Effects of parasitism by *Asobara tabida* (Hymenoptera: Braconidae) on the development, survival and activity of *Drosophila melanogaster* larvae

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Abstract

The impact of parasitism by *Asobara tabida* on *Drosophila melanogaster* larval development, survival features and larval activity has been investigated using two strains of the parasitoid. The successful parasitism rate of the A1 strain was four times greater than that of the WOPV strain. Both strains induced equivalent mortality rates but hosts parasitized by A1 predominantly died as pupae. The time necessary for the host pupariation and emergence, and the larval weight at 72, 96 and 120 h post-parasitization were measured. Parasitized larvae exhibited longer periods of development and lower weights than controls, especially when parasitized by A1. These results suggest that hosts underwent physiological costs varying with respect to the outcome of the parasitoid relationship. Of the parasitoid factors possibly responsible for these costs, we examined venoms for their impact on host mortality. Artificial injections of WOPV venoms induced higher mortality rates than did A1 venoms. Venoms were also found responsible for the induction of a transient paralysis, naturally occurring after parasitization. Again, the strongest effect was observed after parasitization by WOPV or injections of its venoms. This study gives new insights into the intriguing features of *A. tabida* and constitutes the first report of the paralyzing properties of the venoms. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Asobara tabida*; *Drosophila melanogaster*; Parasitoid; Host development; Venoms

1. Introduction

A major constraint for parasitoid Hymenoptera is to make the kinetics of their development compatible with that of their host. This challenge can be achieved by the parasitoids either by adapting to the developmental features of their host, or by altering these features to make them fit with their own developmental requirements (for review see Vinson and Iwantsch, 1980; Strand, 1986; Harvey, 1996). Various physiological effects are induced in hosts upon parasitization, among which modifications of weight gain and/or developmental duration are frequently observed. In a few cases the parasitized host has been reported to gain weight (Hunter and Stoner, 1975; Rahman 1970) although in most models a decrease in host weight is noted (Iwantsch and Smilowitz, 1975; Beckage and Riddiford, 1978; Brewer and King, 1978; Richards and Edwards, 1999). Alterations in the duration of development can affect a decrease in the length of larval life (Jones et al., 1981) or the opposite, a lengthening of the immature stages (Thompson and Adams, 1976). For example, parasitism by the well-studied parasitoid *Cotesia congregata* induces the occurrence of a supernumerary larval instar in its host *Manduca sexta* (Beckage and Templeton, 1986). Such effects are potentially induced by several factors injected by parasitoid females during oviposition (for review, see Lawrence and Lanzrein, 1993). Among these parasitoid factors, venoms have been reported to cause abnormalities of development, with modes of action that vary according to the lifestyle of the parasitoid (Shaw, 1981; Morales-Ramos et al., 1995; Coudron and Brandt, 1996). Ectoparasitoids venoms are often paralytic and may cause a developmental arrest in the host that can be beneficial to the externally developing parasite (Doury et al., 1997). Venoms of endoparasitoids are generally non-paralyzing but can disrupt the endo-
crine system of the host either directly (Shaw, 1981) or in combination with symbiotic viruses that are also produced and injected by the parasitoid females (Tanaka and Vinson, 1991a and b; Gupta and Ferkovitch, 1998; Jones and Wache, 1998).

However most of the effects of parasitoid venoms on the development of their hosts have been described with respect to lepidopteran hosts. Few studies have investigated the impact of parasitoids on the developmental parameters of dipteran hosts. In addition, the degree of involvement of the parasitoid venoms in the effects recorded in the developing hosts remains unknown.

Asobara tabida (Hymenoptera: Braconidae) is a solitary endoparasitoid of several species of Drosophila, including Drosophila melanogaster. Previous studies have demonstrated that intraspecific variation of the successful parasitism rate of A. tabida towards D. melanogaster could be correlated with geographic localization (Kraaijeveld and van Alphen, 1994). The strain called A1 originates in the south of France and develops more successfully on D. melanogaster than the WOPV strain originating from the Netherlands (Eslin and Prévost, 2000).

Although the effects of parasitism by A. tabida on the immune system of its hosts have been recently investigated (Eslin and Prévost, 2000; Moreau et al., 2000), little is known concerning its impact on the development of D. melanogaster.

The objectives of our study are to investigate the effects of parasitism by each strain of A. tabida on the development of D. melanogaster larvae, and to test the capacity of the female’s venom to alter the activity and survival features of the hosts.

2. Materials and methods

2.1. Insects

D. melanogaster and two strains of the parasitoid A. tabida (A1 and WOPV) were reared and experimented on artificial medium in the same conditions of temperature and light (20°C and 13 h light: 11 h dark) as described in Eslin et al. (1996).

Emerging parasitoid wasps were isolated from flies and provided with honey and water until use for experimentation.

2.2. Procedure for controlled parasitization

First instar D. melanogaster larvae (48±1 h post-oviposition) were exposed for 2 h to females from either the A1 or WOPV strain of A. tabida. Parasitization was observed under a stereomicroscope. After a single oviposition by a parasitoid female, each parasitized D. melanogaster larva was removed individually, then placed in a tube containing 3 g of artificial medium prewetted with 150 μl of water. For each replicate, 20 parasitized larvae were pooled, weighed at 72, 96, and 120 h after parasitization or allowed to develop until they emerged.

2.3. Measurement of successful parasitism rate in controlled conditions of parasitization

Successful parasitism rate (SPR) and encapsulation rate (ER) of the parasite by its host were evaluated by counting the number of emerging parasite wasps (SPR) and adult flies bearing a capsule (ER). Total mortality rate (MR) was subdivided into larval (LMR) and pupal (PMR) mortality rates corresponding to larvae failing in pupariate (LMR) and pupae that did not emerge as imagos (PMR). The natural mortality rate was estimated by allowing unparasitized control larvae to complete development.

2.4. Developmental parameters of parasitized larvae

To determine the onset of pupariation of parasitized larvae and the emergence time of flies holding a capsule, parasitized hosts were examined 3 times daily. The mean times required before the onset of pupariation and until emergence were expressed in hours after fly oviposition.

Parasitized hosts were weighed by collecting larvae at 72, 96 and 120 h after parasitization. Larvae were rinsed twice in 0.15 M phosphate buffered saline pH 7.2 (PBS, bioMérieux, Marcy l’Etoile, France) and once in 70% ethanol. They were then dried on filter paper and weighed with an analytical balance (Denver Instrument Company, Arvada, USA) in groups of 10 (at 72 and 96 h) or 5 larvae (at 120 h). The average weight at a given time was expressed in mg per larva.

“Larvae parasitized by WOPV” refer to larvae holding an encapsulated egg of the WOPV strain of A. tabida, whereas “larvae parasitized by A1” consist of larvae hosting a free-developing parasitoid of the A1 strain. The presence of a capsule or of a parasitoid alive within parasitized larvae was systematically checked by dissection after the experiment.

2.5. Collection of A. tabida venom extracts for injections

Three to 5 day-old females from each strain of A. tabida were anesthetized using ethyl acetate. Venom apparatuses (i.e., secreting glands and reservoir) were dissected under a stereomicroscope in 0.15 M PBS pH 7.2, transferred into a new PBS droplet stored on ice and manually squeezed with minutin pins. Samples were then centrifuged at 10 000 g for 5 min at 4°C to discard cellular residues from the supernatant. The protein concentration of the supernatant was spectrophotometrically determined at 595 nm by the Coomassie Blue dye bind-
ing method using bovine serum albumin (SIGMA) as a standard (Bradford, 1976).

2.6. Injection procedure

The effects of the venom of each strain of A. tabida on the survival and activity of D. melanogaster larvae were observed on larvae injected with fresh venom extracts. Second instar larvae (96±1 h post-oviposition) were rinsed once in PBS, twice in 70% ethanol, then once in PBS. Injections of 1, 5, 10, 50 or 100 ng of venom extract proteins in a final volume of 30 nl in PBS were performed using a glass microneedle (25 μm diameter). Uninjected larvae and larvae injected with 30 nl PBS only were used as controls. After injection larvae were kept on fresh medium until they completed their development in the rearing conditions.

2.7. Survival of larvae injected with A. tabida venom extracts

Larvae injected with PBS or venom extracts were checked daily for number of pupariating larvae and emerging flies and compared to uninjected individuals treated and kept in the same conditions. Mortality rates (MR) of injected D. melanogaster were evaluated and subdivided into larval (LMR) and pupal (PMR) mortality.

2.8. Evaluation of larval activity upon parasitization

D. melanogaster first instar larvae (48±1 h post-oviposition) were exposed to A1 or WOPV female wasps as described before. After a single oviposition, each individual parasitized larva was carefully removed and placed into a well of a 24-well plate, each well containing 300 μl of artificial medium (agar 1.2 % (w/v), sucrose 10 % (w/v), and malted yeast 0.2 % (w/v)). Each parasitized larva was then observed individually during a 2 h period, every 5 min during the first 30 min, then every 15 min during the next 90 min. Special attention was given to inactive larvae. Inactivity was considered when absolutely no mandibular nor muscular movements could be observed during the time of observation (approximately 20 sec per larva). Active larvae that exhibited crawling and feeding behaviors are not presented in this study but were taken into account for the calculation of the mean percentages of inactive larvae at each time interval for the first 30 min and the following 90 min of the experiment. These values were compared with those obtained for parasitized larvae and control unparasitized larvae.

2.9. Evaluation of larval activity upon injection of venom extracts

D. melanogaster second instar larvae (96±1 h after fly oviposition) were injected with A1 or WOPV fresh venom extracts and submitted to the same experimental procedure as described above after parasitization. The activity of larvae injected with venom was compared to the activity of larvae injected with PBS only and uninjected larvae.

2.10. Statistical analysis

Statistical analyses were performed using one-way ANOVA and χ² test carried out using Stat View II software (Abacus Concept, Inc.). The acceptance level of statistical significance was p<0.05 for ANOVA analysis and p<0.1 for χ² tests. For tests, parameters (SPR, ER, MR, LMR, PMR) were normalized with the arcsine function according to Rao (1951) and differences between means were analysed by the Fisher test (Positive Least Significant Difference).

3. Results

3.1. Successful parasitism and mortality rates associated with parasitism of D. melanogaster by the A1 or WOPV strain of A. tabida

In our experimental conditions of controlled parasitization, the two strains of A. tabida showed significant differences in their successful parasitism rate (SPR) on D. melanogaster larvae (ANOVA; d.f.=6; F=0.3; p=0.0027) and induced significantly different encapsulation rates (ER) in their hosts (ANOVA; d.f.=6; F=0.29; p=0.0025) (Table 1). Most of the eggs laid in host larvae by females of the A1 strain successfully completed their development (SPR=74.0±2.6%) and few of them became encapsulated by their host (ER=7.5±3.2%). On the other hand, the SPR of the WOPV strain (SPR=18.8±8.8%) was approximately four times lower than that of the A1 strain, with more than half of the WOPV parasitoids being encapsulated by the hosts immune cells (ER=58.8±10.1%).

Mortality rates (MR) of parasitized hosts were significantly higher than those of unparasitized controls (MR=5.8±2.4%) (ANOVA; d.f.=11; F=0.12; p=0.0019), but no significant difference could be observed between the mortalities of hosts parasitized by A1 (MR=18.5±1.2%) or WOPV (MR=22.5±3.2%) (ANOVA; p=0.3042). However, larval mortality rates (LMR) of parasitized larvae and controls were not significantly different (ANOVA; p=0.1738), and only the pupal mortality rates (PMR) of controls and hosts parasitized by A1 were significantly different (ANOVA; d.f.=11; F=0.15; p=0.0341). In fact, parasitism by WOPV induced mortality equally in larval and pupal stages (LMR=11.3±4.3%; PMR=11.3±4.7%), whereas mortality due to parasitism by A1 affected the hosts
mostly during the pupal stage (LMR=3.8±3.8%; PMR=14.8±3.4%).

3.2. Effects of parasitism by each strain of A. tabida on the development of D. melanogaster larvae

3.2.1. Average times to complete pupariation and emergence

Whether parasitized by the A1 or WOPV strain, parasitized larvae showed a significant increase in their larval developmental time (193.8±2.1 h and 184.4±2.2 h after fly oviposition, respectively) when compared to control unparasitized larvae (178.1±1.4 h after fly oviposition) (ANOVA; d.f.=140; F=4.9 and F=5.31, respectively; p=0.0001) (Table 2A). Parasitism by A1 significantly induced the greatest increase in the duration of the larval stage (ANOVA; d.f.=84; F=6.07; p=0.0028).

Emergence of D. melanogaster adults that succeeded in encapsulating an A1 or a WOPV parasitoid occurred significantly later (337.4±2.6 h and 336.2±1.6 h after fly oviposition, respectively) compared to control unparasitized individuals (327.9±1.3 h) (ANOVA; d.f.=123; F=7.08 and F=4.01, respectively; p=0.0001) (Table 2B).

3.2.2. Average weights of parasitized larvae

Parasitized larvae were significantly lighter than unparasitized ones whenever they were weighed (ANOVA72h : d.f.=9; F=0.11; p=0.0001; ANOVA96h : d.f.=19; F=0.1; p=0.0001; ANOVA120h : d.f.=17; F=0.2; p=0.0002) (Table 3). In addition, larvae hosting a free developing A1 parasitoid showed significantly more reduced weight than larvae holding an encapsulated egg of the WOPV strain when measured at 72, 96 or 120 h after parasitization (ANOVA72h : d.f.=4; F=0.08; p=0.0001; ANOVA96h ; d.f.=10; F=0.12; p=0.0242; ANOVA120h : d.f.=7; F=0.14; p=0.0161).

We tested whether venom alone could be involved in host mortality. We therefore performed injections of different doses of venom extracts and measured their effect on pupariation and emergence rates of D. melanogaster.

3.3. Survival features of D. melanogaster larvae injected with venom extracts from the A1 or WOPV strain of A. tabida

Five different quantities of venom extract proteins (1, 5, 10, 50, and 100 ng of protein per larva) from either A1 or WOPV were injected into 4 day-old second instar D. melanogaster larvae (Table 4). No significant effect on the survival of D. melanogaster could be observed by injection of PBS (χ² tests; d.f.=1; p=0.7768 for larval mortality rates (LMR) and p=0.9775 for pupal mortality rates (PMR)).

Only injection of the highest dose of venom proteins from the A1 strain (100 ng/larva) significantly increased the mortality rate of D. melanogaster larvae compared to un.injected controls (χ² test; d.f.=1; χ²=6.28; p=0.0122) or PBS injected larvae (χ² test; d.f.=1; χ²=5.07; p=0.0243), and no significant effect could be noted on host PMR (χ² tests; p<0.1).

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Table 1
Outcomes of the parasitic relationship between D. melanogaster larvae and the A1 or WOPV strain of A. tabida

<table>
<thead>
<tr>
<th></th>
<th>Successful parasitism rate (SPR) (%)</th>
<th>Encapsulation rate (ER) (%)</th>
<th>Mortality rate (MR) (%)</th>
<th>Larval mortality rate (LMR) (%)</th>
<th>Pupal mortality rate (PMR) (%)</th>
<th>No. of larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unparasitized larvae</td>
<td>–</td>
<td>–</td>
<td>5.8 ± 2.4 a</td>
<td>2.5 ± 1.7 a</td>
<td>3.3 ± 1.7 a</td>
<td>123</td>
</tr>
<tr>
<td>Larvae parasitized by A1</td>
<td>74.0 ± 2.6 a</td>
<td>7.5 ± 3.2 a</td>
<td>18.5 ± 1.2 b</td>
<td>3.8 ± 3.8 a</td>
<td>14.8 ± 3.4 b</td>
<td>81</td>
</tr>
<tr>
<td>Larvae parasitized by WOPV</td>
<td>18.8 ± 8.8 b</td>
<td>58.8 ± 10.1 b</td>
<td>22.5 ± 3.2 b</td>
<td>11.3 ± 4.3 a</td>
<td>11.3 ± 4.7 ab</td>
<td>80</td>
</tr>
</tbody>
</table>

* Values (% ± SE) within a column followed by different letters indicate a significant difference (ANOVA, p<0.05).

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Table 2
A. Time required before the onset of pupariation of D. melanogaster larvae parasitized by the A1 or WOPV strain of A. tabida

<table>
<thead>
<tr>
<th></th>
<th>Time required (h)</th>
<th>No. of larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unparasitized larvae</td>
<td>178.1 ± 1.4 a</td>
<td>57</td>
</tr>
<tr>
<td>Larvae parasitized by A1</td>
<td>193.8 ± 2.1 b</td>
<td>59</td>
</tr>
<tr>
<td>Larvae parasitized by WOPV</td>
<td>184.4 ± 2.2 c</td>
<td>54</td>
</tr>
</tbody>
</table>

*D. melanogaster* larvae were reared on artificial diet until complete development. The number of pupae was checked three times-a-day. Values are expressed in hours after fly oviposition (mean ± SE). Values within a column followed by different letters indicate a significant difference (ANOVA, p<0.05).

B. Time required for emergence of D. melanogaster adults parasitized by the A1 or WOPV strain of A. tabida

<table>
<thead>
<tr>
<th></th>
<th>Time required (h)</th>
<th>No. of adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unparasitized controls</td>
<td>327.9 ± 1.3 a</td>
<td>58</td>
</tr>
<tr>
<td>Hosts parasitized by A1</td>
<td>337.4 ± 2.6 b</td>
<td>11</td>
</tr>
<tr>
<td>Hosts parasitized by WOPV</td>
<td>336.2 ± 1.6 b</td>
<td>50</td>
</tr>
</tbody>
</table>

*D. melanogaster* larvae were reared on artificial diet until complete development and the number of emerging adults was checked three times-a-day. Values are expressed in hours after fly oviposition (mean ± SE). Values within a column followed by different letters indicate a significant difference (ANOVA, p<0.05).
On the contrary, injections of venom proteins from WOPV significantly increased both LMR and PMR compared to un.injected or PBS injected controls ($\chi^2$ tests; $p<0.1$), except for the lowest dose (1 ng/larva) that did not significantly increase the LMR compared to control or PBS injected larvae ($\chi^2$ tests; $p=0.4072$ and $p=0.5582$, respectively). When 50 and 100 ng of venom extract proteins from WOPV were injected in D. melanogaster larvae, very few individuals completed their pupariation (LMR were 75.0 and 95.0%, respectively), and none reached the adult stage (PMR=100%).

The experiments revealed that host larvae exhibited transient inactivity during the first 2 h after parasitization or venom injection. We thus decided to measure the larval activity immediately after parasitization or injection to test for potential short-term effects of parasitism and venom.

### 3.4. Activity of D. melanogaster larvae upon parasitization by the A1 or WOPV strain of A. tabida

D. melanogaster first instar larvae were exposed to A1 or WOPV females of A. tabida. Their activity was observed during a 120 min period after oviposition by the parasitoid female, then compared to unparasitized controls (Fig. 1).

During the first 30 min after parasitization, a significant number of larvae parasitized by the WOPV strain underwent total body paralysis immediately after oviposition (mean percentage of 32.3±4.1% of inactive larvae); such larvae lacked mandibular or muscular movement. Over the same period, parasitization by the A1 strain also induced larval immobility, although the proportion of inactive larvae (13.5±1.7%) was significantly less than induced after parasitization by WOPV (ANOVA; d.f.=10; $F=9.88$; $p=0.0017$).

Proportions of inactive larvae observed upon parasitization by the two strains of A. tabida were significantly higher than observed for controls (3.6±0.9%) (ANOVA; d.f.=15; $F=7.88$; $p=0.0001$). The small fraction of controls exhibiting reduced activity during the first 30 min of observation (3.6±0.9% of inactive larvae) could possibly be related to a stress-inducing effect of the experimental conditions because most of the controls became fully active during the following 90 min of the experiment (0.5±0.5% of inactive larvae during this period).

During this same period (30 to 120 min after parasitization by WOPV or A1) inactive parasitized larvae could still be observed (17.0±4.5% and 9.8±0.8% for larvae parasitized by WOPV and A1, respectively). These proportions were not significantly different (ANOVA; $p=0.1356$), but both were significantly greater than the controls (ANOVA; d.f.=18; $F=7.84$; $p=0.0013$).

This experiment showed that parasitism of D. melanogaster by A. tabida induced inactivity in a significant proportion of host larvae. This effect occurred immediately after the oviposition process by the parasitoid female, was manifested as total paralysis, and lasted at least 30 min and up to 120 min for some of the hosts.

### 3.5. Activity of D. melanogaster larvae upon injection of venom extracts from each strain of A. tabida

Four day-old D. melanogaster larvae injected with 5, 10, 50 or 100 ng of venom proteins from the A1 or WOPV strain of A. tabida were observed for their activity under the same conditions as described for the parasitized ones (Fig. 2A and B). The injection of PBS did not induce a significant effect compared to control, un.injected larvae during the first 30 min (ANOVA; $p=0.5665$) and the following 90 min (ANOVA; $p=0.8732$) of observation.

As with parasitization, a significant fraction of larvae injected with venom was found paralysed during the first 30 min post-injection (Fig. 2A). The mean percentage of inactive larvae observed during this period was strongly correlated to the dose of venom proteins injected for both strains ($r=0.95$, $p<0.05$ for larvae injected with venom from WOPV; $r=0.91$, $p<0.05$ for larvae injected with venom from A1). The highest percentage of paralysed larvae was observed after injection of 100 ng of venom proteins from WOPV (84.1±7.9% of inactive larvae), which was significantly greater than the one induced by the injection of the same dose of A1 extracts (52.0±9.3%...


Table 4

Mortality rates of *D. melanogaster* larvae injected with different quantities of venom proteins from the A1 or WOPV strain of *A. tabida* a

<table>
<thead>
<tr>
<th></th>
<th>Mortality rate (MR) (%)</th>
<th>Larval mortality rate (LMR) (%)</th>
<th>Pupal mortality rate (PMR) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninjected larvae (81)</td>
<td>12.3</td>
<td>9.9</td>
<td>2.5</td>
</tr>
<tr>
<td>PBS injected larvae (80)</td>
<td>13.8</td>
<td>11.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Larvae injected with venom extracts from A1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ng (40)</td>
<td>17.5</td>
<td>10</td>
<td>7.5</td>
</tr>
<tr>
<td>5 ng (40)</td>
<td>22.5</td>
<td>17.5</td>
<td>5.0</td>
</tr>
<tr>
<td>10 ng (40)</td>
<td>15</td>
<td>12.5</td>
<td>2.5</td>
</tr>
<tr>
<td>50 ng (41)</td>
<td>14.6</td>
<td>14.6</td>
<td>0</td>
</tr>
<tr>
<td>100 ng (40)</td>
<td>35</td>
<td>27.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Larvae injected with venom extracts from WOPV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ng (40)</td>
<td>35</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>5 ng (40)</td>
<td>80</td>
<td>42.5</td>
<td>37.5</td>
</tr>
<tr>
<td>10 ng (40)</td>
<td>60</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>50 ng (40)</td>
<td>100</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>100 ng (40)</td>
<td>100</td>
<td>95</td>
<td>5</td>
</tr>
</tbody>
</table>

a Injected and control *D. melanogaster* larvae were reared on artificial diet until complete development and the number of new pupae and new adults was checked daily. Numbers in brackets indicate the number of larvae used for each experimental category. Values were compared with a \( \chi^2 \) test with 1 degree of freedom. Significant differences \( (p<0.1) \) are given in the results section.
Fig. 1. Mean percentages of inactive *D. melanogaster* larvae observed after parasitization by the A1 or WOPV strain of *A. tabida*. Results are given in mean percentages of inactive larvae observed every 5 min during the first 30 min (white bars) or every 15 min from 30 to 120 min (black bars) after parasitization by the A1 or WOPV strain of *A. tabida*. Values measured for control unparasitized larvae are given for comparison with parasitized ones. Statistical analysis of the differences between the categories was made by ANOVA, and significant differences are reported in the results section.

of inactive larvae) (ANOVA; d.f.=10; *F*=27.29; *p*=0.0255).

From 30 to 120 min after injection of venom proteins from A1 or WOPV, the mean percentages of paralysed larvae decreased (Fig. 2B). Notably, injection of venom proteins from A1 did not significantly increase the mean percentage of inactive larvae compared to larvae injected with PBS or uninjected controls (ANOVA; *p*=0.4229). However, during the same period, high percentages of inactive larvae could still be observed after injection of 50 or 100 ng of venom proteins from WOPV (36.6±3.1% and 36.8±3.9% of inactive larvae, respectively). A good correlation between the dose of WOPV venom extracts injected and the paralysis observed was noted (*r*=0.89, *p*<0.05).

Together with the previous experiments, these results indicate that the reduction of host larvae activity recorded after parasitization by *A. tabida* is due to the venom injected by the ovipositing parasitoid female.

4. Discussion

This study shows that parasitism by *A. tabida* induces significant effects on the development of *D. melanogaster* larvae and that venoms on their own affect the survival and activity of host larvae.

Our experimental conditions of controlled parasitization allowed us to measure precisely the survival and encapsulation rates for each parasitoid egg laid by an *A. tabida* female and the mortality rates for each parasitized host. It is clearly shown that the french A1 strain of the parasitoid has a higher successful parasitism rate (SPR) on *D. melanogaster* than the dutch WOPV strain. These results confirm the virulent character of the A1 strain, previously studied in several works (Monconduit and Prévost, 1994; Kraaijeveld and van Alphen, 1994; Eslin et al., 1996; Eslin and Prévost, 1998). An interesting finding is that the SPR we measured in this study for A1 (74%) is greater than the one reported by Eslin and Prévost (1998) (58.6%) and obtained under partially controlled conditions, with first instar host larvae exposed by groups of thirty to one A1 female during 24 h. Additionally, we observed a lower mortality rate associated with parasitism by this strain (18.5%) than the one previously reported by these authors (36.2%). These differences may reflect the negative impact of superparasitism that is likely to occur, under partially controlled conditions on the survival chances of the parasitoid and its host.

The mechanism by which eggs from southern european strains of *A. tabida* can develop more successfully than northern ones on *D. melanogaster* has been partially investigated (Kraaijeveld and van Alphen, 1994; Eslin et al., 1996; Eslin and Prévost, 2000). The exochorion of these eggs is made of a fibrous layer responsible for their adhesiveness to the host tissues, thus protecting them from encapsulation by circulating hemocytes. This feature is generally considered to be the main virulence mechanism of *A. tabida*. Recently, we found that the A1 strain induces a significant reduction of hemolymph phenoloxidase activity in parasitized hosts during the first 72 h after parasitization (Moreau et al., 2000). As phenoloxidase constitutes an essential enzyme of the host immune system (Nappi et al., 1991), this alteration suggests that the virulence of the A1 strain could rely, at least partially, on other mechanisms than eggs adhesiveness. In addition, the finding in our present
study that approximately 19% of the WOPV eggs, devoid of a “sticky” chorion (Kraaijeveld and van Alphen, 1994; personal observations), can successfully develop does suggest the existence in A. tabida of a still unknown strategy of avoidance of the host immune system.

Considering the overall mortality associated with parasitism, we found that both strains induced mortality rates that were equivalent, but occurring at different stages during host development. Indeed, the A1 strain has a lethal effect mainly during the pupal stage of the host, whereas parasitism by WOPV induces mortality equally in larval and pupal stages. These results could be explained by the different physiological states between larvae succeeding or not in encapsulating their parasitoid. The free development of a parasitoid, as well as its elimination, are known to induce some important energetic costs for the host, and this has been studied in several interactions between D. melanogaster and its parasitoids (Vass and Nappi, 2001). The significant reductions of larval weight and increases in larval developmental time we observed in hosts parasitized by A1 or WOPV would, in fact, constitute some consequences for energy costs. Contrary to other parasitoid species, A. tabida cannot prevent the triggering of the immune reaction of its host, even in the case of virulent strains (Eslin and Prévost, 2000). As the A1 strain induced the most severe effects on the development of D. melanogaster, we can hypothesize that larvae hosting such a parasitoid may accumulate the costs associated both to their immune response and to the parasite’s development, leading to an energetic deficiency at the time they pupariate. Contrary to this, larvae parasitized by the non-virulent WOPV strain would mainly suffer from the meta-
bolic investment in their immune reaction. Thus, the differences in the mortality rates we measured may, to some extent, be related to the occurrence of these different costs.

Aside from directly competing for host nutrients, some parasitoid larvae have been shown to secrete factors modifying host hormonal balance and development (Brown and Reed-Larsen, 1991; Brown et al., 1993; Doury et al., 1995; Pfister-Wilhelm and Lanzrein, 1996; Alleyne et al., 1997; Reed and Brown, 1998; Hochuli et al., 1999). One could hypothesize that A. tabida larvae were involved in some of the effects we observed on host development and mortality. However, significant reductions of weight in parasitized larvae were noted as soon as 72 h post-parasitization and may occur before, whereas most of the A. tabida larvae hatch 24 to 48 h later (Kraaijeveld and van Alphen, 1994; Moncondrut and Prévost, 1994). If the involvement of the parasitoid larvae in the delay of host larval and pupal developments cannot be ruled out, it seems unlikely that they can directly cause the effect noted on host weight at 72 h post-parasitization.

Injections of venom extracts from the WOPV strain led to high mortality rates, which were not obtained when venom extracts from A1 were injected, except at the highest dose. This indicates that a direct involvement of WOPV venoms in the mortality observed upon parasitization by this strain cannot be completely excluded. The way by which A. tabida venoms could act is unknown and until now, their precise role in interactions between A. tabida and D. melanogaster remains to be determined. For the first time we present here evidence for their involvement in the induction of paralysis in host larvae immediately after parasitization, a transient phenomenon described by van Alphen (1982). Indeed, injection of venoms from A1 or WOPV induced, in a dose-depandent manner, the same effect as the one observed after parasitization. Interestingly, the strongest effect was induced by the non virulent WOPV strain, both after parasitization and venom injections. To our knowledge, this is the first report of intraspecific variation in the ability of a parasitoid to induce paralysis. It would be of interest to determine which venom components can cause such strain-specific effects, and whether the transient depletion of activity could be related to the mortality observed in hosts both after venom injections and natural parasitizations. Additional work is also needed to evaluate the effect of A. tabida venom on host development.

Asobara tabida can be distinguished from most of the other braconid endoparasitoids by several features involving different effects on the physiology of its hosts. Firstly, A. tabida is naturally deprived of endosymbiotic virus-like particles unlike any other braconid endoparasitoid studied (Eslin et al., 1996). Furthermore, this species also seems to lack teratocytes (personal observations), which are cells derived from the serosal membrane of the parasitoid egg and observed in several hosts endoparasitized by braconid species (Dahman and Vinson, 1993). Both these factors are known to greatly contribute to the survival of the parasitoid as they can notably affect the development of the host (Strand and Dover, 1991; Pennacchio et al., 1994; Nogushi et al., 1995; Gupta and Ferkovitch, 1998; Qin et al., 2000). For example, it has been shown that polydnavirus from the braconid Microplitis demolitor causes delayed development in the hosts Pseudoplusia includens and Heliotis virescens, associated with a decline in juvenile hormone esterase (Strand and Dover, 1991; Dover et al., 1995). Additionally, injection of teratocytes or teratocyte-secreted proteins from Microplitis croceipes into H. virescens larvae reduces protein synthesis and proliferation of the host fat body to the benefit of the developing parasitoid (Zhang et al., 1997; Schepers et al., 1998). Our results suggest that despite the absence of these important regulatory factors, A. tabida also has a significant impact on the development of its hosts. The present finding of a paralytic effect of A. tabida venom, even though transient, also differs from what has been shown in other endoparasitoid braconids, whose venoms are commonly considered as non-paralysing (Coudron, 1991). In contrast, several ectoparasitoid venoms have been reported to induce host developmental arrest (Rivers and Denlinger, 1995; Coudron and Brandt, 1996; Weaver et al., 1997) and most of them cause paralysis (Bocchino and Sullivan, 1981; Morales-Ramos et al., 1995; Doury et al., 1997). Taken together, the intriguing features of A. tabida suggest that this species may share some properties with ectoparasitoid braconids (ability to delay development in parasitized hosts and to induce paralysis) even though its lifestyle is clearly endoparasitic. This view is in accordance with Dowton et al. (1998) who suggested on the basis of molecular phylogeny that the Asobara genus, which belongs to a predominantly ectoparasitic clade, would have reverted to endoparasitism. By its unusual aspects, the A. tabida/D. melanogaster system thus appears as a useful model to investigate the complex relationships between endoparasitoids and their hosts and may provide new insights on the evolution of parasitic wasps.

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