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Resistance of *Drosophila suzukii* to the larval parasitoids *Leptopilina heterotoma* and *Asobara japonica* is related to haemocyte load

MATHILDE POYET[1,3], SEBASTIEN HAVARD[2], GENEVIEVE PREVOST[1], OLIVIER CHABRERIE[1], GERALDINE DOURY[1], PATRICIA GIBERT[3] and PATRICE ESLIN[1]

[1]Unité Ecologie et Dynamique des Systémes Anthropisés, Université de Picardie Jules Verne, Amiens, France, [2]Laboratoire Diversité, Génomes et Interactions Microorganismes-Insectes, Université de Montpellier 2, Montpellier, France and [3]Laboratoire de Biométrie et Biologie Evolutive, Université Lyon, Lyon 1, France

Abstract. Unlike other *Drosophila* species, the invasive *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) shows a remarkable pest status. Among the physiological traits that may explain the high level of resistance to parasitoids of *Drosophila* larvae, the haemocyte load is shown repeatedly to play an important role. To determine whether haemocyte load can explain immunity resistance of *D. suzukii* to parasitoids, the haemocytes of parasitized and healthy larvae are quantified in two Japanese and three French populations of *D. suzukii*. Parasitization tests are conducted with two larval parasitoids: the paleartic *Leptopilina heterotoma* Thomson (Hymenoptera: Figitidae) and the Asian *Asobara japonica* Belokobylskij (Hymenoptera: Braconidae). Based on morphological and functional criteria, *D. suzukii* has classes of haemocytes similar to those described in *Drosophila melanogaster*. However, healthy larvae of the five populations tested possess particularly large numbers of haemocytes compared with *D. melanogaster*. Haemocyte load is also higher in larvae from the French populations than in the Japanese strains. The ability of *D. suzukii* larvae to encapsulate eggs of *L. heterotoma* is associated with a particularly high load of circulating haemocytes. However, it is notable that *A. japonica* induces a strong depression of the haemocyte population in this resistant host associated with an inability to encapsulate parasitoid eggs. The results show that the cellular immune system plays a major role in the failure of larval parasitoids to develop in most instances in larvae of *D. suzukii*, possibly contributing to the success of this species as an invader.

Key words. *Asobara japonica*, *Drosophila suzukii*, haemocyte concentration, immune resistance, *Leptopilina heterotoma.*

Introduction

The increase of invasive insect species has been recorded worldwide within the past decade (Huang *et al*., 2011). Although invasions are known to be largely a result of human activities through the development of international trade and intercontinental transportation, local causes of the success of individual species are still a matter of debate. Two major and complementary hypotheses contribute to our understanding of the success of invasive species: (i) the enemy release hypothesis (Keane & Crawley, 2002) considers that the success of a species outside its native range is the result, at least partly, of the absence or the reduced efficiency of natural enemies, and (ii) facing this lack of enemy, the evolution of increased competitive ability hypothesis (Blossey & Nötzold, 1995) predicts that non-indigenous species will be more productive and thus successful in habitats into which they are introduced compared

Correspondence: Patrice Eslin, Laboratoire de Bio-écologie des Insectes Phytophages et Entomophages, Université de Picardie Jules Verne, 33 rue Saint Leu, F-80039 Amiens Cedex, France. Tel.: +33 (0)3 22 82 75 47; e-mail: patrice.eslin@u-picardie.fr

with their native habitats. Therefore, the success of an invasion depends not only on environmental factors of the colonized habitat (invasibility), but also on the biological characteristics of the invasive species (invasiveness). These two hypotheses are tested mainly in plants (Keane & Crawley, 2002; Blumenthal, 2006; Facon *et al*., 2006; Liu & Stiling, 2006) but are less studied in animals, especially insects. According to the European DAISIE (Delivering Alien Invasive Species Inventories in Europe) programme conducted between 2005 and 2008, 85% of the 1517 exotic invertebrate species already established on the European continent are insects (DAISIE, 2009). Nevertheless, the biological traits determining the success of these invasions are still poorly understood.

Drosophila suzukii Matsumura (Diptera: Drosophilidae) is a *Drosophila* species belonging to the *melanogaster* group. Native to Asia, it has recently colonized the American (Hauser *et al*., 2009) and European (Calabria *et al*., 2010) continents. The fly infests a variety of commercial fruits, including raspberry, strawberry, grape and tree fruits (cherry, kiwi, fig, apple, plum, peach) (Hauser *et al*., 2009; Steck *et al*., 2009). Its invasion is extremely fast and raises many concerns from American and European producers of red fruit facing important economic losses (Goodhue *et al*., 2011; Walsh *et al*., 2011). *Drosophila suzukii* represents an excellent biological model for understanding better the conditions and factors allowing a successful biological invasion. Researchers already benefit from a huge knowledge on *Drosophila* biology, genetics and ecology regarding competitive interactions and the selective pressure exerted by natural enemies (Hemmat & Eggleston, 1988; Fleury *et al*., 2009; Verspoor & Haddrill, 2012). The same data on *D. suzukii* would provide a greater insight into the mechanisms underlying successful biological invasions. From a more applied perspective, this would allow the opportunities available to halt the spread of this pest to be better identified.

Among natural enemies, parasitoids constitute a group whose impact on insect communities is now well described. They play a key role by controlling insect pests and affecting invasive populations (Magal *et al*., 2008). In host insects, the process of encapsulation is the main physiological defence described against endoparasitoids. The parasite is enclosed within a capsule, which undergoes a progressive blackening as a result of melanization (Carton & Nappi, 1997). The interaction between frugivorous *Drosophila*, especially *D. melanogaster*, and their parasitoids is the subject of many studies (Prévost, 2009). In the larval stages of *D. melanogaster*, three main types of haemocytes are described that play a role in the formation of capsules. The plasmatocytes represent the most abundant haemocyte type circulating in the haemolymph. These small rounded cells, also called *Drosophila* plasmatocytes (Ribeiro & Brehélin, 2006), are not only involved in phagocytosis, but also in the initial and terminal steps of encapsulation (Russo *et al*., 1996). Crystal cells, the least abundant haemocyte type, contain elements (substrate and enzymes) of the phenoloxidase cascade. They are involved in the melanization of the capsules, as well as in clotting and the production of cytotoxic radicals (Evans *et al*., 2003; Meister, 2004; Carton *et al*., 2008). The third haemocyte type is the lamellocyte, whose structure and function are very similar to those exhibited by lepidopteran plasmatocytes (Ribeiro & Brehélin, 2006). Lamellocytes are considered as the major capsule-forming haemocyte type in *Drosophila* (Brehélin & Duvic, 1999; Havard *et al*., 2009). These cells are large and round flattened, and represent approximately 6% of the total haemocyte count in non-immune-challenged *Drosophila* larvae (Eslin & Prévost, 1996, 1998; Lanot *et al*., 2001). They participate in the encapsulation reaction against intruders that exceed the size limit for phagocytosis by plasmatocytes (Eslin *et al*., 2009). Both the number and maturity of the haemocytes circulating at the time of parasitization may be important factors influencing the success of the encapsulation process in *Drosophila* species (Eslin & Prévost, 1998, 2000).

Recent studies show that different strains of *D. suzukii* [a French one (Chabert *et al*., 2012) and two American ones (Kacsoh & Schlenke, 2012)] are resistant to the majority of the larval parasitoids tested. Increased resistance against larval parasitoids also appears to be associated with a high haemocyte load in *D. suzukii* (Kacsoh & Schlenke, 2012). Kacsoh & Schlenke (2012) compare the amounts of constitutive haemocytes between *D. suzukii* and *D. melanogaster* larvae either after a wound inflicted by a sterile needle or after parasitization by a strain of the parasitoid *Leptopilina boulardi* (Hymenoptera: Figitidae) that is considered avirulent to *D. melanogaster* host.

In the present study, the haemocytes of *D. suzukii* larvae parasitized by the Paleartic parasitoid species *Leptopilina heterotoma* Thompson (Hymenoptera: Figitidae) or the Asian species *Asobara japonica* Belokobylskij (Hymenoptera: Braconidae) are described and quantified. Both of these larval parasitoid species are known to be highly virulent to *D. melanogaster* and their effects on the regulation of the host larval immune system are known (Rizki & Rizki, 1984; Mabiala-Moundoungou *et al*., 2010). Five strains of *D. suzukii* (three collected in France recently and two others from Japan) are used to test whether *D. suzukii* suffers a decline of its circulating haemocyte population after parasitization, and also whether this may be linked to the success or failure of the development of the parasitoid.

Materials and methods

Insects

Five strains of *D. suzukii* were tested. The three French strains were collected using banana traps. One strain was collected in Sainte-Foy-les-Lyon (latitude: 45°7′) in 2010, whereas the other two were collected in 2011 in Gotheron (latitude: 44°4′) and Compiègne (latitude: 49°24′). The two Japanese strains were collected in Tokyo (strain E-15014 OGH06-03) in 2006 and in Tsushima (strain E-15017 TSM92) in 2008, and were provided by Masayoshi Watada (Ehime University, Japan). For phagocytosis assay, one strain of *D. melanogaster* originating from Sainte-Foy-les-Lyon, France (collected in 1994), was used. All strains were mass reared at 20 °C and were fed a regular banana *Drosophila* diet (Chabert *et al*., 2012) and maintained under an LD 13 : 11 h photocycle.

Two species of *Drosophila* larval parasitoids were used in this experiment: the cynipid *L. heterotoma* Thomson (Hymenoptera: Figitidae) was collected in October 2010 in France (Rhône valley) using banana traps, and the Japanese strain of *A. japonica* Belokobylshij (Hymenoptera: Braconidae) was graciously provided by Professor J. van Alphen (Leiden University, The Netherlands). Both parasitoid species were mass reared in the laboratory at 20 °C on *D. melanogaster* under a LD 13 : 11 h photocycle.

Procedure for controlled parasitization

To ensure that *Drosophila* larvae would develop synchronously, eggs of *D. suzukii* and *D. melanogaster* were collected after a 4-h oviposition period. The second-instar larvae were then exposed to females of either *L. heterotoma* or *A. japonica* for 96 h. Parasitization was observed thereafter under a stereomicroscope. After a single oviposition by a parasitoid female, each parasitized *D. suzukii* larva was removed individually and placed in a tube containing artificial banana *Drosophila* diet.

Phagocytosis assay

The production of haemocytes was stimulated by controlled parasitization of third-instar larvae (7 days old at 20 °C) by *L. heterotoma* 72 h before phagocytosis assay. For phagocytosis assays, 20 nL of fluorescent polystyrene latex microspheres (approximately 11 000 beads; Fluoresbrite® – YG Microspheres, Polysciences Europe GmbH, Germany) were microinjected into each larva (Havard *et al*., 2012). Thirty minutes after the injection of the latex beads, the haemolymph was collected and the haemocytes were observed using phase contrast microscopy under indirect ultraviolet illumination.

Haemocyte counts

Ninety larvae per *Drosophila* strain were bled. Thirty of the larvae were parasitized by *A. japonica* and another group of 30 larvae were parasitized by *L. heterotoma*. The remaining 30 larvae were unparasitized (control). Haemolymph was collected from *Drosophila* larvae by sectioning the body posteriorly to the mandibules with opthalmic scissors (Eslin & Prévost, 1996). After bleeding, each parasitized larva was dissected to check for the presence of a parasitoid egg or larva and the status of parasitization success in terms of encapsulation. Hosts containing an encapsulated parasitoid (resistant hosts) or a non-encapsulated egg or larva (susceptible hosts) were separated. The haemocyte counts were made 72 h after parasitization when parasitized and nonparasitized larvae (7-day-old third-instar larvae raised at 20 °C) provided sufficient haemolymph (approximately 0.2 μL) to be applied without dilution onto a Thoma haemocytometer slide (Eslin & Prévost, 1996). Haemocyte counts were obtained by applying haemolymph onto this slide and then immediately counting the number of cells. A total haemocyte count (i.e. total number of immune cells) and a differential haemocyte count (i.e. number of plasmatocytes, lamellocytes and crystal cells) were performed. Haemocyte counts were expressed as the number of cells per mm^3 of haemolymph.

Development experiment

In this experiment, parasitized and nonparasitized larvae were allowed to complete their development at 20 °C until emergence as an adult *Drosophila* or parasitoid. Parasitization was achieved under controlled conditions as described above. In each strain of *D. suzukii*, 100 repetitions were established for each treatment. Adult *Drosophila* and parasitoids were counted at emergence. The mean number of flies emerging from the unparasitized control gave an estimate of the viability of each *D. suzukii* strain in the absence of parasitism. *Drosophila* adults carrying a capsule were counted as hosts resistant to parasitism. The number of emerged *A. japonica* or *L. heterotoma* adults gave an estimate of the percentage of susceptible larvae hosting a developing parasitoid. The difference between the number of flies emerging from the unparasitized control and the total number of insects emerging from the parasitized treatment gave an estimate of the number of parasitized larvae that died during development.

The total encapsulation rate (TER), the successful parasitism development rate (SPR) and the mortality rate of parasitized larvae (MR) were estimated as follows:

- TER = (number of *Drosophila* with a capsule/number of parasitized *Drosophila*) × 100
- SPR = (number of *Drosophila* permitting parasitoid development/number of parasitized *Drosophila*) × 100
- MR = (number of parasitized larvae that died during development/number of parasitized *Drosophila*) × 100

Statistical analysis

For each strain of *D. suzukii*, the number of haemocyte cells was compared between parasitized and unparasitized (control) larvae using analysis of variance (ANOVA; $P < 0.05$). ANOVA was also used to compare the number of haemocytes between control and parasitized larvae for each of the five strains of *D. suzukii*. Normality and homosedasticity assumptions were checked before the analyses. The relationship between encapsulation rates and mean total haemocyte counts was assessed using linear regressions. Statistical analyses were performed using Statistica, version 6.1 (StatSoft, Inc., Tulsa, Oklahoma).

Results

D. suzukii plasmatocytes show phagocytosis ability

Larvae previously immunostimulated by *L. heterotoma* parasitization produced a large quantity of haemocytes. As

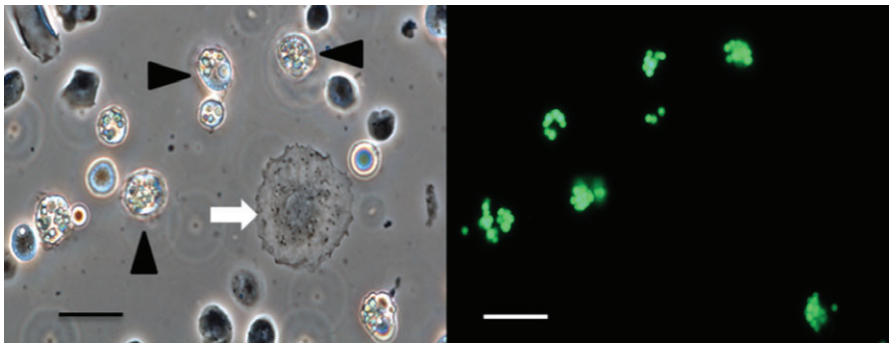

Fig. 1. Phagocytosis by haemolymph plasmatocytes in third-instar larvae of *Drosophila suzukii* at 72 h post-parasitization by the parasitoid *Leptopilina heterotoma* and 30 min post-injection of 20 nL fluorescent polystyrene latex microspheres into each larva. Phase contrast images are shown on the left. Although lamellocytes are devoid of latex beads, plasmatocytes have engulfed the foreign bodies. Black arrowhead, plasmatocyte; white arrow, lamellocyte. Scale bar = 20 μm.

in *D. melanogaster*, most of the plasmatocytes of *D. suzukii* larvae were found to engulf the injected fluorescent latex microscopic beads, whereas no fluorescence was observed in the lamellocytes (Fig. 1).

D. suzukii larvae have a very high load of circulating haemocytes

The three types of *D. suzukii* haemocytes (plasmatocytes, lamellocytes and crystal cells) were morphologically similar to those of *D. melanogaster*. By contrast to *D. melanogaster* (total haemocyte count of approximatelly 5000 cells per mm^3 of haemolymph; Eslin & Prévost, 1998), *D. suzukii* showed a very high haemocyte load (Fig. 2). Total haemocyte counts revealed that the Japanese strains of *D. suzukii* produced up to five-fold more haemocytes (up to 25 000 cells) than *D. melanogaster* (data for unparasitized larvae). Also, the French strains of *D. suzukii* had almost twice as many haemocytes (over 40 000 cells per mm^3 of haemolymph) as the Japanese strains ($F = 11.89$, d.f. = 145, $P < 0.0001$). The data also show that the haemocyte loads of the French *D. suzukii* strains are eight-fold higher than the mean for the *D. melanogaster* strain. The results from the haemocyte counts (Table 1) showed that these observations were consistent for the three categories of haemocytes: plasmatocytes, lamellocytes and crystal cells.

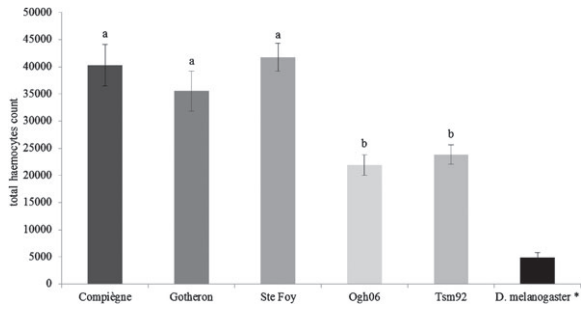

Fig. 2. Total haemocyte counts (mean ± SE) in unparasitized *Drosophila suzukii* larvae from three French strains (Compiègne, Gotheron and Sainte-Foy-les-Lyon) and two Japanese strains (Ogh 06 and Tsm 92). Different letters indicate a significant difference between strains ($P < 0.05$). For comparison, data showing haemocyte numbers in unparasitized *Drosophila melanogaster* larvae were obtained from Eslin & Prévost (1998) (*).

Encapsulation of L. heterotoma eggs is associated with high total haemocyte count in D. suzukii larvae

After parasitization by *L. heterotoma*, the larval development of the host *D. melanogaster* was never completed, whereas larvae of all strains of *D. suzukii* showed the ability to encapsulate the eggs of this parasitoid species (Table 2). In particular, the French strains of *D. suzukii* showed a significantly higher ability to encapsulate this parasitoid than the Japanese strains.

Unexpectedly, *L. heterotoma*, a virulent parasitoid of *D. melanogaster* was unable to parasitize *D. suzukii* larvae successfully. The total haemocyte counts recorded in the three French *D. suzukii* strains were much higher than those recorded in the two Japanese strains (Fig. 3). The three French strains showed very high counts of circulating haemocytes after parasitization by *L. heterotoma* (up to 160 000 cells per mm^3 of haemolymph), and counts that were significantly higher ($F = 5.68$, d.f. = 145, $P < 0.0001$) than for the two Japanese strains (up to 91 000 cells). One of the more remarkable observations is that parasitization by *L. heterotoma* induced a decrease in the number of circulating haemocytes in *D. melanogaster*, whereas it led to a large increase in the total haemocyte counts of *D. suzukii*.

Figure 4 shows that total haemocyte concentrations recorded in larvae 72 h post-parasitization by *L. heterotoma* and encapsulation rates were highly correlated ($r^2 = 0.93$, $P = 0.008$). Observations on lamellocytes of *D. suzukii* showed that larvae parasitized by *L. heterotoma* were characterized by bipolar or elongated cells, whereas unparasitized (control) larvae and those parasitized by *A. japonica* exhibited discoidal shaped cells. Also, the percentage of bipolar lamellocytes was lower in the Japanese strains (Table 1). Finally, in all strains of *D. suzukii*, only the numbers of crystal cells were significantly

Table 1. Differential and total haemocytes counts (THC) (mean ± SE) in *Drosophila suzukii* larvae from three French strains (Compiègne, Gotheron and Sainte-Foy-les-Lyon) and two Japanese strains (Ogh 06 and Tsm 92) unparasitized (control) or parasitized by *Leptopilina heterotoma* or *Asobara japonica*.

		Total number of haemocytes per mm^3 of haemolymph			
Drosophila suzukii strains	Conditions	Plasmatocytes	Lamellocytes (% bipolar lamellocytes)	Crystal cells	Number of larvae
Compiègne	Controls	36 369 ± 3621	2281 ± 502	1703 ± 166	30
	Parasitized by *Leptopilina heterotoma*	128 489 ± 8122	33 458 ± 2416 (10.1 ± 1.5)	1031 ± 174	30
	Parasitized by *Asobara japonica*	13 989 ± 1411	1093 ± 285	229 ± 76	30
Gotheron	Controls	33 151 ± 3667	1265 ± 151	1114 ± 175	30
	Parasitized by *Leptopilina heterotoma*	100 234 ± 7416	29 515 ± 2968 (11 ± 1.4)	671 ± 109	30
	Parasitized by *Asobara japonica*	13 479 ± 1042	1604 ± 257	406 ± 105	30
Ste Foy	Controls	36 489 ± 2128	3229 ± 713	2072 ± 288	30
	Parasitized by *Leptopilina heterotoma*	130 572 ± 6180	29 750 ± 1180 (8.8 ± 1.8)	1260 ± 175	30
	Parasitized by *Asobara japonica*	10 385 ± 1359	1489 ± 623	333 ± 79	30
Ogh 06	Controls	18 671 ± 1847	1940 ± 228	1520 ± 181	30
	Parasitized by *Leptopilina heterotoma*	63 580 ± 3244	22 005 ± 1705 (7.8 ± 2.1)	39 ± 31	30
	Parasitized by *Asobara japonica*	13 345 ± 676	2187 ± 189	427 ± 89	30
Tsm 92	Controls	20 812 ± 1682	1484 ± 333	1854 ± 194	30
	Parasitized by *Leptopilina heterotoma*	68 872 ± 5967	22 008 ± 1536 (6.5 ± 2.1)	489 ± 103	30
	Parasitized by *Asobara japonica*	11 875 ± 1024	2137 ± 295	350 ± 82	30
Drosophila melanogaster	Controls[a]	4528 ± 856	28 ± 18	337 ± 88	21
	Parasitized by *Leptopilina heterotoma*[b]	4129 ± 726	111 ± 31	127 ± 34	20
	Parasitized by *Asobara japonica*[c]	2552 ± 320	158 ± 25	22 ± 10	52

For comparison, data for *D. melanogaster* larvae in the same conditions were obtained from: [a]Eslin & Prévost (1998), [b]Havard *et al*. (2012) and [c]Mabiala-Moundoungou *et al*. (2010) (analysis of variance; $P < 0.05$).

Table 2. Total encapsulation rate, successful parasitism rate and mortality rate in *Drosophila suzukii* larvae from three French strains (Compiègne, Gotheron and Sainte-Foy-les-Lyon) and two Japanese strains (Ogh 06 and Tsm 92) parasitized by *Leptopilina heterotoma.*

Drosophila suzukii strains	Total encapsulation rate (%)	Successful parasitism rate (%)	Mortality rate (%)
Compiègne	87 ± 0.2	0	13 ± 0.2
Gotheron	77 ± 0.4	0	23 ± 0.4
Ste Foy	80 ± 0.7	0	20 ± 0.7
Ogh 06	62 ± 0.7	0	38 ± 0.7
Tsm 92	59 ± 0.4	0	41 ± 0.4
Drosophila melanogaster	0	85	15

Results were recorded after the insects (parasitoids and resistant hosts) had completed their development. Percentages (mean ± SE) were estimated from 100 larvae in each *Drosophila* strain. For comparison, data for *D. melanogaster* larvae were obtained from Havard *et al*. (2012).

reduced in larvae parasitized by *L. heterotoma* compared with unparasitized larvae ($F = 9.903$, d.f. = 145, $P < 0.0001$).

Parasitism by A. japonica generates a significant drop of the total haemocyte count in D. suzukii

Although larvae of *D. melanogaster* were totally unable to encapsulate eggs of *A. japonica*, the five strains of *D. suzukii* were able to encapsulate them, although encapsulation occurred at different rates (Table 3). Among the five *D. suzukii* strains tested, the Compiègne strain showed the greatest ability to encapsulate *A. japonica* eggs (ER = 26%) (Table 3). Host larvae of both *D. melanogaster* and *D. suzukii* were also able to support the successful development of *A. japonica*, although the success of parasitoid development (SPR) was higher in *D. melanogaster* (76%) than in *D. suzukii* (50–63%) (Table 3).

In all five strains of *D. suzukii*, parasitization by *A. japonica* generated a significant drop in the total number of haemocytes (Fig. 3). This was observed for the three haemocyte types: plasmatocytes, lamellocytes and crystal cells. No significant difference in the number of haemocytes was observed between the five strains of *D. suzukii* infested by *A. japonica* (approximately 12 000 and 15 000 cells). However, the number of circulating haemocytes was five-fold greater in *D. suzukii* than in *D. melanogaster* when larvae were parasitized by *A. japonica.*

Discussion

The haemocytes of *D. suzukii* are morphologically and functionally similar to those described in *D. melanogaster* (Brehélin, 1982; Kacsoh & Schlenke, 2012). As in *D. melanogaster* (Lanot *et al*., 2001; Lavine & Strand, 2002; Meister, 2004), plasmatocytes represent the main, most abundant haemocytic cell type in unparasitized *D. suzukii*

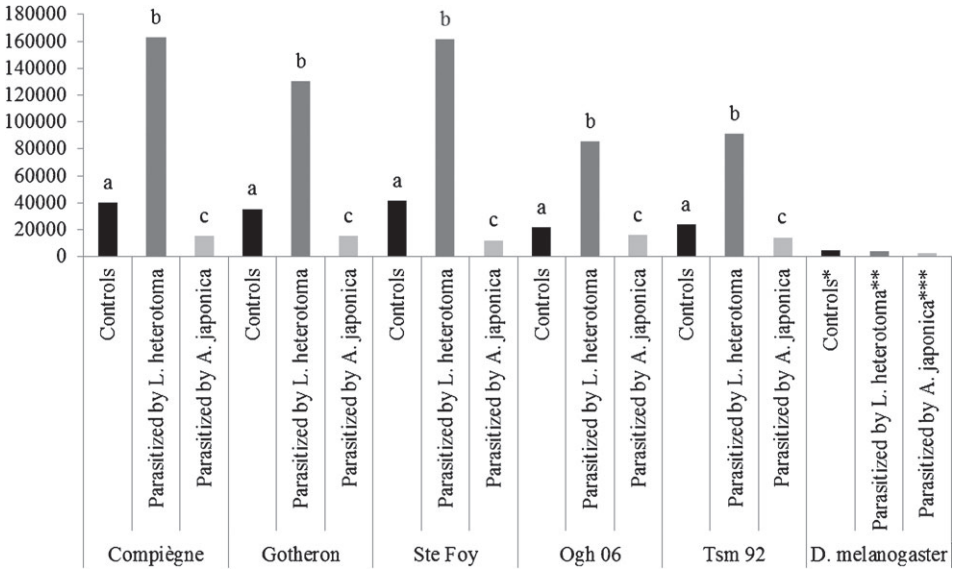

Fig. 3. Total haemocytes counts (mean ± SE) in *Drosophila suzukii* larvae from three French strains (Compiègne, Gotheron and Sainte-Foy-les-Lyon) and two Japanese strains (Ogh 06 and Tsm 92) unparasitized (control) or parasitized by *Leptopilina heterotoma* or *Asobara japonica*. Different letters indicate a significant difference between blocks ($P < 0.05$). For comparison, haemocyte numbers in *Drosophila melanogaster* larvae under the same conditions were obtained from Eslin & Prévost (1998) (*), Havard *et al*. (2012) (**) and Mabiala-Moundoungou *et al*. (2010) (***) (analysis of variance; $P < 0.05$).

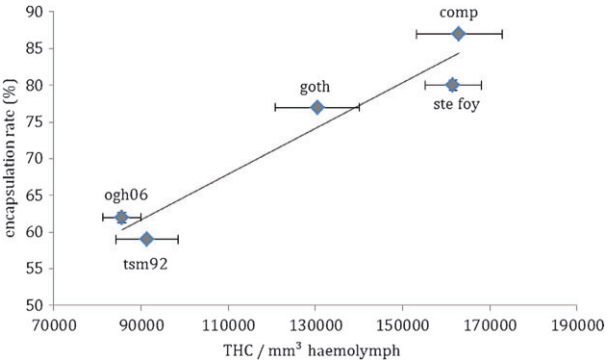

Fig. 4. Correlation between encapsulation rate (TER) and the total haemocyte count (THC; mean ± SE) in three French strains (Compiègne, Gotheron and Sainte-Foy-les-Lyon) and two Japanese strains (Ogh 06 and Tsm 92) of *Drosophila suzukii* larvae parasitized by *Leptopilina heterotoma* ($r^2 = 0.9267$, $P = 0.008$).

Table 3. Total encapsulation rate, successful parasitism rate and mortality rate in *Drosophila suzukii* larvae from three French strains (Compiègne, Gotheron and Sainte-Foy-les-Lyon) and two Japanese strains (Ogh 06 and Tsm 92) parasitized by *Asobara japonica.*

Drosophila suzukii strains	Total encapsulation rate (%)	Successful parasitism rate (%)	Mortality rate (%)
Compiègne	26 ± 0.5	50 ± 1.1	24 ± 1.4
Gotheron	10 ± 1.1	63 ± 0.5	27 ± 1.3
Ste Foy	8 ± 0.4	56 ± 0.6	36 ± 0.8
Ogh 06	7 ± 0.2	56 ± 0.9	37 ± 0.8
Tsm 92	6 ± 0.3	60 ± 2.1	34 ± 1.9
Drosophila melanogaster	0	76.8 ± 3.6	23.2 ± 3.6

Results were recorded after the insects (parasitoids and resistant hosts) had completed their development. Percentages (mean ± SE) were estimated from 100 larvae in each *Drosophila* strain. For comparison, data for *D. melanogaster* larvae were obtained from Mabiala-Moundoungou *et al*. (2010).

larvae, although crystal cells and lamellocytes are also found. In unparasitized larvae of *D. suzukii*, the lamellocytes range from 4% of the total number of circulating haemocytes (in the Gotheron strain) to 10% (in the OGH 06 strain), a result that is consistent with what is already known for the *melanogaster* subgroup of *Drosophila* (Eslin *et al*., 2009) but different from the results reported in other subgroups (Havard *et al*., 2009, 2012). By contrast, the total haemocyte counts measured in *D. suzukii* are quite unusual. To the best of current knowledge, the numbers of circulating haemocytes in *D. suzukii* larvae are the highest ever recorded for *Drosophila* larvae of the *melanogaster* group (for a comparison, see Eslin & Prévost, 1998). The Japanese strains of *D. suzukii* carry four- to five-fold more haemocytes than *D. melanogaster*, whereas the French strains have eight-fold higher haemocyte counts than *D. melanogaster*. Such differences between the French and the Japanese or American geographical strains may be related to genetic variations, which may contribute to the success of biological invasions (Lindholm *et al*., 2005), at the same time as reflecting the rapid evolutionary changes likely to occur among invasive species (Sakai *et al*., 2001).

Larvae of the five *D. suzukii* strains show a prompt haemocytic response after parasitization by *L. heterotoma*.

The high haemocyte load and the ability to trigger an increase in circulating plasmatocytes and lamellocytes quickly when challenged by *L. heterotoma* are correlated with the immune resistance to parasitoids. A correlation between the concentration of circulating haemocytes and the aptitude to form a haemocytic capsule is demonstrated for species in the *melanogaster* subgroup (Eslin & Prévost, 1998). The present study shows that such a correlation not only applies to another host-parasitoid association (*D. suzukii – L. heterotoma*) but also is true at the intraspecific level as well, considering variations among local strains of *D. suzukii*. Crystal cells are the only haemocyte type to show a decline in number after parasitization by *L. heterotoma*. These cells are considered to be main carriers of some of the enzymes of the phenoloxidase system (Meister, 2004). A quantitative decrease in the circulating crystal cells could either be the result of cell lysis releasing opsonizing factors in the early stages of the defence reaction or a parasitoid-induced disruption of the functioning of the phenoloxidase system (Nappi *et al*., 2009). Bipolar lamellocytes range from approximately 6.5% (Tsm92 strain) to 11% (Gotheron strain) in parasitized larvae of *D. suzukii*. Virulent strains of *L. boulardi* and *L. heterotoma* exert an active depressive effect on the immune system of their *Drosophila* hosts (Rizki & Rizki, 1991; Dubuffet *et al*., 2009). The proportion of affected lamellocytes can represent up to 68% of haemocytes in *D. melanogaster* larvae parasitized by *L. heterotoma* (Rizki & Rizki, 1991). The molecular factors responsible for this effect in *D. melanogaster* are located in the wasp's venom and have been identified (Chiu *et al*., 2006; Dubuffet *et al*., 2009; Gueguen *et al*., 2011). The results of the present study suggest that the immunosuppressive factors of *L. heterotoma* could act on some haemocytes of *D. suzukii* larvae, in particular the lamellocytes and possibly the crystal cells, although this effect may not be sufficient to prevent the encapsulation reaction. One possible explanation for the observed difference between the immune response towards *L. heterotoma* in *D. suzukii* and *D. melanogaster* (Rizki & Rizki, 1991) could be that European populations of *L. heterotoma* are not adapted to this new exotic host. This hypothesis is consistent with the predictions of the enemy release hypothesis.

Drosophila suzukii appears to be the first species of the *melanogaster* subgroup to resist the immunosuppressive properties of the parasitoid *L. heterotoma* by encapsulating its eggs. Encapsulation of *A. japonica* by *D. suzukii* remains at a low level, as also reported by Chabert *et al*. (2012) and Kacsoh & Schlenke (2012). The present study shows that the number of circulating haemocytes is consistently reduced in the five *D. suzukii* strains 72 h post-parasitization by *A. japonica*. Parasitism by *A. japonica* also affects haemocyte load and the phenoloxidase activity of *D. melanogaster* (Mabiala-Moundoungou *et al*., 2010). Therefore, *A. japonica*, which exists in sympatry with *D. suzukii* in Japan, appears to exert immunosuppressive effects on larvae of *D. suzukii* that appear to be similar to those described in *D. melanogaster*. However, *D. suzukii* is able to encapsulate eggs of *A. japonica* eggs at rates ranging from 6% (Tsm 92 strain) to 26% (Compiègne strain), which are never observed in *D. melanogaster* larvae. The exceptionally high haemocyte load of *D. suzukii* larvae at the time of parasitization is considered as likely contributing to their resistance towards the highly virulent *A. japonica*. The same phenomenon is reported for the interaction between the closely-related parasitoid *Asobara citri* and host *Drosophila simulans* (Moreau *et al*., 2005).

Cynipids from the *Leptopilina* genus and Braconids from the *Asobara* genus are the most studied larval endoparasitoids of *Drosophila* to date. Over the last 20 years, an extensive knowledge has accumulated on the genetics and physiology of the interactions between hosts of the *melanogaster* subgroup and their larval parasitoids. Molecular data regarding the virulence strategies of these parasitoids have also been characterized (Chiu *et al*., 2006; Colinet *et al*., 2007; Dubuffet *et al*., 2009; Prévost *et al*., 2011). This knowledge should facilitate our understanding of the interactions between *D. suzukii* and potential larval parasitoids. Ongoing studies on the molecular basis of the virulence of *A. japonica* towards *D. suzukii* are expected to bring some insight to both fundamental and applied research, in particular regarding the control of this invasive fruit fly.

In conclusion, the results of the present study support the hypothesis that the cellular immune system of *D. suzukii* plays a major role in the failure, in most instances, of larval parasitoids to develop in larvae of this species. The results suggest that *D. suzukii* has evolved a more potent cellular immune response than any other *Drosophila* species (i.e. tested to date), providing resistance to the guild of parasitoids potentially present in its habitat. High numbers of haemocytes may allow better protection from several physiological constraints and could improve the ability to respond quickly to environmental changes. The rapid dissemination of *D. suzukii* in North America and Europe demonstrates the remarkable invasion capability of this species. In addition to other life-history traits that are presently under study, the high haemocyte load of *D. suzukii* suggests that this species is particularly well armed for successful invasion. Identifying the biological factors involved in the success of an invasion is an important step towards defining suitable measures for invader management. According to the evolution of increased competitive ability hypothesis (Blossey & Nötzold, 1995), a host could reallocate resources from defence mechanisms into growth and development in the absence of effective natural enemies. Populations might be evolving towards an opposite direction. By contrast, it appears that *D. suzukii*, which is invading Europe, avoids the competitor species that it may have in its exotic range because it is the only *Drosophila* species using unripe fruits. Such a gain of energy could be reallocated into the production of haemocytes, therefore improving the resistance of the fly to parasitoids, a condition that would certainly contribute to the success of its invasion.

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