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Short communication

Effects of *Asobara japonica* venom on larval survival of host and non-host *Drosophila* species

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Right running title**:** *Effects of* Asobara japonica *venom*

Left running title: S. X. Furihata and M. T. Kimura

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Abstract. Ovipositing *Asobara japonica* females inject venom (containing paralysis-inducing factors) immediately after the insertion of their ovipositors into *Drosophila* larvae, and lay eggs a little later. Interruption of their oviposition behaviour before egg laying causes high larval mortality in host *Drosophila* species, while normal oviposition does not. This suggests that venom of this parasitoid is toxic to larvae of these host species but its toxicity is suppressed by factor(s) provided by parasitoid females at the time of laying egg or by parasitoid embryos developing in the hosts. On the other hand, venom does not show toxicity to larvae of non-host *Drosophia* species. Possible functions of venom are discussed.

Key words. *Asobara*, *Drosophila*, parasitism, toxicity, venom.

Introduction

Parasitoids provide hosts with various substances, viruses or virus-like particles to modify the host's behaviour, metabolism or development (Vinson & Iwantsch, 1980; Strand, 1986). Venom is an important source of such substances and viruses and has diverse functions such as induction of paralysis, interference of host development and/or suppression of host immune responses (Vinson & Iwantsch, 1980; Strand, 1986; Moreau & Guillot, 2005). In addition, venom sometimes shows toxicity to host and/or non-host insects (Rivers *et al*., 1993; Ergin *et al*., 2006). An example is seen in *Asobara tabida*. Host (*Drosophila melanogaster*) larvae artificially injected with only venom of this parasitoid (the WOPV strain from the Netherlands) suffer high mortality at the larval stage (Moreau *et al*., 2002). However, host larvae naturally parasitized by this parasitoid usually develop to pupae, although they are eventually killed by parasitoid larvae before eclosion. This suggests that this parasitoid provides hosts with some factors that suppress the toxicity of venom.

In this paper, we report effects of *Asobara japonica* Belokobylskij (Hymenoptera: Braconidae) venom on host and non-host *Drosophila* (Diptera: Drosophilidae) species. *Asobara japonica* is a larval-pupal parasitoid (i.e., adult parasitoid oviposits into host larva and parasitoid larva kills host after its pupariation), and uses a variety of *Drosophila* species as hosts (Mitsui *et al*., 2007; Ideo *et al*., 2008). Its populations in the main islands of Japan are parthenogenetic, whereas those in the southwestern islands are sexual (Mitsui *et al*., 2007).

Materials and methods

Experimental strains and rearing

Experiments were made with a parthenogenetic *A. japonica* strain and five *Drosophila* species, *D. simulans* Sturtevant, *D. lutescens* Okada, *D. auraria* Peng, *D. bipectinata* Duda and *D. ficusphila* Kikkawa et Peng. The first three *Drosophila* species are natural hosts of *A. japonica*, whereas the last two species are non-hosts (Mitsui *et al*., 2007; Ideo *et al*., 2008). The larval (egg to pupariation) period is about 6 days in *D. simulans*, *D. lutescens* and *D. bipectinata* and 7 days in *D. auraria* and *D. ficusphila* at 23 °C.

The parasitoid strain and the *D. simulans*, *D. lutescens*, and *D. auraria* strains originated from females collected in Tokyo (35.7 °N in latitude), and the *D. bipectinata* and *D. ficusphila* strains from those collected in Iriomote-jima (24.4 °N), a subtropical island of Japan. These strains were maintained for a few years in the laboratory. The *Drosophila* strains were reared with cornmeal-malt medium, and the parasitoid strain was reared using *D. simulans* larvae as host. Maintenance and experiments were carried out at 23 °C.

Oviposition behaviour and experiments

Drosophila larvae inserted withthe ovipositor of *A. japonica* females become paralysed within a few seconds. Venom that contains paralysis-inducing factor(s) would be injected immediately after the insertion of the ovipositor. Some seconds after *Drosophila* larvae are paralysed, parasitoid females vibrate their ovipositors for a few seconds and then withdraw them. Vibration of the ovipositor indicates that an egg has been laid.

In the present experiments, female parasitoids (aged as two or three days after eclosion) were placed with *Drosophila* larvae (aged as two or four days after they were oviposited) in Petri dishes (3 cm in diameter) with small amounts of food medium, and observed for oviposition behaviour under a stereoscopic microscope. After parasitoids had inserted their ovipositors to *Drosophila* larvae and paralysed them, the insects were drawn apart using forceps before the parasitoids started to vibrate their ovipositors (‘interrupted’ group). In another group, parasitoids were allowed to complete their oviposition behaviour without interruption (‘un-interrupted’ group). *Drosophila* larvae were then dissected under a stereoscopic microscope and examined for the presence or absence of parasitoid eggs. In addition, *Drosophila* larvae of ‘interrupted’ and ‘un-interrupted’ group were reared in vials with food, and examined for pupariation and emergence of adult parasitoids or flies. Control (untreated) *Drosophila* larvae were also examined for pupariation and adult emergence. Thirty-100 *Drosophila* larvae were used for each treatment.

In addition, survival time was examined for *D. simulans* larvae. Larvae of

the ‘interrupted’ group were prepared as above, placed in Petri dishes with food, and examined for survival 1, 2, 4, 8, 16 and 32 h after the treatment. Larvae were judged as dead if they did not move even upon touching with the tip of a forceps. Control (untreated) *D. simulans* larvae were also examined for survival under the same conditions.

Statistical analyses were performed using a JMP statistical package (version 4; SAS Institute Inc.).

Results and Discussion

In all *Drosophila* species tested, larvae of both ‘interrupted’ and ‘un-interrupted’ groups always became paralysed (data not shown). However, no parasitoid egg was laid in larvae of the ‘interrupted’ group, whereas parasitoid eggs were found in 87- 97 % of larvae of the ‘un-interrupted’ group (Table 1). These results indicate that venom is injected soon after parasitoid females inserted their ovipositors to *Drosophila* larvae and egg is laid after they vibrated their ovipositors. Parasitoid females did not show any difference in behaviours toward host (*D. simulans*, *D. lutescens* and *D. auraria*) and non-host (*D. bipectinata* and *D. ficusphila*) larvae (personal observation).

Survival of *Drosophila* larvae differed according to treatment, and also between host and non-host species. In *D. simulans*, a natural host species of *A. japonica*, 97 % (*N*=100) of the ‘interrupted’ larvae died before pupariation, whereas

76 % (*N*=100) of the ‘un-interrupted’ larvae pupariated, when they were treated 2 days after they were oviposited (Fig. 1a). When treated 4 days after they were oviposited, 100 % (*N*=50) of the ‘interrupted’ larvae died before pupariation, whereas 72 % (*N*=50) of the ‘un-interrupted’ larvae pupariated (Fig. 1b). In control larvae, 93 % (*N*=100) and 98 % (*N*=50) pupariated, when treated 2 and 4 days after they were oviposited, respectively. Differences in the rate of pupariation between two- and four-day-old host larvae were statistically insignificant (χ^2-test, $P > 0.05$).

Survival time of the ‘interrupted’ larvae (two-day old) of *D. simulans* was shown in Fig. 2; 95 % (*N*=21) died within 16 h of the treatment. On the other hand, 85 % of control (untreated) larvae pupariated. Difference in survival time between them was statistically significant (Kaplan-Meier method, $P < 0.01$).

Another host species, *D. lutescens* and *D. auraria*, showed similar results with *D. simulans*; 100 % (*N*=50) of *D. lutescens* and 94 % (*N*=50) of *D. auraria* larvae of the ‘interrupted’ group died before pupariation, whereas 96 % (*N*=50) of *D. lutescens* and 82 % (*N*=50) of *D. auraria* larvae of the ‘un-interrupted’ group pupariated (Fig. 3a and b).

By contrast, non-host species, *D. bipectinata* and *D. ficusphila*, showed different results (Fig. 3c and d); only 18 % (*N*=50) of *D. bipectinata* and 10 % (*N*=50) of *D. ficusphila* larvae of the ‘interrupted’ group died before pupariation, and 98 % (*N*=50) of *D. bipectinata* and 78 % (*N*=50) of *D. ficusphila* larvae of the ‘un-interrupted’ group grew up to not only pupae but also adults.

Thus, venom of *A. japonica* has a lethal effect on host species (*D. simulans*, *D.*

lutescens and *D. auraria*), but has not on non-host species (*D. bipectinata* and *D. ficusphila*). Moreau *et al*. (2002) also observed that venom of *A. tabida* is toxic to a host species, *D. melanogaster*. In the present study, it is also suggested that the lethal effect of venom on host species is suppressed by substance(s) provided by parasitoid females at the time of egg laying or by parasitoid embryos developing in the host larvae. At present, nothing is known about the nature of *A. japonica* venom, but there are some possible hypotheses on its toxicity.

One hypothesis is that paralysis-inducing substances themselves or their derivatives are toxic to host species. *Drosophila* larvae injected with venom become paralysed within a few seconds and recover usually within 1 h (personal observation). There are two possible processes for their recovery; 1) paralysis-inducing substances in venom are degraded or modified, and 2) paralysis-inducing substances are not degraded or modified, but the target of these substances was modified or desensitized. In the first case, derivatives of the paralysis-inducing substances could be toxic to host species, and the paralysis-inducing substances themselves could be toxic in the second case. In either case, non-host species have mechanisms to suppress the toxicity of venom. On the other hand, such mechanisms are useless for host species, because parasitized host larvae are killed (i.e., fed) eventually by parasitoid larvae at the pupal stage even if they suppress the toxicity of venom. Then, host species have not evolved such suppression mechanisms, and therefore parasitoids are required to provide hosts with factors that suppress the toxicity of the substances or their derivatives to allow host larvae to grow up to pupae.

Another hypothesis is that factors causing paralysis and mortality are not related. One of candidates for mortality factors is a substance that suppresses host immune systems. In this hypothesis, host larvae injected only with venom are killed by microbial infection, and substances provided later by the parasitoid females or parasitoid embryos have antimicrobial activity. However, it is questionable whether microbes proliferate to kill host larvae within 8 or 16 h after injection of venom (see Fig. 2).

To assess the likelihood of the above hypotheses, it is important to identify and characterize the substances that are provided by parasitoids. It has so far been reported that parasitoids provide hosts with immune suppressive factors and antibacterial and antifungal factors (Vinson & Iwantsch, 1980; Strand, 1986; Strand & Pech, 1995; Dani *et al*., 2003; Moreau & Guillot, 2005; Li *et a*l., 2007), but almost nothing is known about how these factors function in the hosts. Also, little is known about the nature of paralysis-inducing substances and their degradation pathways. Further study is needed on these topics.

It is also questioned whether the toxicity of venom is adaptive or not. Immune suppressive factors apparently have a function to protect parasitoid eggs or embryos from host immune systems. In the other cases, the toxicity may be only a side effect.

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Table 1. Percentage of host larvae with parasitoid eggs in the 'interrupted' and 'un-interrupted' groups.

Host	Interrupted	Un-interrupted
D. simulans (two day old)	0 (30)	91 (100)
D. simulans (four day old)	0 (30)	93 (30)
D. lutescens (two day old)	0 (30)	97 (30)
D. auraria (two day old)	0 (30)	87 (30)
D. bipectinata (two day old)	0 (30)	93 (30)
D. ficusphila (two day old)	0 (30)	93 (30)

The number in parentheses refers to the number of larvae examined.

Legends to Figures

Fig. 1. Percentage of *D. simulans* larvae died at the larval (■) and pupal (■) stages, produced adult parasitoids (■) and survived until emergence as adult flies (□) in the 'un-interrupted' (U), 'interrupted' (I) and control (C) groups. *Drosophila* larvae were treated at the age of two or four days after they were oviposited.

Fig. 2. Survivorship of *D. simulans* larvae of the 'interrupted' group (solid line). *Drosophila* larvae were treated at the age of two days they were oviposited. Survivorship of control *D. simulans* larvae is also shown (broken line).

Fig. 3. Percentage of *D. lutescens*, *D. auraria*, *D. bipectinata* and *D. ficusphila* larvae that died at the larval (■) and pupal (■) stages, produced adult parasitoids (■) and survived until emergence as adult flies (□) in the 'un-interrupted' (U), 'interrupted' (I) and control (C) groups. *Drosophila* larvae were treated at the age of two days

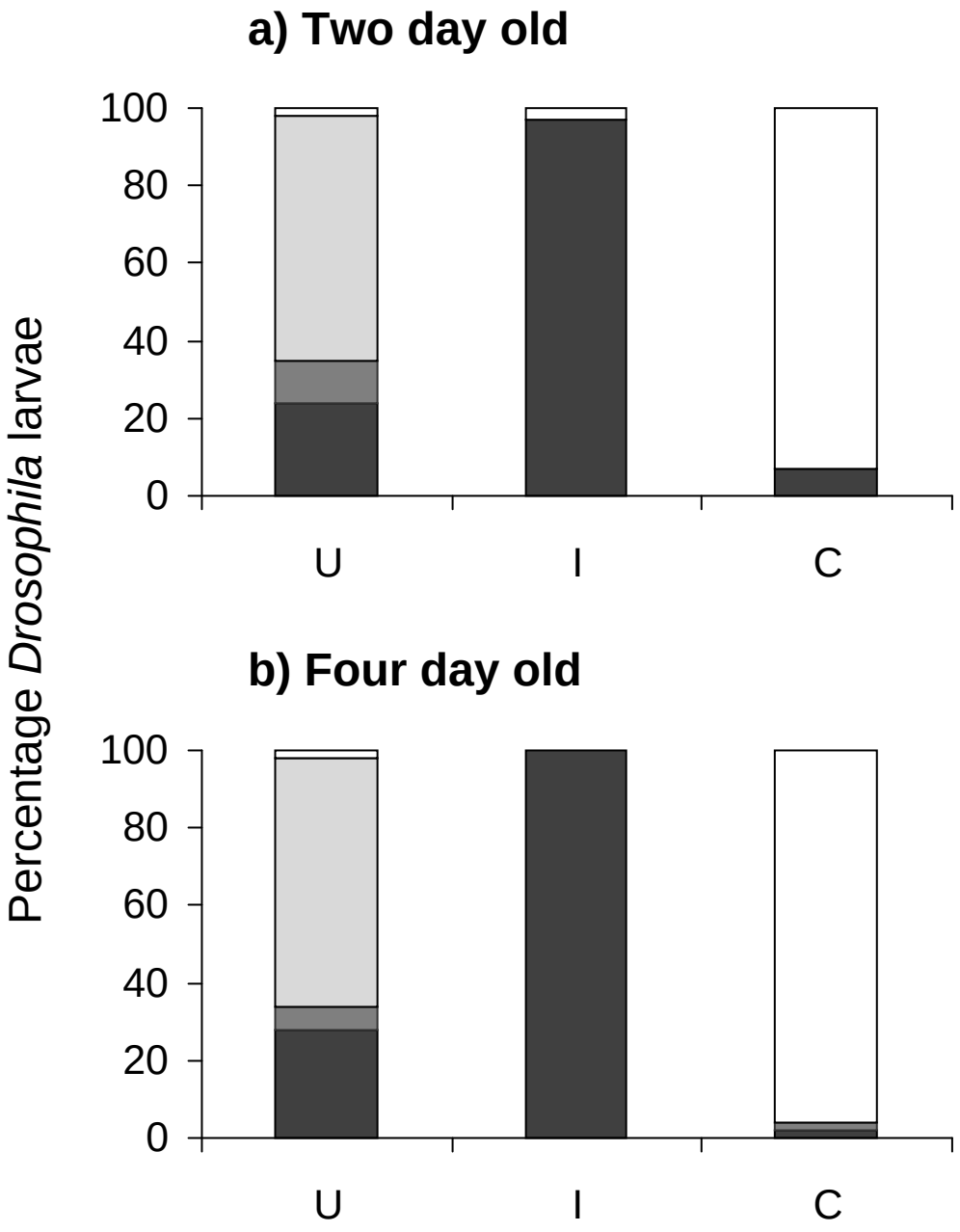
a) Two day old
100
80
60
40
20
0
U
I
C
b) Four day old
100
80
60
40
20
0
U
I
C
Percentage Drosophila larvae

Fig. 1

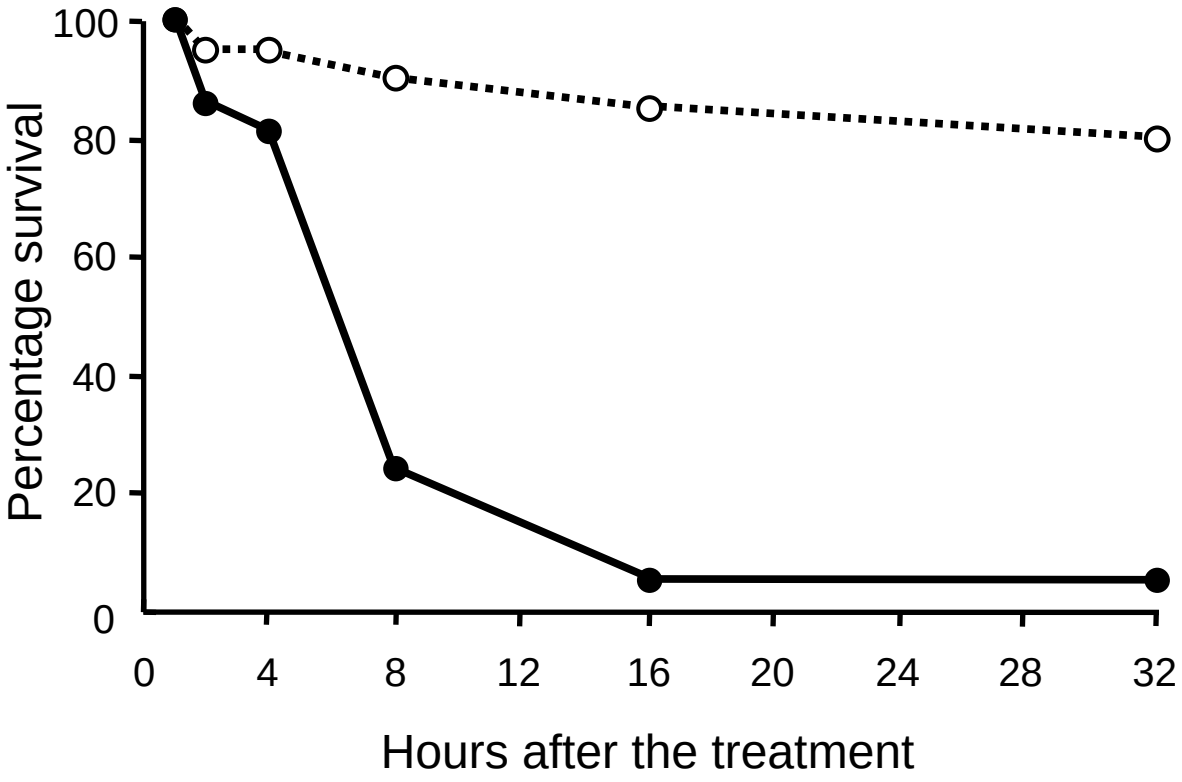
Percentage survival
100
80
60
40
20
0
0
4
8
12
16
20
24
28
32
Hours after the treatment

Fig. 2

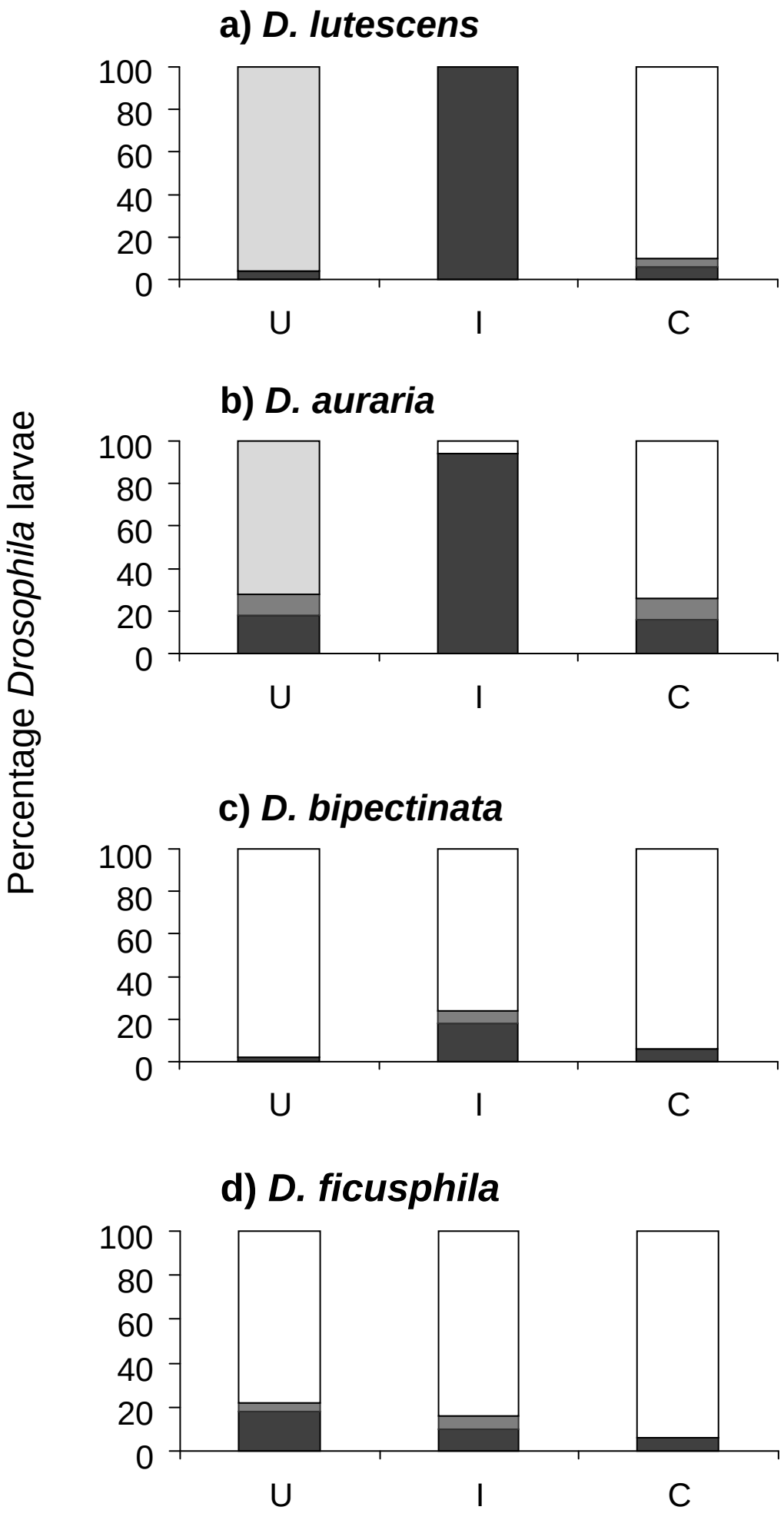
a) D. lutescens
b) D. auraria
c) D. bipectinata
d) D. ficusphila
Percentage Drosophila larvae
100
80
60
40
20
0
U
I
C

Fig. 3