bait traps placed in orchards, as described in Delava *et al.* (2014).
147 Eggs from the northern population failed to develop in our strains of *D. melanogaster* – this
148 parasitoid strain is thus later called “non-virulent field strain” - while development in this host
149 species occurred for the southern strain, later called “virulent field strain” (Fig. 1). The main
150 difference in ecological factors between these two populations is the presence in the southern
151 population and the absence in the northern population of a competitor species, *Leptopilina boulardi*
152 (Delava *et al.*, 2014) which is known to influence evolution of life history traits of *Asobara tabida*
153 (Vayssade *et al.*, 2012)

154 All parasitoid strains were reared on *D. subobscura* larvae placed into rearing tubes with
155 fermented diet at 20±1°C, LD 16:8, RH: 66%.

156

157 *Artificial selection of virulent and non-virulent strains of Asobara tabida*

158 Two strains were selected from the original lab strain for their virulence or non-virulence at
159 the end of 2009. The virulent strain, later called “virulent lab strain”, was established first by rearing
160 parasitoids on a resistant strain of *Drosophila melanogaster*, as did Kraaijeveld *et al.* (2001), during
161 six generations and secondly by selecting females from this generation which laid 100% of eggs
162 found embedded in host tissues (sticky eggs). The non-virulent strain, later called “non-virulent lab
163 strain”, was established by selecting females laying no adhesive eggs. First, we selected females
164 from the original lab strain which had laid a high percentage of eggs found floating free in the
165 hemolymph of *D. melanogaster* larvae. After rearing their descendants for seven generations on
166 *D. subobscura*, females from this seventh generation which laid 100% of non-sticky eggs floating free
167 in *D. melanogaster* hemolymph were selected to initiate the non-virulent strain.

168

169 *Virulence of lab and field A. Tabida strains*

170 Prior to measurement of life history traits, the proportion of sticky eggs laid by females from
171 the three lab strains and the two field ones was calculated. Eight *A. tabida* females per lab strain and
172 six females per field strain were placed separately with twenty second instar *D. subobscura* larvae
173 and their behaviour was observed under a microscope. When oviposition occurred, attacked larvae
174 were transferred into a tube containing fermented medium and placed in rearing conditions for 24
175 h. Larvae were then washed into three successive PBS baths and dissected under a microscope to
176 determine if the parasitoid egg was adhesive to host tissues or free in host haemocoel. The
177 percentage of sticky eggs per strain was then calculated.

178 The emergence rates of the five parasitoid strains were calculated after development in a
179 resistant strain of *D. melanogaster*. Eight females per strain were allowed to parasitize second instar
180 *D. melanogaster* larvae and their behaviour was observed until twenty hosts had been attacked.
181 Those larvae were removed and placed at 20°C until emergence of either a *Drosophila* or a parasitic
182 wasp. The number of emerged *A. tabida* wasps and *Drosophila* flies allowed us to calculate the
183 parasitoid emergence rate.

184

185 *Life history traits*

186

187 A set of *A. tabida* life-history traits linked to morphology, maintenance, reproduction and
188 locomotion were measured. Tibia length, fecundity, egg volume, starvation resistance and wing
189 area, were measured in females from the original lab strain, the two selected strains and the two
190 field strains. Walking activity and metabolic rate were measured in females from the two selected
191 strains and the two field strains. These experiments were performed a year after obtaining the
192 selected lab strains, i.e. 15 *A. tabida* generations, and six months after sampling the two field strains,
193 i.e. 7 *A. tabida* generations.

194

195 In order to obtain the adult parasitoid females used for measurements of life-history traits,
196 *Drosophila subobscura* females were allowed to lay eggs on a 1 mm thick layer of fermented
197 medium spread on a microscope slide in a rearing tube for 4h at 20 °C. The slide was then
198 transferred to another tube and maintained in rearing conditions for 48 h, i.e. until *Drosophila* eggs
199 had hatched and larvae had reached the second instar. The slide with the larvae was then placed in a
200 Petri dish (9 mm diameter) in the presence of a five days-old mated *Asobara tabida* female for 8
hours. Parasitized *Drosophila* larvae were then transferred to a tube containing fermented medium

201 and maintained in rearing conditions until parasitoid emergence. Female parasitoid wasps were used
202 for the experiments described below.

203

204 **Reproductive traits** *Asobara tabida* is a synovigenic species, i.e. females emerge with a set of
205 mature eggs and mature additional eggs during adult life. Thus, we measured initial egg load just
206 after emergence as a proxy of resources invested in reproduction during development, and egg load
207 at 5 days as an estimate of total fecundity. For each strain, thirty virgin females aged of less than one
208 hour were placed at -80 °C while 30 other females were isolated in a tube at 20°C and provided *ad*
209 *libitum* with honey and water before being frozen 5 days later. Females were then dissected in a
210 drop of PBS solution under a binocular (×40, Leica M165C) linked to a camera (Leica DFC290).
211 Ovaries were both removed and the number of mature eggs was counted using the software
212 Photoshop CS5. After counting, eggs were photographed and the length (L) and width (w) of ten
213 eggs per ovary were measured with the analysis software ImageJ. Volume of eggs was then
214 calculated as the volume of two identical cones linked by their bases: $V = 1/12\pi Lw^2$ (Vayssade *et al.*,
215 2012). The left hind tibia was also photographed and measured using the analysis software ImageJ to
216 be used as a proxy for body size.

217

218 **Starvation resistance and wing area.** We measured females longevity under starvation, but
219 with access to water, to estimate the amount of resources extracted from the host and available for
220 adult life. At emergence, 30 females per strain were placed individually in small tubes containing a
221 moist cotton ball and maintained under rearing conditions. Mortality was checked twice daily (at
222 9:00 am and 7:00 pm) and dead individuals were frozen at -80°C. Individuals were then
223 photographed as described above and the length of their left hind tibia and the area of their left

224 wing were measured using the analysis software ImageJ. Left wing area was later used to evaluate
225 flight capacity, considering tibia length as a covariate (Gilchrist & Huey, 2004).

226

227 **Walking activity.** The behavioural data acquisition and analysis software Ethovision (Noldus
228 Information Technology, 1997) was used to record and analyse walking activity, which can be
229 associated with both locomotion and foraging in parasitoids (Suverkropp *et al.*, 2001). This activity
230 was measured on 30 females per strain. Virgin females were individually placed at emergence in
231 small tubes and fed with honey diluted in water for 24h. Wasps were then placed separately in Petri
232 dishes (diameter 4 cm) on a white paper sheet 30 min before the start of a recording, which started
233 at 10:00 am. For each run, twelve females belonging to the four strains were simultaneously filmed
234 (camera: Panasonic CCTV) for 10 hours, in a windowless room under artificial white light. The
235 contrast between individuals and the white background used to detect females and their position
236 was automatically recorded 12 times per minute. The total distance covered and the average
237 walking speed were automatically calculated from the track records by the software Ethovision.
238 After recording, females were individually placed back in their tube and provided with honey. Their
239 metabolic rate was measured the next day.

240

241 **Metabolic rate.** Metabolic rate is a central physiological trait used to understand trade-offs
242 between life history traits since it determines the rates of resources acquisition and allocation
243 between traits (Brown *et al.*, 2004). Routine metabolic trait – that corresponds to basal metabolic
244 rate plus some contribution from activity – was measured at 20°C using flow-through respirometry
245 described in Le Lann *et al.* (2014). Females were individually placed in small cylindrical chambers for
246 six hours in the dark during the photophase of their rearing conditions (10:00 am–04:00 pm). The air,

247 drawn off from the environment, entered the chambers after passing through Drierite and Ascarite
248 used to remove water and CO₂. A Sierra mass flow controller maintained constant flow rates of dry
249 CO₂-free air. CO₂ production was measured at the exit of chambers with an infrared CO₂ analyzer
250 (CA-10A Carbon Dioxide Analyser; Sable Systems International). Four recordings of 5 min each were
251 performed per individual and, because only one tube could be measured at a time, there was a lag
252 of 80 min (5 min per tube) between two successive recordings. One tube was left empty as a control
253 and the value measured in this control chamber was subtracted to the values measured in chambers
254 with wasps. The first recording allowed individuals to get used to their new environment and was
255 not considered for analysis. We considered the average of the last three recordings as a measure of
256 metabolic rate for each sample. All individuals were placed at -80°C after this protocol. Females
257 were then weighted with a microbalance (Metler Toledo XP2U; ±0.0001 mg) to correct metabolic
258 rate for body mass and the length of their hind tibia and the area of their wings were measured as
259 described above.

260

261 *Statistical analyses*

262 We compared the proportions of sticky eggs laid by female wasps and wasp emergence rates
263 using a GLM with strains as fixed factor and considering a binomial distribution for these data.

264 Two-ways ANOVAs were performed to compare tibia length and wing area between strains
265 using “origin” (i.e. lab versus field) and “virulence status” (i.e. virulent versus non-virulent versus
266 intermediate for the original lab strain) as factors. Moreover, we used an ANCOVA to compare wing
267 area between strains with tibia length as a covariate to test for differences in allometry. Initial egg
268 load and egg load at 5 five days were compared between strains using a GLM, with “origin” and
269 “virulence status” as fixed factors and tibia length as a covariate, considering a Poisson distribution

270 for these data. Differences in egg volume were tested using an ANCOVA with tibia length as a
271 covariate.

272 We used the survival package provided by R software to test for differences in longevity
273 under starvation between the five strains, using a Weibull distribution.

274 In insects, metabolic rate (Brown *et al.*, 2004) and walking speed (Hurlbert *et al.*, 2008) are known to
275 increase with body size so we performed ANCOVA with respectively fresh body mass and tibia length
276 as covariates to compare these traits between strains.

277 All the statistical analyses were carried out using R software version 3.2.1 (R Development
278 Core Team, 2008). Post-hoc tests were performed after ANCOVA and GLM using the multcomp
279 package provided by R software.

280

281 RESULTS

282 *Strain selection and virulence estimation*

283

284 We recorded significant differences between strains in the percentage of sticky eggs laid by
285 *A. tabida* females in *D. subobscura* larvae (GLM, $z = 6.572$, $P < 0.001$) and in the emergence rate of
286 parasitoids developing in *D. melanogaster* (GLM, $z = 5.053$, $P < 0.001$). Both lab and field virulent
287 strains laid a higher proportion of sticky eggs and showed a higher emergence rate than non-virulent
288 ones. The original lab strain had an intermediate virulence status compared to the virulent and non-
289 virulent lab strains (Fig. 1).

290

292 **Morphological trait** There was no significant difference in tibia length between strains
293 (ANOVA, $F = 0.074$, $P = 0.23$) (Table1).

294
295 **Reproductive traits** We observed a significant interaction between origin (i.e. lab versus
296 field) and virulence status (i.e. virulent versus non-virulent versus intermediate) for initial egg load
297 (GLM, $z = 5.178$, $P < 0.001$). The non-virulent lab strain had fewer eggs than the virulent lab strain
298 and also than both the field strains. The initial number of eggs in females from the original lab strain
299 was highly variable and not significantly different from that of the other strains (Fig. 2). The same
300 pattern was observed for egg load at 5 days (GLM, $z = 3.453$, $P < 0.001$) (Table 1).

301 The interaction between origin and virulence status had a significant effect on the egg
302 volume at emergence (ANCOVA, $F = 0.207$, $P < 0.05$), the non-virulent lab strain laying smaller eggs
303 than the other strains, which laid eggs of similar same sizes (ANCOVA, $F = 0.017$, $P = 0.564$). The
304 same pattern was observed for 5-days old females (ANCOVA, $F = 0.318$, $P < 0.05$) (Table 1).

305
306 **Maintenance traits** There was a significant interaction between origin and virulence status
307 for starvation resistance (Survival analysis, $z = 63.29$, $P < 0.001$). When deprived of food, only the
308 non-virulent lab strain survived longer than the other strains, (Fig. 3).

309 Metabolic rate was significantly influenced by origin (ANCOVA, $F = 5.370$, $P < 0.05$) but not
310 by virulence status (ANCOVA, $F = 0.012$, $P = 0.312$). The field strains had a higher metabolic rate than
311 the lab strains (Table 1).

312

313 *Locomotion associated traits*

314 Virulence status had a significant effect on wing area, females from the virulent lab and field
315 strains having smaller wings than females from the non-virulent strains (ANOVA, $F = 12.422$, $P <$
316 0.001). The original lab strain did not differ from the other strains (Tukey test, $P > 0.17$) (Fig. 4).
317 When corrected for body size, the difference between field strains was still observed (ANCOVA, $F =$
318 3.071 , $P < 0.05$) but it disappeared between lab strains (ANCOVA, $F = 0.073$, $P = 0.31$).

319 We observed a significant effect of origin (ANCOVA, $f = 6.033$, $P = 0.014$), virulence status
320 (ANCOVA, $f = 3.221$, $P < 0.05$) and of the interaction between these two factors (ANCOVA, $f = 4.172$,
321 $P < 0.05$) for the total distance walked during recording. Origin (ANCOVA, $f = 4.993$, $P < 0.05$),
322 virulence status (ANCOVA, $f = 2.780$, $P < 0.05$) and the interaction between these two factors also
323 significantly influenced walking speed (ANCOVA, $f = 3.284$, $P < 0.05$). These two traits were
324 significantly higher in the field strains than in the lab strains and virulent females walked more and
325 faster than non-virulent females (Fig. 5).

326

327

DISCUSSION

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We investigated effects of virulence selection on several life history traits in the *Drosophila* parasitoid *Asobara tabida* by comparing virulent and non-virulent lab and field strains. Strong differences were observed between selected lab strains (i.e. the non-virulent strain had fewer and smaller eggs than the virulent strain but female wasps lived longer) whereas only differences in traits related to locomotion were observed between field strains (i.e. the non-virulent strain had larger wings but walked less and more slowly than the virulent strain). This pilot study provides new insights on the evolution of life history traits in response to selection for virulence in parasitoids.

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Virulence mechanisms In *Asobara tabida*, virulence is generally associated with oviposition of sticky eggs which adhere to host tissues and are thus protected from encapsulation (Kraaijeveld & van Alphen, 1994; Eslin *et al.*, 1996). In our experiment, selection for a high (99%) and low proportion (0%) of sticky eggs laid by parasitoid females resulted respectively in a developmental success of 78% and 0% in *D. melanogaster* larvae. Moreover, the field strain which laid the highest proportion of sticky eggs (89% versus 15%) developed more frequently in *D. melanogaster* (41% versus 1%). These results support previous evidence of the major role of the adhesive eggs in the counter-defense strategy exhibited by *Asobara tabida* (Eslin & Prévost, 2000).

345

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348

Laboratory strains Using a standard selection protocol, we were able to rapidly select, respectively in six and seven generations, a virulent strain and a non-virulent strain of *Asobara tabida*. This artificial selection was rather fast and strong in comparison with the study of Kraaijeveld *et al.* (2001b) who observed an increase from 8% to 37% of sticky eggs over 10 generations when

349 selecting *Asobara tabida* for virulence on *D. melanogaster*. This difference is probably the
350 consequence of the high degree of variation in the percentage of sticky eggs laid by females
351 observed in our original lab strain (from 25% to 100%) which favoured a rapid selection.

352 This artificial selection resulted in strong differences between virulent and non-virulent
353 strains. We observed increased egg load and egg volume in the virulent strain compared to the non-
354 virulent strain, but the latter lived on average 45% longer. These results contrast with the study of
355 Kraaijeveld *et al.* (2001b) who did not find any difference in egg load or in other life history traits
356 between virulent and non-virulent strains of *A. tabida*, except for the egg stage that was on average
357 2.5 h longer in virulent strains.

358 Longevity and metabolic rate are generally negatively correlated, as described in the “rate of living”
359 theory (Brown *et al.*, 2004), but this pattern could not explain the longer lifespan under starvation in
360 the non-virulent strain since there was no difference for CO₂ production between lab strains. Thus,
361 the better starvation resistance of non-virulent parasitoids probably results from a higher
362 investment of resources in this trait, which was paid back by a lower investment in reproduction.

363 Our results do not clearly demonstrate the existence of costs incurred by the selection of virulence
364 since virulent females invested more resources in reproduction than non-virulent ones. It is rather
365 likely that two alternative strategies associated with different virulence status exist, the non-virulent
366 females investing more in longevity and starvation resistance, and the virulent females investing
367 more in reproduction. The classical trade-off observed between longevity and reproduction (Roff,
368 1992; Ellers *et al.*, 2000) would thus explain differences between our lab strains. In nature, non-
369 virulent females probably encounter less oviposition opportunities than virulent females because
370 they are unable to successfully parasitize resistant host species. Increased longevity and starvation
371 resistance may thus be adaptive for these females since they need more time to find non-resistant

372 hosts (Jervis *et al.*, 2008). On the other hand, virulent females are able to successfully parasitize
373 more hosts and thus encounter more oviposition opportunities. An adaptive strategy would be to
374 invest resources in reproductive traits, as predicted by the model of Jervis *et al.* (2008). Parasitoids
375 would thus limit hypothetical costs of virulence by adapting their life histories to their ability to
376 parasitize either few or many hosts.

377

378 **Field strains** We observed a geographic variation in virulence over 90 km in South-eastern
379 France, the southern population being more virulent than the northern population. This result is
380 consistent with the study by Kraaijeveld and van Alphen (1994) who observed such a variation at a
381 continental scale. These authors explained this pattern by the predominance of the resistant host
382 species *D. melanogaster* in southern Europe and the abundance of the non-resistant host species *D.*
383 *subobscura* in northern Europe. Such a difference in *Drosophila* community composition was also
384 observed between our two populations, *D. subobscura* being only present in the northern sampling
385 site (E. Delava, personal communication). The geographical limit between virulence and non-
386 virulence may thus be located between these two sites but may evolve in the next decades since the
387 composition of *Drosophila* and parasitoid communities has been changing rapidly with global
388 warming in this area (Vuarin *et al.*, 2012; Delava *et al.*, 2014).

389 Females from the non-virulent strain had larger wings, proportionally to their body size, but
390 walked more slowly and less than the virulent strain, while the two strains were similar for other life
391 history traits. Climate is known to affect wing size in insects (e.g. Imasheva *et al.*, 1994; Gilchrist *et*
392 *al.*, 2001). Huey *et al.* (2000) observed for example an increase in wing length with latitude in
393 *Drosophila subobscura*. Gilchrist and Huey (2004) proposed that larger wings may compensate for
394 the lower wingbeat frequency occurring in cold climate. In our study, it is unlikely that climate may

395 explain variation in wing length since there is only a slight difference in temperature between the
396 two field strains sampling sites (the difference in annual mean temperature for the 2005 – 2010
397 period is of 0.9 °C: MétéoFrance data), while observations of latitudinal variations in wing length
398 have been made over large distances at a continental scale with strong differences in temperature
399 (e.g. Gilchrist *et al.*, 2001).

400 Alternatively to the climate, larger wings may have been selected in response to relative abundance
401 of the different *Drosophila* host species. Virulent parasitoid females are able to rapidly find
402 *D. melanogaster*, the most abundant host species for *A. tabida* in orchards of the Rhône-Saône
403 valley (Delava *et al.*, 2014) while non-virulent females probably have to fly over larger distances to
404 find the alternative and rare host species, *D. subobscura*. This last host is preferentially attacked
405 when the two *Drosophila* larvae species are proposed to parasitoid females originating from non-
406 virulent populations (Kraaijeveld *et al.*, 1995). Investing resources in better flying ability may thus be
407 adaptive for non-virulent parasitoid females. On the opposite, virulent females which can attack *D.*
408 *melanogaster* are able to rapidly find an important quantity of hosts over small distances in orchards
409 (Moiroux *et al.*, 2013; Delava *et al.*, 2014). They may thus walk more frequently rather than fly, a
410 costly mode of locomotion (Nation, 2008), as suggested by the significant differences observed
411 between natural strains in walking speed and total distance covered during our experiment, and also
412 by differences between virulent and non-virulent artificial strains. Therefore, our results on
413 locomotion associated traits do not support the hypothetical existence of virulence costs but rather
414 suggest that virulent and non-virulent populations may adopt different foraging strategies.

415 Contrarily to the lab strains, we did not observe significant differences for reproductive traits and
416 starvation resistance between virulent and non-virulent field strains. This absence of difference may
417 be the consequence of ecological factors which would counterbalance selection for longer longevity

418 and starvation resistance in non-virulent populations and for higher fecundity in non-virulent
419 populations. Our field populations differed in virulence but were also exposed to different
420 competitor species, the southern virulent population competing for hosts with an invasive
421 *Drosophila* parasitoid, *Leptopilina boulardi*, but not the northern non-virulent population. Presence
422 of this competitor is known to induce selection for higher fecundity in *Asobara tabida* (Vayssade *et*
423 *al.*, 2012). It is thus unlikely that the composition of parasitoid community may counterbalance
424 evolution of reproductive traits in response to selection for virulence since these two factors would
425 induce selection for a higher fecundity in the southern population. Other ecological factors than
426 climate or composition of parasitoids community may thus explain the absence of differences
427 between our virulent and non-virulent field strains. Differences in host distribution which is a major
428 selection pressure for parasitoids (Moiroux *et al.*, 2013), may for example counterbalance influence
429 of virulence selection on life history traits.

430

431 **Conclusion**

432

433 Sasaki and Godfray (1999) developed a model to predict co-evolution of host resistance and
434 parasitoid virulence which mainly depends on costs of these two traits. If costs of host resistance
435 have been clearly documented (Kraaijeveld & Godfray, 2009), costs of virulence have been mainly
436 hypothesized (Kraaijeveld *et al.*, 2001b). Our pilot study add evidence that virulence may not be so
437 costly in parasitoids, but rather suggests that different strategies associated with different levels of
438 virulence may co-exist at inter- and intra-population levels. More strains should however be
439 investigated to confirm this hypothesis. If costs of virulence were not observed in a parasitoid using
440 a passive virulence mechanism, they may however occur for species using active virulence

441 mechanisms and should be further investigated. *Asobara japonica* and *A. citri* (Prévost *et al.*, 2005,
442 2009; Mabiála-Moundoungou *et al.*, 2010) may for instance represent good models for such a study.

443

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451

452 **References**

453 van Baalen, M. (1998) Coevolution of recovery ability and virulence. *Proceedings of the Royal Society*
454 *of London B: Biological Sciences*, 265, 317–325.

455 Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. and West, G.B. (2004) Toward a metabolic theory
456 of ecology. *Ecology*, 85, 1771–1789.

457 Delava, E., Allemand, R., Léger, L., Fleury, F. and Gibert, P. (2014) The rapid northward shift of the
458 range margin of a Mediterranean parasitoid insect (Hymenoptera) associated with regional
459 climate warming. *Journal of Biogeography*, 41, 1379–1389.

-
- 460 Ebert, D. (2008) Host–parasite coevolution: Insights from the *Daphnia*–parasite model system.
461 *Current Opinion in Microbiology*, 11, 290–30.
- 462 Ellers, J., Driessen, G. and Sevenster, J.G. (2000) The shape of the trade-off function between egg
463 production and life span in the parasitoid *Asobara tabida*. *Netherlands Journal of Zoology*, 50,
464 29–36.
- 465 Eslin, P., Giordanengo, P., Fourdrain, Y. and Prévost, G. (1996) Avoidance of encapsulation in the
466 absence of VLP by a braconid parasitoid of *Drosophila* larvae: an ultrastructural study.
467 *Canadian Journal of Zoology*, 74, 2193–2198.
- 468 Eslin, P. and Prévost, G. (2000) Racing against host's immunity defenses: a likely strategy for passive
469 evasion of encapsulation in *Asobara tabida* parasitoids. *Journal of Insect Physiology*, 46, 1161–
470 1167.
- 471 Fellowes, M.D.E., Kraaijeveld, A.R. and Godfray, H.C.J. (1999). Association between feeding rate and
472 parasitoid resistance in *Drosophila melanogaster*. *Evolution*, 53, 1302–1305.
- 473 Gerritsma, S., de Haan, A., van de Zande, L. and Wertheim, B. (2013) Natural variation in
474 differentiated hemocytes is related to parasitoid resistance in *Drosophila melanogaster*.
475 *Journal of Insect Physiology*, 59, 148–158.
- 476 Gilchrist, G.W., Huey, R.B. and Serra, L. (2001) Rapid evolution of wing size clines in *Drosophila*
477 *subobscura*. *Genetica*, 112, 273–286.
- 478 Gilchrist, G.W. and Huey, R.B. (2004) Plastic and genetic variation in wing loading as a function of
479 temperature within and among parallel clines in *Drosophila subobscura*. *Integrative and*
480 *Comparative Biology*, 44, 461–470.

-
- 481 Gilchrist, M.A. and Sasaki, A. (2002) Modeling host–parasite coevolution: a nested approach based
482 on mechanistic models. *Journal of Theoretical Biology*, 218, 289–308.
- 483 Godfray, H.C.J. (1994) *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press.
- 484 Green, D. M., Kraaijeveld, A. R. and Godfray, H. C. J. (2000) Evolutionary interactions between
485 *Drosophila melanogaster* and its parasitoid *Asobara tabida*. *Heredity*, 85, 450–458.
- 486 Gwynn, D.M., Callaghan, A., Gorham, J., Walters, K.F.A. and Fellowes, M.D.E. (2005) Resistance is
487 costly: trade-offs between immunity, fecundity and survival in the pea aphid. *Proceedings of*
488 *the Royal Society of London B: Biological Sciences*, 272, 1803–1808.
- 489 Huey, R.B., Gilchrist, G.W., Carlson, M.L., Berrigan, D. and Serra, L. (2000) Rapid evolution of a
490 geographic cline in size in an introduced fly. *Science*, 287, 308–309.
- 491 Hurlbert, A.H., Ballantyne, F. and Powell, S. (2008) Shaking a leg and hot to trot: the effects of body
492 size and temperature on running speed in ants. *Ecological Entomology*, 33, 144–154.
- 493 Imasheva, A.G., Bubli, O.A. and Lazebny, O.E. (1994) Variation in wing length in Eurasian natural
494 populations of *Drosophila melanogaster*. *Heredity*, 72, 508–514
- 495 Jalvingh, K.M., Chang, P.L., Nuzhdin, S.V. and Wertheim, B. (2014) Genomic changes under rapid
496 evolution: selection for parasitoid resistance. *Proceedings of the Royal Society of London B:*
497 *Biological Sciences*, 281, 20132303.
- 498 Janzen, D.H. (1980) When is it coevolution. *Evolution*, 34, 611–612.
- 499 Jervis, M.A., Ellers, J. and Harvey, J.A. (2008) Resource acquisition, allocation, and utilization in
500 parasitoid reproductive strategies. *Annual Review of Entomology*, 53, 361–385.

-
- 501 Kapranas, A., Tena, A. and Luck, R.F. (2012) Dynamic virulence in a parasitoid wasp: the influence of
502 clutch size and sequential oviposition on egg encapsulation. *Animal Behaviour*, 83, 833–838.
- 503 Kraaijeveld, A. R. and van Alphen, J.J.M. (1994) Geographical variation in resistance of the parasitoid
504 *Asobara tabida* against encapsulation by *Drosophila melanogaster* larvae: the mechanism
505 explored. *Physiological Entomology*, 19, 9–14.
- 506 Kraaijeveld, A.R. and Godfray, H.C.J. (1999) Geographic patterns in the evolution of resistance and
507 virulence in *Drosophila* and its parasitoids. *The American Naturalist*, 153, S61–S74.
- 508 Kraaijeveld, A.R. and Godfray, H.C.J. (2009) Evolution of host resistance and parasitoid counter-
509 resistance. *Advances in Parasitology*, 70, 257–280.
- 510 Kraaijeveld, A.R., Hutcheson, K.A., Limentani, E.C. and Godfray, H.C.J. (2001b) Costs of
511 counterdefenses to host resistance in a parasitoid of *Drosophila*. *Evolution*, 55, 1815–1821.
- 512 Kraaijeveld, A.R., Limentani, E.C. and Godfray, H.C.J. (2001a) Basis of the trade-off between
513 parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Proceedings of*
514 *the Royal Society of London B: Biological Sciences*, 268, 259–261.
- 515 Kraaijeveld, A. R., Nowee, B. and Najem, R. W. (1995) Adaptive variation in host-selection behaviour
516 of *Asobara tabida*, a parasitoid of *Drosophila* larvae. *Functional Ecology*, 9, 113–118.
- 517 Kraaijeveld, A.R. and van der Wel, N.N. (1994) Geographic variation in reproductive success of the
518 parasitoid *Asobara tabida* in larvae of several *Drosophila* species. *Ecological Entomology*, 19,
519 221–229.
- 520 Lambrechts, L., Fellous, S. and Koella, J.C. (2006) Coevolutionary interactions between host and
521 parasite genotypes. *Trends in Parasitology*, 22, 12–16.

-
- 522 Le Lann, C., Visser, B., Mériaux, M., Moiroux, J., Van Baaren, J., van Alphen, J.J.M. and Ellers, J. (2014)
523 Rising temperature reduces divergence in resource use strategies in coexisting parasitoid
524 species. *Oecologia*, 174, 967–977.
- 525 Mabilia-Moundougou, A.D.N., Doury, G., Eslin, P., Cherqui, A. and Prévost, G. (2010) Deadly venom
526 of *Asobara japonica* parasitoid needs ovarian antidote to regulate host physiology. *Journal of*
527 *Insect Physiology*, 56, 35–41.
- 528 Maure, F., Thomas, F., Doyon, J. and Brodeur, J. (2016) Host nutritional status mediates degree of
529 parasitoid virulence. *Oikos*, doi: 10.1111/oik.0294.
- 530 May, R.M. and Anderson, R.T. (1983) Epidemiology and genetics in the coevolution of parasites and
531 hosts. *Proceedings of the Royal Society of London B: Biological Sciences*, 219, 281–313.
- 532 Moiroux, J., Delava, E., Fleury, F. and Baaren, J. (2013) Local adaptation of a *Drosophila* parasitoid:
533 habitat - specific differences in thermal reaction norms. *Journal of Evolutionary Biology*, 26,
534 1108–1116.
- 535 Moiroux, J., Le Lann, C., Seyahooei, M.A., Vernon, P., PIERRE, J.S., van Baaren, J. and van Alphen, J. J.
536 (2010) Local adaptations of life-history traits of a *Drosophila* parasitoid, *Leptopilina boulardi*:
537 does climate drive evolution? *Ecological Entomology*, 35, 727–736.
- 538 Nappi, A.J., Vass, E., Frey, F. and Carton, Y. (1995) Superoxide anion generation in *Drosophila* during
539 melanotic encapsulation of parasites. *European Journal of Cell Biology*, 68, 450–456.
- 540 Nation, J.L. (2008) *Insect Physiology and Biochemistry*. CRC press.

-
- 541 Nomano, F.Y., Mitsui, H. and Kimura, M.T. (2015) Capacity of Japanese *Asobara* species
542 (Hymenoptera; Braconidae) to parasitize a fruit pest *Drosophila suzukii* (Diptera;
543 Drosophilidae). *Journal of Applied Entomology*, 139, 105–113.
- 544 Prévost, G., Doury, G., Moundoungou-Mabiala, A.D.N., Cherqui, A. and Eslin, P. (2009) Strategies of
545 avoidance of host immune defense in *Asobara* species. *Advances in Parasitology*, 70, 235–255.
- 546 Prévost, G., Eslin, P., Doury, G., Moreau, S.J. and Guillot, S. (2005) *Asobara*, braconid parasitoids of
547 *Drosophila* larvae : unusual strategies to avoid encapsulation without VLPs. *Journal of Insect*
548 *Physiology*, 51, 171–179.
- 549 Price, P.W. (1974) Strategies for egg production. *Evolution*, 28, 76–84.
- 550 Roff, D.A. (1992) *Evolution of Life Histories: Theory and Analysis*. Springer Science & Business Media.
- 551 Sanders, A.E., Scarborough, C., Layen, S.J., Kraaijeveld, A.R. and Godfray, H.C.J. (2005) Evolutionary
552 change in parasitoid resistance under crowded conditions in *Drosophila melanogaster*.
553 *Evolution*, 59, 1292–1299.
- 554 Sasaki, A. and Godfray, H.C.J. (1999) A model for the coevolution of resistance and virulence in
555 coupled host–parasitoid interactions. *Proceedings of the Royal Society of London B: Biological*
556 *Sciences*, 266, 455–463.
- 557 Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford: Oxford University Press.
- 558 Strand, M.R. and Pech, L.L. (1995) Immunological basis for compatibility in parasitoid-host
559 relationships. *Annual Review of Entomology*, 40, 31–56.

560 Suverkropp, B.P., Bigler, F. and van Lenteren, J.C. (2001) Temperature influences walking speed and
561 walking activity of *Trichogramma brassicae* (Hym., Trichogrammatidae). *Journal of Applied*
562 *Entomology*, 125, 303–307.

563 Takigahira, T., Suwito, A. and Kimura, M.T. (2014) Assessment of fitness costs of resistance against
564 the parasitoid *Leptopilina victoriae* in *Drosophila bipectinata*. *Ecological research*, 29, 1033–
565 1041.

566 van Valen, L. (1973) A new evolutionary law. *Evolutionary Theory*, 1, 1–30.

567 Vayssade, C., Martel, V., Moiroux, J., Fauvergue, X., van Alphen, J.J.M. and van Baaren, J. (2012) The
568 response of life-history traits to a new species in the community: a story of *Drosophila*
569 parasitoids from the Rhône and Saône valleys. *Biological Journal of the Linnean Society*, 107,
570 153–165.

571 Vinson, S.B. (1990) How parasitoids deal with the immune system of their host: an overview.
572 *Archives of Insect Biochemistry and Physiology*, 13, 3–27.

573 Vuarin, P., Allemand, R., Moiroux, J., Van Baaren, J. and Gibert, P. (2012) Geographic variations of life
574 history traits and potential trade-offs in different populations of the parasitoid *Leptopilina*
575 *heterotoma*. *Naturwissenschaften*, 99, 903–912.

576

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580 **Table 1.** Life-history traits (mean±s.e.) measured on *Asobara tabida* females from the five strains.

581 Different letters in the same row indicate significant differences between strains.

582

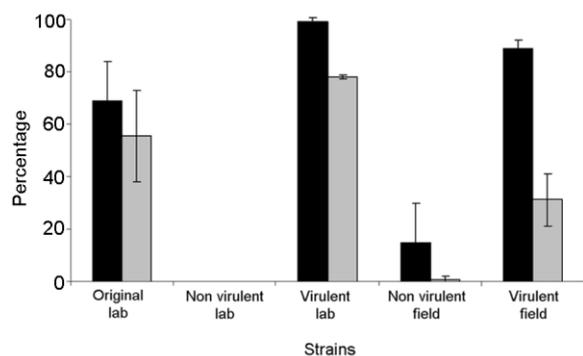
Strains	Lab			Field	
	Original	Non virulent	Virulent	Non virulent	Virulent
Tibia length (mm)	0.729 ± 0.021 a	0.756 ± 0.026 a	0.721 ± 0.023 a	0.772 ± 0.035 a	0.748 ± 0.027 a
Egg load at five days	268 ± 16 ab	241 ± 19 a	277 ± 20 b	302 ± 23 b	283 ± 17 b
Egg volume at emergence (10 ⁻⁴ mm ³)	0.112 ± 0.006 a	0.075 ± 0.005 b	0.116 ± 0.005 a	0.102 ± 0.004 a	0.091 ± 0.006 a
Egg volume at five days (10 ⁻⁴ mm ³)	0.107 ± 0.005 a	0.081 ± 0.006 b	0.111 ± 0.004 a	0.097 ± 0.007 a	0.089 ± 0.006 ab
Metabolic rate (mL CO ₂ /h/g)	NA	0.380 ± 0.030 a	0.410 ± 0.020 a	0.610 ± 0.05 b	0.570 ± 0.060 b

583

584

585 **Figure legends**

586

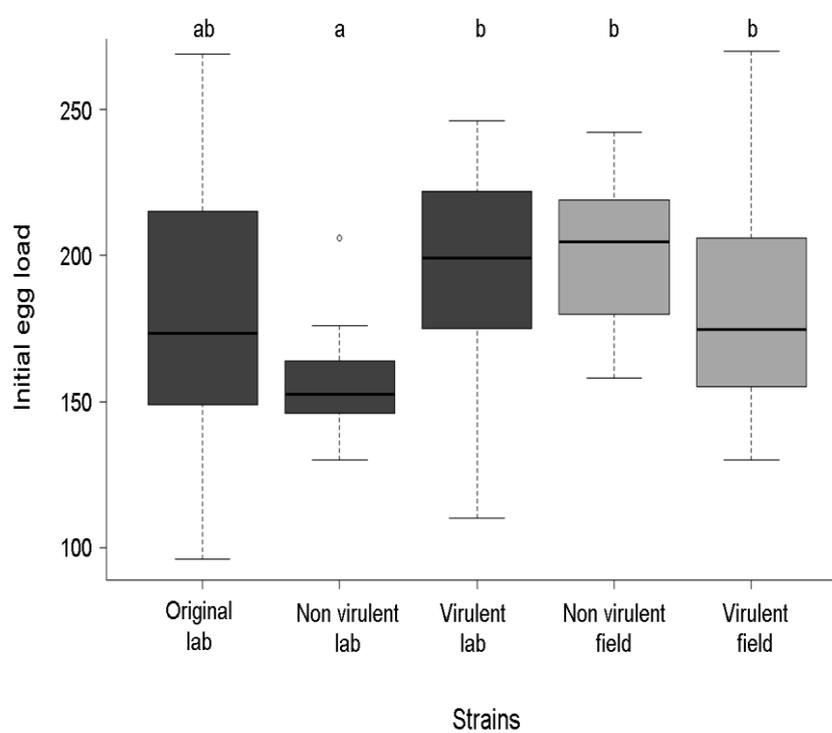


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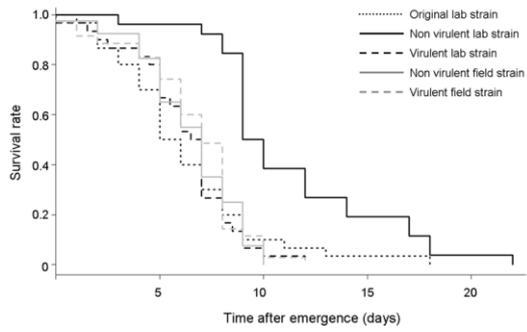
Figure 2. Boxplot of initial egg load measured in ovaries of emerging *Asobara tabida* females from the five tested strains. Different letters mean significant differences between strains.



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Figure 3. Survivorship curves under starvation of the five *Asobara tabida* tested strains.

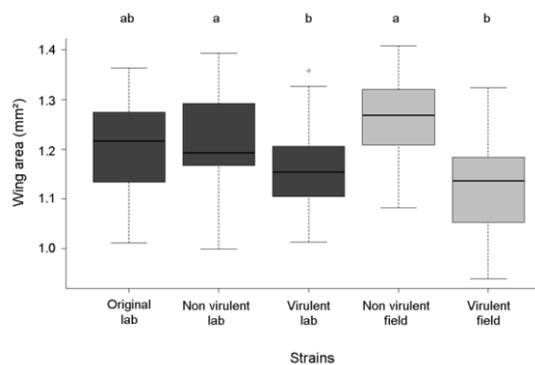


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Figure 4. Boxplot of wing area measured on emerging *Asobara tabida* females from the five tested strains. Different letters mean significant differences between strains.

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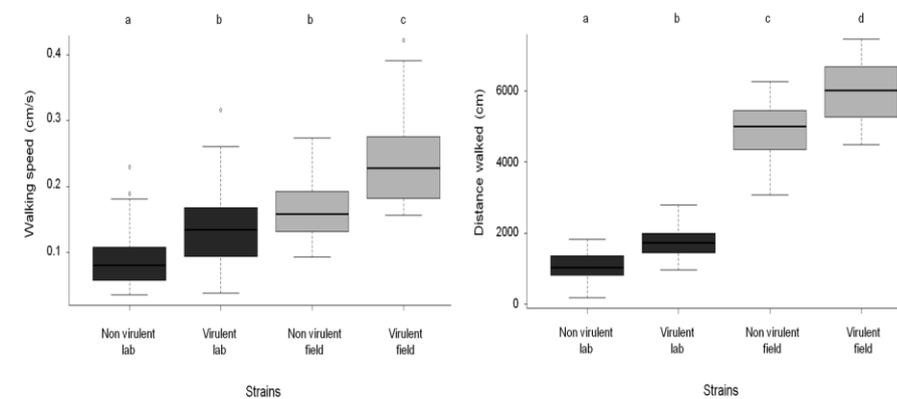
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Figure 5. Boxplot of locomotion related traits, i.e. walking speed on the left and distance walked on the right, measured on *Asobara tabida* females from the four tested strains recorded for ten hours. Different letters mean significant differences between strains.

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