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**Masahito T. Kimura**

**Prevalence of exotic frugivorous *Drosophila* species (Diptera: Drosophilidae) and its effects on local parasitoids in Sapporo, northern Japan**

Graduate School of Environmental Earth Science, Hokkaido University, Sapporo,  
Hokkaido 060-0810, Japan

Correspondence: Masahito T. Kimura, E-mail: [mtk@ees.hokudai.ac.jp](mailto:mtk@ees.hokudai.ac.jp), Tel:  
+81-11-706-2236, Fax: +81-11-706-2225

Running title: Prevalence and parasitism of exotic *Drosophila*

**Abstract** The prevalence of exotic species has various effects on native biological communities, e.g. the diversity and relative abundance of preys, predators or competitors. Here, I investigated associations of native and exotic frugivorous *Drosophila* species with local parasitoids in Sapporo, northern Japan, to understand the influence of exotic species on a local *Drosophila*-parasitoid community. Two exotic species, *D. simulans* and *D. immigrans*, comprised approximately 90% of *Drosophila* individuals that emerged from banana-baits, and two native species, *D. auraria* and *D. bauraria*, comprised approximately 9%. Seven larval parasitoid species were recorded from *Drosophila* larvae occurring in banana-baits, and six of them were examined for host use by laboratory experiments. Either or both of the two native *Drosophila* species were favorable hosts for four of the six larval parasitoids. Two other parasitoids seemed to use herbage- or fungus-feeding *Drosophila* species as major hosts. *D. simulans* was a favorable host at least for four larval parasitoids, but *D. immigrans* was not favorable for most of the larval parasitoids. Nevertheless, more than one third of parasitoid individuals emerged from *D. immigrans* in the field study, probably because of its extreme abundance. Thus, exotic species are assumed to affect the abundance and distributions of native parasitoid species. On the other hand, it is not certain whether the parasitoid species mediate the effects of exotic *Drosophila* species on native *Drosophila* species, because the parasitism rate was usually low.

**Keywords** Abundance – exotic *Drosophila* – habitats – parasitoid – host interaction.

## Introduction

The prevalence of exotic species could affect the diversity and abundance of native preys, predators or competitors and cause degradation of biodiversity and ecosystem functions (Collard 1996). Various factors could cause the population increase or explosion of exotic species, and the lack of effective local enemies is considered as one of important factors (Vincent et al. 2007). However, exotic species are not always free from enemies, but most of them are more or less attacked by local predators and parasitoids. In general, parasitoids are believed to be less effective enemies of exotic species than predators, because parasitoid's host range is narrower than predator's prey range. Nevertheless, exotic insects are frequently attacked by native parasitoids. In *Drosophila*, for example, the two cosmopolitan domestic species *D. melanogaster* Meigen and *D. simulans* Sturtevant are attacked by local parasitoid species, e.g., *Leptopilina heterotoma* (Thomson) and *Asobara tabida* Nees von Esenbeck in Europe and *A. japonica* Belokovylskij in Japan (Allemand et al. 1999; Fleury et al. 2004; Mitsui et al. 2007; Ideo et al. 2008; Mitsui and Kimura 2010). Thus, colonization of exotic species may cause the change of host use in local parasitoids, and further affect the abundance of native host species by a mechanism called "apparent competition" where parasitoids or predators play a mediating role.

This study investigates the abundance and habitat selection frugivorous *Drosophila* species and their parasitoids in Sapporo (northern Japan) where the two exotic species *D. simulans* and *D. immigrans* Sturtevant are abundant (Mitsui et al. 2007), and further examines the parasitoid's host ranges by laboratory experiments, to understand how exotic *Drosophila* species affect native *Drosophila* and parasitoid species. *Drosophila* and their parasitoids have been used as model systems in many

studies of coevolution (Fleury et al. 2009; Kraaijeveld and Godfray 2009; Dupas et al. 2009). Particularly, the exotic species *D. melanogaster* has been a major target of these studies. However, little is understood on the effects of exotic host species on local *Drosophila*-parasitoid communities.

## **Materials and methods**

### Field survey

Collections were conducted from July 6 to September 21 in 2012 in a domestic area (around houses in the central area of city), a grove area in Botanical Garden located in the center of city, and a forest area located about 4 km southwest of Botanical Garden in Sapporo, northern Japan. Three traps baited with banana (30 g) were placed in each environment, and banana in the traps was collected after a week. At that time, fresh banana was placed in each trap. Collected banana was brought to the laboratory and placed in plastic boxes with clothes. When drosophilid larvae in banana pupariated, they were collected, identified to species and then placed on wet paper in Petri dishes. When flies or parasitoids emerged from these puparia, they were collected and identified to species.

### Laboratory experiments

Laboratory populations were established for the following drosophilid and parasitoid species with several females collected in and near Sapporo, *Drosophila simulans*, *D. auraria* Peng, *D. biauraria* Bock & Wheeler, *D. bifasciata* Pomini, *D. immigrans*, *D.*

*nigromaculata* Okada and *D. orientacea* Grimaldi, James & Jaenike, *Asobara japonica*, *A. rossica* Belokobylskij, *A. rufescens* Förster, *Leptopilina heterotoma*, *L. longipes* (Hartig) and *L. japonica* Novković & Kimura. Among these *Drosophila* species, *D. simulans* and *D. immigrans* are exotic, while the others are native. Among the native species, *D. auraria* and *D. biauaria* mainly exploit fermenting fruits, *D. bifasciata* is basically a tree-sap feeder but sometimes exploits fruits, *D. nigromaculata* is a generalist using fermenting fruits, decayed herbaceous plants and mushrooms, and *D. orientacea* is fungivorous (Kimura et al. 1977). The *Drosophila* populations were maintained with *Drosophila* medium (ingredients: 50 g cornmeal, 50 g wheat germ, 50 g sugar, 40 g dry yeast, 10 g agar and 5 mL propionic acid in 1 L water). All of the parasitoid species are native. The laboratory population of *A. japonica* was maintained with *D. lutescens* as host, those of *A. rossica* and *A. rufescens* with *D. auraria*, and those of the three *Leptopilina* species with *D. simulans*. Parasitism experiments were done within three months after the collection of the laboratory populations except the *L. japonica* population that was used approximately four years after the collection. Parasitoids used in experiments were 3-6 days old, mated, fed on *Drosophila* medium and given experience on patches of hosts. Maintenance of the populations and experiments were carried out under 15 h light:9 h dark at 23 °C.

*Host suitability.* Host suitability for parasitoid development was determined as follows. Approximately 50 two-day-old larvae of each drosophilid species and several (2-5) females of each parasitoid species were placed in a Petri dish (3 cm in diameter) containing a small amount of *Drosophila* medium, and parasitoid females were monitored for oviposition under a stereoscopic microscope. Characteristic oviposition behavior such as full extension of the ovipositor after contact with the host and longer insertions of the ovipositor into larvae (>10 s) were taken as indicators of

successful oviposition (Vet and Bakker 1985; van Lenteren et al. 1998; Dubuffet et al. 2006). When oviposition was confirmed, parasitized fly larvae were transferred into vials containing *Drosophila* medium. The vials were later checked for the emergence of flies and/or parasitoids. Approximately 30 parasitized larvae were obtained per fly species. In some cases, no or only few parasitized larvae were obtained due to the lack or low rates of oviposition. In such case, the oviposition test was conducted to ascertain the host acceptance of parasitoids for oviposition. Approximately 30 second instar larvae of each drosophilid species and five females of each parasitoid species were placed in a Petri dish containing a small amount of *Drosophila* medium for 4 hours. After removal of parasitoids, fly larvae were dissected under a stereoscopic microscope and checked for the presence/absence of parasitoid eggs. The oviposition rate was calculated as the number of parasitized larvae per total number of larvae checked.

In *L. longipes*, it was difficult to determine whether females oviposit or not, because they took only few seconds for oviposition and show no obvious sign. In this species, therefore, the parasitism capacity was examined as follows. Approximately 25 *L. longipes* females were placed in a vial (100 ml in volume) containing *Drosophila* medium and 100-300 two-day-old larvae of a drosophilid species for two days. When parasitoids and/or flies emerged from the vial, they were counted. One to three replicates were used.

## Statistics

The rate of successful parasitism (i.e. number of parasitoid emergence/(number of host larvae parasitized) in the parasitism experiments was compared by  $\chi^2$  test using Jmp ver.

6.1 (SAS Institute, Cary, USA).

## Results

### Field survey

A total of 27,761 drosophilid pupae were collected in this survey (Table 1: 12448 from the domestic area, 8276 from the grove area and 7037 from the forest area). The two exotic species *D. simulans* and *D. immigrans* comprised approximately 90% in all environments; *D. simulans* did 71.3, 16.9 and 2.0% in the domestic, grove and forest areas, respectively, and *D. immigrans* did 20.0, 71.2 and 87.4%, respectively. Among the native species, *D. auraria* and *D. bauraria* comprised 7.5-11.2%. These two species are indistinguishable at the pupal stage but distinguishable at the adult stage. According to the adult data, individuals collected from the domestic and grove areas were mostly *D. auraria*, and those from the forest area are mainly *D. bauraria* (data not shown).

A total of 377 parasitoid individuals comprising eight species emerged from 27,761 drosophilid pupae (Table 2). The parasitism rate is only 1.5%. Among the major *Drosophila* species, *D. auraria* (and *D. bauraria*) was rather frequently parasitized (4.9%). Except *L. japonica* and *Opius* sp. SP1 that were rarely collected, *A. rossica* emerged mainly from *D. auraria* (*D. bauraria* in the forest area), *D. rufescens* from *D. auraria* (*D. bauraria* in the forest area), *D. immigrans* and *D. simulans*, *A. japonica* from *D. simulans* and *D. auraria*, *L. heterotoma* mainly from *D. immigrans* and probably *D. bauraria*, and *L. longipes* and *P. vindemmiae* from *D. simulans* and *D. immigrans*. The three parasitoid species *A. japonica*, *L. longipes* and *P. vindemmiae* were mainly collected from the domestic area, *L. heterotoma* from the grove and forest



areas, and *A. rossica* and *A. rufescens* from all environments.

### Laboratory experiments

The present parasitoid species usually oviposited well in the *Drosophila* species used in the experiments, but *A. japonica* rarely oviposited in *D. immigrans*, *A. rossica* in *D. orientacea* and *D. immigrans*, and *A. rufescens* in *D. nigromaculata*. In the oviposition test, *A. japonica* oviposited in one out of 25 *D. immigrans* larvae, *A. rossica* did in one out of 27 *D. orientacea* larvae and none out of 30 *D. immigrans* larvae, and *A. rufescens* did in five out of 30 *D. nigromaculata* larvae (Table 3).

Six parasitoid species showed species-specific host use (Tables 3 and 4). *A. japonica* significantly more successfully parasitized *D. simulans*, *D. auraria*, *D. bifasciata* and *D. orientacea* at least than *D. immigrans* and *D. nigromaculata* ( $P < 0.05$ ); *A. rufescens* did *D. auraria* than the others except *D. bifasciata* ( $P < 0.05$ ); *A. rossica* did *D. auraria* and *D. biauraria* than the others ( $P < 0.05$ ); *L. heterotoma* did *D. simulans*, *D. bifasciata*, *D. nigromaculata* and *D. orientacea* than the others ( $P < 0.05$ ); and *L. japonica* did *D. simulans* and *D. biauraria* than the others ( $P < 0.05$ ). *L. longipes* parasitized all of the seven *Drosophila* species tested, although *D. bifasciata* and *D. orientacea* were less frequently parasitized (Table 4).

## Discussion

### Abundance of exotic and native *Drosophila* species

In the present survey, the two exotic species *D. simulans* and *D. immigrans* comprised

approximately 90% of *Drosophila* individuals that emerged from banana-baits, although their frequency was lower (approximately 60%) in our preliminary study (Mitsui et al. 2007). These two species differed in habitats; *D. simulans* was highly restricted to domestic areas, but *D. immigrans* was found even in the forest area at a high frequency (also see Parsons and Stanley 1981). A high migration ability of *D. immigrans* may enable it to move into the forest. Among native species, *D. auraria*, *D. bauraria* and *D. bifasciata* emerged from banana-baits more frequently than the others. Among them, *D. bifasciata* breeds on fruits and also tree sap, whereas *D. auraria* and *D. bauraria* mainly breed on fruits (Kimura et al. 1977). These two native fruit feeders differ in habitats; *D. auraria* mainly occurs domestic areas and open lands, whereas *D. bauraria* is restricted to the forest (Kurokawa 1967; Kimura 1987).

As mentioned above, the two exotic species *D. simulans* and *D. immigrans* are the major species that emerged from banana-baits in Sapporo, but the frequency of exotic species in *Drosophila* communities was not so high at lower latitudes. In Tokyo (35.7 °N), *D. simulans* and *D. immigrans* were also the major exotic species and comprised 60-82% of *Drosophila* individuals in the domestic area, although only 2-26% in the forest, central Japan (Mitsui & Kimura 2000a, 2000b 2010). In Iriomote-jima (24.3 °N), *D. melanogaster* and *D. ananassae* were the major exotic frugivorous species and they comprised only 2% even in the domestic area (Novković et al. 2012). In Bogor (6.5 °S), Indonesia, *D. ananassae* was the only cosmopolitan species and comprised only 0.5% in the domestic area (Kimura and Suwito 2012). Conversely, the abundance of native frugivorous *Drosophila* species is lower at higher latitudes, probably reflecting the decrease of fruit diversity and abundance with increasing latitude. As well, the diversity of native frugivorous species decreases with latitude; the number of native species that frequently breed on banana baits was 9-10 in Bogor (Kimura and Suwito

2012), 7-8 in Iriomote-jima (Novković et al. 2012) 5-6 in Tokyo (Mitsui and Kimura 2010) and 2 in Sapporo (this study). In tropics and subtropics, the prevalence of native frugivorous species may prevent the colonization of exotic frugivorous species through their competitive interactions. In temperate regions, on the other hand, the scarceness of native frugivorous species may allow the prevalence of exotic frugivorous species, especially in the domestic areas where cultivated fruits or waste fruits would be abundantly supplied by human activities but underexploited by native fruit-feeders.

#### *Drosophila*-parasitoid association

In this survey, one pupal parasitoid (*Pachycrepoideus vindemmiae*) and seven larval parasitoids (the others) were recorded from Sapporo. Among them, the *Opius* species (*O.* sp. SP1) was firstly recorded from *Drosophila* species in Northern Hemisphere. The other larval parasitoids have been recorded from the Oriental Region (*Asobara japonica*, *A. rossica* and *Leptopilina japonica*) or the Palearctic region (*A. rufescens*, *L. heterotoma* and *L. longipes*), while *P. vindemmiae* is cosmopolitan (Carton et al. 1986; Nordlander 1980; Mitsui et al. 2007; Novković et al. 2011).

Four parasitoids, *A. japonica*, *A. rossica*, *A. rufescens* and *A. japonica* were highly successful in parasitizing either or both of the two major native frugivorous species *D. auraria* and *D. bauraria*. Indeed, these two *Drosophila* species were parasitized more frequently than *D. bifasciata* or the exotic species *D. simulans* and *D. immigrans* in the field study. *A. rufescens* from Europe has also been reported to parasitize *Scaptomyza pallida* (Zetterstedt) that breeds on decaying plant materials (Vet et al. 1984), but the use of this drosophilid species by *A. rufescens* has not been examined in Sapporo. On the other hand, *L. heterotoma*, and *L. longipes* were

successful in parasitizing the major mycophagous species (*D. orientacia*) and/or herbage-feeding species (*D. nigromaculata*). At least *L. heterotoma* has been reported to parasitize mycophagous drosophilids, *D. orientacea*, *D. kuntzei* and *D. phalerata* in Sapporo and Europe (Driessen et al. 1990; Kasuya et al. 2013). Mycophagous and herbage-feeding *Drosophila* species may be their major hosts in Sapporo.

In the parasitism experiments, one of the exotic species *D. simulans* was successfully parasitized by *A. japonica* and the three *Leptopilina* species but not by *A. rossica* or *A. rufescens*. In the field study, this fly species was parasitized by *A. japonica*, *L. longipes* and *P. vindemmiae*, particularly in the domestic areas where these parasitoid and fly species were abundant. In the domestic area, two individuals of *D. simulans* were also parasitized by *A. rossica*, despite *D. simulans* showed relatively high resistance to this parasitoid species in the laboratory experiments. In the laboratory experiments, *D. immigrans* was not or rarely parasitized by all of the tested parasitoids except *L. longipes*. In the field, however, *D. immigrans* was parasitized more frequently by *L. heterotoma* than *D. simulans*. This inconsistency would be due to the difference in their microdistribution. All individuals of *L. heterotoma* were collected in the grove and forest areas, where *D. immigrans* was much more abundant than *D. simulans*. In addition, several individuals of *A. rufescens* emerged from *D. immigrans* in the forest area, despite it did not successfully parasitized this fly species in the laboratory experiments. This inconsistency may be due to the age of fly larvae parasitized. In the experiments, two-day old fly larvae were subjected to parasitization, but younger larvae may be somehow successfully parasitized by *A. rufescens*. In fact, *D. immigrans* larvae soon after hatching were somehow more successfully parasitized at least by *A. japonica* than older larvae (Kimura unpublished data). Anyway, abundant host species could be important resources even if they are less suitable as hosts.

To maintain their populations, it would be beneficial for parasitoids to use frequently encountered species as hosts (Hawkins 1994; Lapchin 2002). Indeed, one or two abundant (i.e., frequently encountered) species are usually included in the host lists of most *Drosophila* parasitoids (Janssen et al. 1988; Yorozya 2006; Ideo et al. 2008; Mitsui and Kimura 2010; Novković et al. 2012; Kasuya et al. 2013; Kimura & Suwito 2014). However, the exotic species *D. immigrans* was an unsuitable or inferior host for all of the tested parasitoid species except *L. longipes*, and the other exotic species *D. simulans* was an inferior host for *A. rossica* and *A. rufescens*, despite their exceeding abundances in Sapporo. The parasitism capacity or virulence may hardly change in evolutionarily short time (probably <100 years), even if parasitoids face novel situations.

The exceeding abundance of these exotic *Drosophila* species would more or less affect the abundance and distribution of parasitoids that use them. A typical case would be the abundance of *L. longipes* in the domestic area. This species would be native in northern Japan because it shows a Palearctic distribution (Nordlander 1980; Wachi et al. 2015). Before the land development by humans, this species might have occurred mainly in the forest and parasitized some native *Drosophila* species such as *D. bauraria*, *D. nigromaculata* and *D. orientacea*. Its occurrence in the domestic area may be attributable to the prevalence of *D. immigrans* and *D. simulans* in the domestic area. In addition to *L. longipes*, *P. vindemmiae* and *A. japonica* were collected mostly from the domestic areas. The former is a cosmopolitan species and has recently colonized Japan (Carton et al. 1986), and the latter is suggested to have recently colonized Sapporo from southern Japan, since its Sapporo population has a relatively weak diapause response that is a characteristic of southern Japanese populations (Murata et al. 2009). Thus, these species may have not yet well adapted to environmental conditions

(forests) of northern Japan.

The two exotic *Drosophila* species could affect the abundance of the two major native frugivorous *Drosophila* species *D. auraria* and *D. bauraria* through resource competition because these four species exploit fruits (Kimura et al. 1977). By contrast, the effect of the exotic species on these native species via parasitoids (i.e., through apparent competition) would be small, because the parasitism rate was low. In addition, the abundance of the major parasitoids attacking the native frugivorous species (*A. rossica* and *A. rufescens*) would not be strongly affected by the abundance of the exotic *Drosophila* species, because these parasitoids have low virulence against them. On the other hand, *L. japonica* has virulence against *D. simulans* and *D. bauraria*, but the influence of *D. simulans* to *D. bauraria* via *L. japonica* would not be large, because *D. simulans* seldom occurs in the forest where *D. bauraria* mainly occurs.

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**Table 1** Number of drosophilid pupae collected from banana-baits in domestic area, Botanical Garden and forest area

	Domestic area	Botanical Garden	Forest area	Total
<i>D. simulans</i>	8985	1400	138	10523
<i>D. immigrans</i>	2801	5895	6148	14844
<i>D. auraria+biauraria</i>	1513	924	525	2962
<i>D. bifasciata</i>	27	26	190	243
<i>D. nigromaculata</i>			16	16
<i>D. tsigana</i>	1	31	19	51
<i>A. conifera takadai</i>	1		1	2
Total	13328	8276	7037	28641

**Table 2** Number of drosophilid pupae collected from banana-baits, number of flies and parasitoids that emerged from these pupae, and parasitism rate (Dead: number of pupae from which neither of them emerged). Their relative frequency at domestic area, Botanical Garden and forest area was also shown

	Pupae	Flies	Dead	Parasitoids								Parasitism (%)
				Ajap	Aros	Aruf	Osp	Lhet	Ljap	Llon	Pvin	
<i>D. simulans</i>	10523	9719	666	72	2	1	-	2	-	18	43	1.4
<i>D. immigrans</i>	14844	13188	1526	-	-	6	5	103	-	7	8	1.0
<i>D. auraria+biauraria</i>	2962	2416	434	16	74	8	1	8	2	2	1	4.4
<i>D. bifasciata</i>	243	206	35	-	-	2	-	-	-	-	-	1.0
<i>D. nigromaculata</i>	16	14	1	-	-	-	-	-	-	1	-	6.7
<i>D. tsigana</i>	51	42	9	-	-	-	-	-	-	-	-	0.0
<i>A. conifera takadai</i>	2	2	-	-	-	-	-	-	-	-	-	0.0
Total	28641	25587	2671	88	76	17	6	113	2	28	52	1.5
No. of individuals per trap	292.26			0.90	0.78	0.17	0.06	1.15	0.02	0.29	0.53	
Relative frequency (%)												
Domestic area	46.5			98.9	51.3	41.2	0	0	0	89.3	98.1	
Botanical Garden	28.9			1.1	39.5	5.9	33.3	78.8	0	7.1	1.9	
Forest area	24.6			0	9.2	52.9	66.7	21.2	100	3.6	0	

Ajap: *Asobara japonica*, Aros: *A. rossica*, Aruf: *A. rufescens*, Osp: *Opius* sp., Lhet: *Leptopilina heterotoma*, Ljap: *L. japonica*, Llon: *L. longipes*, Pvin: *Pachycrepoideus vindemmiae*.

**Table 3** Number of parasitized fly larvae from which flies (F), parasitoids (P) and neither of them (D) emerged in parasitism experiment (N: number of larvae parasitized), and oviposition rate in acceptance experiments. Acceptance experiment was performed when no or only few parasitized eggs were obtained in parasitism experiment

	Parasitism experiment				Acceptance (%)
	N	F	P	D	
<i>A. japonica</i>					
<i>D. simulans</i>	30	1	20	9	-
<i>D. immigrans</i>	-				4 (25)
<i>D. auraria</i>	31	0	19	12	-
<i>D. biauaria</i>	30	1	12	17	-
<i>D. bifasciata</i>	30	0	23	7	-
<i>D. nigromaculata</i>	30	4	5	21	-
<i>D. orientacea</i>	40	0	28	12	-
<i>A. rufescens</i>					
<i>D. simulans</i>	38	13	2	23	-
<i>D. immigrans</i>	28	11	0	17	-
<i>D. auraria</i>	31	2	15	14	-
<i>D. biauaria</i>	33	10	5	18	-
<i>D. bifasciata</i>	32	8	9	15	-
<i>D. nigromaculata</i>	15	13	0	2	17 (30)
<i>D. orientacea</i>	32	8	1	23	-
<i>A. rossica</i>					
<i>D. simulans</i>	31	21	1	9	-
<i>D. immigrans</i>	-				0 (30)
<i>D. auraria</i>	30	2	26	2	-
<i>D. biauaria</i>	30	6	18	6	-
<i>D. bifasciata</i>	30	22	0	8	-
<i>D. nigromaculata</i>	30	26	0	4	-
<i>D. orientacea</i>	3	0	0	3	4 (27)
<i>L. heterotoma</i>					
<i>D. simulans</i>	30	0	18	12	-
<i>D. immigrans</i>	32	2	2	28	-

<i>D. auraria</i>	33	30	0	3	-
<i>D. bauraria</i>	30	6	5	21	-
<i>D. bifasciata</i>	30	10	17	3	-
<i>D. nigromaculata</i>	32	0	20	12	-
<i>D. orientacea</i>	40	0	15	25	-
<i>L. japonica</i>					
<i>D. simulans</i>	31	2	16	12	-
<i>D. immigrans</i>	30	2	0	28	-
<i>D. auraria</i>	30	16	1	13	-
<i>D. bauraria</i>	30	2	14	14	-
<i>D. bifasciata</i>	30	25	0	5	-
<i>D. nigromaculata</i>	11	2	0	9	13(30)
<i>D. orientacea</i>	32	0	6	26	-

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Numbers in parentheses indicate the number of larvae used in experiment.

**Table 4** Number of flies and parasitoids that emerged when *L. longipes* females were introduced into vials with 100-400 two-day old drosophilid larvae. One to three vials were prepared

	Fly	Parasitoids
<i>D. simulans</i>	544	237
<i>D. immigrans</i>	89	31
<i>D. auraria</i>	194	57
<i>D. biauraria</i>	173	64
<i>D. bifasciata</i>	214	3
<i>D. nigromaculata</i>	79	100
<i>D. orientacea</i>	269	27