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

Drosophila* parasitoid, *Asobara tabida

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Abstract.

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

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

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

INTRODUCTION

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

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

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

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

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

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

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

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

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

MATERIAL AND METHODS

STRAINS ORIGIN AND REARING.

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

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

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

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

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.

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

Life history traits

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

In order to obtain the adult parasitoid females used for measurements of life-history traits, *Drosophila subobscura* females were allowed to lay eggs on a 1 mm thick layer of fermented medium spread on a microscope slide in a rearing tube for 4h at 20 °C. The slide was then transferred to another tube and maintained in rearing conditions for 48 h, i.e. until *Drosophila* eggs had hatched and larvae had reached the second instar. The slide with the larvae was then placed in a 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

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

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

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

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

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

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

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

Statistical analyses

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

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

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

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

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

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

RESULTS

Strain selection and virulence estimation

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

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

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

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

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

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

DISCUSSION

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.

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).

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

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

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

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

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

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

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

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

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

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

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

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

Conclusion

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

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

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

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

Strains	Lab			Field	
	Original	Non virulent	Virulent	Non virulent	Virulent
Tibia length (mm)	0.729 \pm 0.021 a	0.756 \pm 0.026 a	0.721 \pm 0.023 a	0.772 \pm 0.035 a	0.748 \pm 0.027 a
Egg load at five days	268 \pm 16 ab	241 \pm 19 a	277 \pm 20 b	302 \pm 23 b	283 \pm 17 b
Egg volume at emergence (10⁻⁴mm³)	0.112 \pm 0.006 a	0.075 \pm 0.005 b	0.116 \pm 0.005 a	0.102 \pm 0.004 a	0.091 \pm 0.006 a
Egg volume at five days (10⁻⁴mm³)	0.107 \pm 0.005 a	0.081 \pm 0.006 b	0.111 \pm 0.004 a	0.097 \pm 0.007 a	0.089 \pm 0.006 ab
Metabolic rate (mL CO₂/h/g)	NA	0.380 \pm 0.030 a	0.410 \pm 0.020 a	0.610 \pm 0.05 b	0.570 \pm 0.060 b

Figure legends

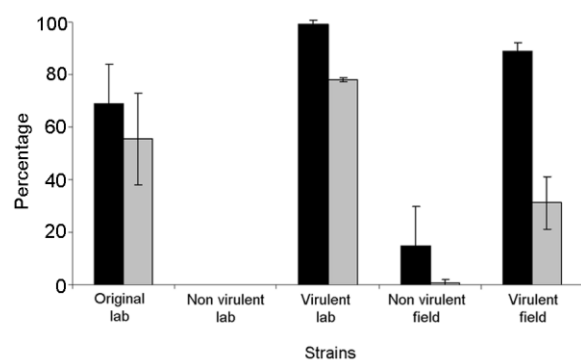


Figure 2. Boxplot of initial egg load measured in ovaries of emerging *Asobara tabida* females from the five tested strains. Different letters mean significant differences between strains.

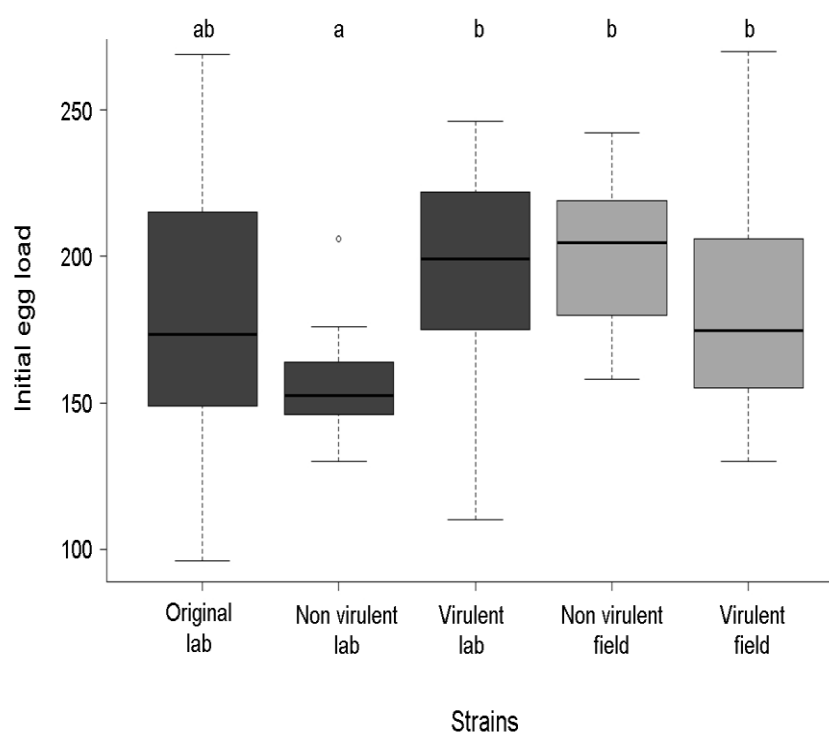


Figure 3. Survivorship curves under starvation of the five *Asobara tabida* tested strains.

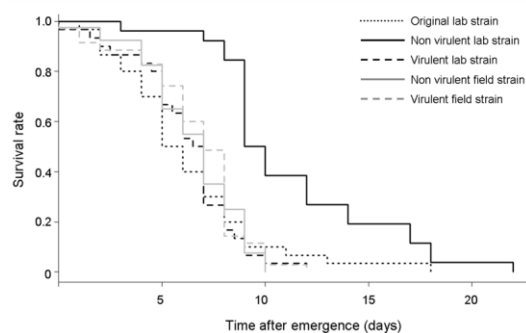


Figure 4. Boxplot of wing area measured on emerging *Asobara tabida* females from the five tested strains. Different letters mean significant differences between strains.

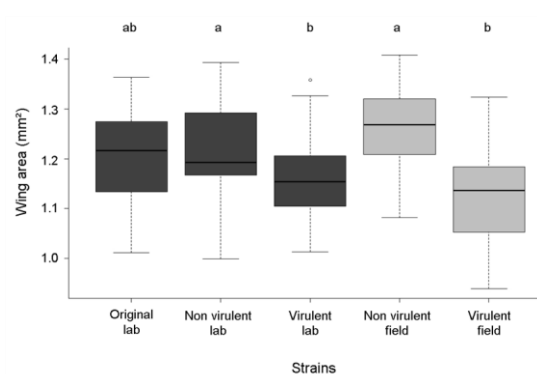


Figure 5. Boxplot of locomotion related traits, i.e. walking speed on the left and distance walked on the right, measured on *Asobara tabida* females from the four tested strains recorded for ten hours. Different letters mean significant differences between strains.

