



## Ecological and genetic interactions in *Drosophila*–parasitoids communities: a case study with *D. melanogaster*, *D. simulans* and their common *Leptopilina* parasitoids in south-eastern France

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**Key words:** field ecology, genotype-by-environment interaction, host–parasite interactions, host suitability, interspecific competition, parasite-mediated coexistence, temperature

### Abstract

*Drosophila* species are attacked by a number of parasitoid wasps, which constitute an important factor of population regulation. Since *Drosophila melanogaster* and *Drosophila simulans* share common parasitoid species, their ecology and evolution can hardly be understood without considering parasitoids. After a short review of data available on *Drosophila*–parasitoid interactions involving *D. melanogaster* and *D. simulans* as hosts, we report field and laboratory experiments investigating the ecological role of *Leptopilina* parasitoids in *Drosophila* communities of southern France. Seasonal survey of species abundance shows that strong interspecific interactions occur at both tropic levels. *D. simulans* progressively replaces *D. melanogaster* in southern areas suggesting competitive displacement. Parasitoids are responsible for very high *Drosophila* mortality (up to 90% in some fruits). Field data emphasize the importance of selective pressure that parasitoids exert on *Drosophila* communities. The two *Leptopilina* parasites (*L. heterotoma* and *L. bouvardi*) have different local abundances, which vary in time, and they also compete for hosts. We show that parasitoids can mediate the coexistence of *D. melanogaster* and *D. simulans* in the laboratory, and thus may contribute to their puzzling coexistence in the field. Conversely, hosts exert selective pressures on parasitoids, and development on either *D. melanogaster* or *D. simulans* strongly affects fitness of adult wasps in a temperature-dependent fashion. Local variation in host species abundance and diversity could thus account for the genetic differentiation we observed in one parasitoid species. Despite laboratory studies cannot fully explain complex field situations, it is clear that the ecology and evolution of *Drosophila* populations and communities, especially *D. melanogaster* and *D. simulans*, are strongly constrained by parasitoids, which should receive more attention.

### Introduction

Most insect species are attacked by a number of natural enemies, among which parasitoids play a major role. Parasitoid insects, mostly wasps, lay their eggs on or into the body of preimaginal stages (eggs, larvae or pupae) of other insects (Godfray, 1994; Quicke, 1997). When early stages of endoparasitoids have escaped or overcome immune rejection of the host, they develop as parasites and consume the tissues of their host until it dies, then pupate and emerge as

free living adults. Because successful parasitization always results in host death, parasitoids constitute an important (perhaps the first) regulator of insect populations (Hawkins, Cornell & Hochberg, 1997). Thus, they behave as powerful biocontrol agents and have stimulated extensive research in population dynamics (Murdoch & Briggs, 1996; Hassell, 2000), behavioral ecology (review in Godfray, 1994; Godfray & Shimada, 1999) and evolutionary biology, particularly co-evolutionary dynamics of host resistance and parasite virulence (reviews in Boulétreau, 1986;

Kraaijeveld, Van Alphen & Godfray, 1998; Dupas, Carton & Poirié, 2003).

Like other insects, *Drosophila* species are attacked at larval and pupal stages by many parasitoids. Carton et al. (1986) have listed 42 Hymenopteran species attacking them, and it is likely that a number of other species remain to be described. During last decades, a lot of laboratory and field investigations have been performed on these parasitoids, mainly on larval parasitoids of the genus *Leptopilina* (Eucoilidae) and *Asobara* (Braconidae). Studies have focused on their biology, behaviour, ecology, genetics, physiology and immunology (e.g., Boulétreau, 1986; Carton et al., 1986; Carton & Nappi, 1997, 2001; Ellers et al., 1998; Kraaijeveld, Van Alphen & Godfray, 1998; Van Dijken & Van Alphen, 1998; Eijs & Van Alphen, 1999; Fauvergue et al., 1999; Eslin & Prévost, 2000; Fellowes & Godfray, 2000; Fleury et al., 2000; Vavre et al., 2000). In all these fields, parasitoids of *Drosophila* have proved suitable biological models because of the extensive knowledge available on *Drosophila* biology, and owing to the easiness of experimental and field studies. Moreover, results have clearly evidenced that the ecology and evolution of *Drosophila* can hardly miss out parasitoids, and that conversely the ecology and evolution of parasitoids tightly depend on the composition of the host community. We focus here on relationships between the two cosmopolitan sibling species *Drosophila melanogaster* and *Drosophila simulans*, and two of their main larval parasitoids, the Eucoilidae *Leptopilina boulandi* Barbotin, Carton & Kelner-Pillault and *L. heterotoma* Thompson.

We first review available data on the role of *D. melanogaster* and *D. simulans* as hosts of parasitoid wasps. Few works have compared these two host species and we present data on field ecology, behavioural ecology, physiological and immunological host–parasitoid relationships. In a second part, we present field and laboratory experiments that better document the ecological and evolutionary interactions between *D. melanogaster*, *D. simulans* and their parasitoids. We provide ecological data on the organization and functioning of *Drosophila*–parasitoids communities, concentrating on geographic and seasonal variation in species abundance and richness as well as parasitization rate in south of France. The possible role of parasitoids as mediators of interacting *D. melanogaster* and *D. simulans* populations was investigated under laboratory conditions. We also compare the suitability of *D. melanogaster* and *D. simulans* to

parasitoids. Despite their ecological and phylogenetic relatedness, *D. melanogaster* and *D. simulans* have quite different values with regard to their quality as hosts, and we demonstrate that wasps' fitness strongly depends on complex interactions between their own genotype, host species, and temperature.

### ***D. melanogaster* and *D. simulans* as hosts for parasitoids: the background**

All over their geographic area, *D. melanogaster* and *D. simulans* have proved attacked by parasitoid wasps (Carton et al., 1986; Nordlander, 1988; Schilthuisen et al., 1998; Allemand et al., in press). However, the diversity of parasitoid species and their impact on *Drosophila* populations remain poorly documented in most areas, particularly in native afro-tropical sites. Under temperate and Mediterranean climates (including South Europe, North and South Africa, California and Florida, Australia), frugivorous *Drosophila* species are most commonly attacked by the solitary parasitoids *L. heterotoma* and *L. boulandi*. *L. heterotoma* is a generalist species with a world-wide geographic distribution, which attacks *Drosophila* species living in various fermenting substrates such as fruits, plants, fungi or sap flux. *L. boulandi* is specialized in parasitizing mainly (but not exclusively) *D. melanogaster* and *D. simulans*. The braconid *Asobara tabida* (Nees) can be found with sporadic local abundance in fruits, but cannot develop in *D. simulans*. *Drosophila* pupae can be attacked by Chalcids (*Pachycrepoideus dubius*, *Spalangia* species) and Diapriidae (*Trichopria*), which have lower host specificity.

### *Field ecology*

The natural impact of parasitism on *Drosophila* communities has only been documented in a few cases. High levels of parasitoid-induced mortality have been reported in mycophagous communities (Janssen et al., 1988; Driessen & Hemerik, 1991). Frugivorous *Drosophila* communities have received more attention in North Africa (Rouault, 1979; Carton et al., 1987; Boulétreau, Fouillet & Allemand, 1991). In the Nasrallah oasis (60 km south of Kairouan, Tunisia), *D. melanogaster*, *D. simulans* and *L. boulandi* are coexisting from October to February on a unique resource: prickly pears, the fruits of *Opuntia*. *D. buzzatii* and the parasitoid *L. heterotoma* can also be found at

lower abundance and for a few weeks only. Dissection of field-collected *Drosophila* larvae have shown that *D. simulans* dominates (at least 60% of the *Drosophila* community) and that *Drosophila* larvae are heavily parasitized: from 21 to 41% for *D. melanogaster*, and 14 to 40% for *D. simulans*, with wide variation among fruits. Both field and laboratory data suggest that parasitoids preferentially attack *D. melanogaster* (Rouault, 1979; Carton et al., 1987, 1991). That was confirmed by Boulétreau, Fouillet and Allemand (1991) on the basis of number of adult insects emerging from fruits collected in this Tunisian site. The apparent level of parasitization, calculated for each fruit as the proportion of wasps among emerging adult insects (wasps + flies), can reach 68%, and the higher the infestation rate, the lower the proportion of *D. melanogaster* among flies. That suggests that *D. melanogaster* suffers higher parasitoid-induced mortality than *D. simulans*. Boulétreau, Fouillet and Allemand (1991) have also shown that number of insects (*Drosophila* and wasps) emerging from naturally infested *Opuntia* fruits collected in Tunisia do not differ significantly from maximum numbers of flies that can emerge from *Opuntia* fruits after experimental colonization by excess numbers of egg-laying flies. That strongly suggests that numbers of larvae breeding on fermenting *Opuntia* fruits in the field are very high, close to resource exhaustion, and that *Drosophila* larvae do suffer within- or between-species competition. The outcome of competition depends on the intrinsic competitive ability of *Drosophila* larvae and on the effect of parasitization on their competitiveness (Prévost, 1985), and finally determines the relative abundance of species at emergence.

#### *Host searching strategy*

Infestation of *Drosophila* larvae relies on the behavior of wasps (Godfray, 1994), and possible differential infestation of species depends on choice by foraging females. Extensive studies have been performed on host searching strategies by *Drosophila* parasitoids, including use of host chemical cues (Van Alphen, Nordlander & Eijs, 1991; Vet & Dicke, 1992; Vet et al., 1998), patch time allocation and host detection (Vet & Bakker, 1985; Van Alphen & Vet, 1986; Haccou et al., 1991; Van Dijken & Van Alphen, 1998), oviposition decision into already parasitized hosts (Van Alphen & Visser, 1990; Visser, 1991, 1995). *D. melanogaster* and *D. simulans* are similarly attractive to parasitoids through their own kairomones

(Vet et al., 1993; Hedlund, Vet & Dicke, 1996). However, when given a choice *L. bouleardi* females parasitize more *D. melanogaster* than *D. simulans* and distribute more regularly their eggs among *D. melanogaster* larvae, thus reducing super-parasitism (Carton et al., 1987). Host selection by *Drosophila* parasitoids deserves further studies in several fields, such as the role of developmental host species on further host choice and fidelity, or the role of host species and habitat patchiness on infestation strategies.

#### *Immunological interactions*

In response to parasitism, *Drosophila* larvae are able to develop an immune haemocyte-mediated response which quickly encapsulates parasitoid eggs or young larvae within a multi-layered melanotic capsule and kills them (see Carton & Nappi, 1997 for a review). In return, parasitoids can circumvent the host immune reaction owing to different counter-defence mechanisms such as molecular mimicry, concealment of eggs within host tissues, or active immune depression mediated by virus-like particles (Rizki & Rizki, 1990; Dupas et al., 1996; Eslin et al., 1996; Eslin & Prévost, 2000). A number of studies have investigated the whole encapsulation process, and considered various functional and evolutionary aspects such as genetic basis and physiological path (Carton, Frey & Nappi, 1992; Carton & Nappi, 1997; Orr & Irvin, 1997; Poirié et al., 2000); heritability of host resistance and response to selection (Boulétreau & Fouillet, 1982; Carton & Boulétreau, 1985; Hughes & Sokolowski, 1996; Kraaijeveld & Godfray, 1997; Fellowes, Kraaijeveld & Godfray, 1998); heritability of parasite virulence (Carton, Capy & Nappi, 1989), cost of the immune reaction and possible pleiotropic effects of resistance (Kraaijeveld & Godfray, 1997; Fellowes, Kraaijeveld & Godfray, 1998, 1999; Tiën et al., 2001; Hoang, 2002), cost of virulence (Kraaijeveld et al., 2001). These data have provided good insight into the understanding of co-evolutionary interactions between *Drosophila* and their parasitoids (Boulétreau, 1986; Kraaijeveld et al., 1998; Kraaijeveld & Godfray, 1999; Fellowes & Godfray, 2000). However, few studies have tackled this question at the population level and considered genetic differentiation and local adaptation of populations (but see Kraaijeveld & Van Alphen, 1995). All studies have been performed on *D. melanogaster*, and data on resistance of *D. simulans* are quite scarce. The immune reaction appears stronger in

*D. simulans* than in *D. melanogaster* but it depends on host strain. For sympatric Mediterranean strains (Tunisia), encapsulation rates of *L. boulardi* eggs in experimental infestations vary from 0 in *D. melanogaster* to 10.9% in *D. simulans* (Carton et al., 1991). Some parallel appears between the capacity to encapsulate and the number of circulating haemocytes:  $4894 \pm 913$  cell/mm<sup>3</sup> in *D. melanogaster* versus  $18943 \pm 2679$  in *D. simulans* (Eslin & Prévost, 1998). By contrast, rates of encapsulation are higher in African strains (Congo): 49.2% in *D. melanogaster* and 25.5% in *D. simulans* (Carton & Nappi, 1991). Such geographical differences can be explained by considering that the expression of immune reaction in the field depends on complex interplay between host resistance and parasitoid virulence, which both depend on the genetic status of insects. We have very few indication about levels of resistance in the field, geographic patterns of *Drosophila* resistance and parasite virulence, possible local adaptations and seasonal dynamics of genes involved (see Boulétreau, 1986; Kraaijeveld & Van Alphen, 1995; Kraaijeveld & Godfray, 1999). Owing to the co-evolutionary arm race that is likely to take place between the two genetic systems, we can expect the allelic systems responsible for resistance and virulence to vary in frequency, resulting in quite contrasted outcome among local populations that have experienced different histories.

#### Host suitability

Success of parasitoid larvae that have escaped or overcome the host immune defence depends on the overall 'suitability of the host' (Vinson & Iwantsch, 1980), that conditions both the development of parasitoids and the fitness of adults, and which encompasses a number of host features and parasitoid traits. With regard to their suitability to parasitoids, *D. melanogaster* and *D. simulans* larvae appear quite unequal (Carton & Kitano, 1981; Carton et al., 1987; Boulétreau, Fleury & Fouillet, 1994). For example, developmental success of *L. boulardi* is 89.8% in *D. melanogaster* larvae, only 60.5% in *D. simulans* (Carton et al., 1987). These results depart from those of Janssen (1989), who found higher survival of *L. heterotoma* in *D. simulans* than in *D. melanogaster* (65.3% v.s. 26.1%). Egg-to-adult development of both *L. boulardi* and *L. heterotoma* takes almost two days more on *D. simulans* at 22°C (Fleury, 1993). Fitness traits of adult wasp also depend on developmental hosts: Kopelman and Chabora (1986)

have demonstrated that *L. boulardi* females produce more offspring after development on *D. melanogaster* ( $293.4 \pm 10.1$ ) than on *D. simulans* ( $184.4 \pm 5.2$ ). Few works have considered effects of the developmental host species on the fitness of parasitoids and their further host choice (Janssen, 1989; Kraaijeveld & Van der Wel, 1994).

Clearly, using different host species results in difference in parasite's fitness, and wasps seem to exploit preferentially and more efficiently host species that maximize their offspring production, according to prediction of optimal host selection models. *D. melanogaster* is generally taken as a better host than *D. simulans*, but data are lacking consistency. Moreover, several studies have revealed important genetic variation within and among *D. melanogaster* populations as for their suitability to parasitoids, and have also evidenced the influence of the nutritional status of host larvae (Wajnberg, Prévost & Boulétreau, 1985; Boulétreau, 1986; Boulétreau & Wajnberg, 1986). We describe later how complex interactions between wasp's genotype, host species and environmental factors can account for these apparent discrepancies.

### Ecological interactions in *Drosophila*–parasitoids communities

#### Materials and methods

*Field experiments.* In south-east of France, frugivorous communities involve several *Drosophila* species (mainly *D. melanogaster*, *D. simulans*, *D. immigrans*, *D. subobscura*, *D. hydei*), three larval parasitoids (*L. heterotoma*, *L. boulardi*, *A. tabida*) and two pupal ones (*P. dubius* and *Trichopria cf drosophilae*). We performed thorough seasonal survey of field communities in three orchards regularly distributed along the north–south axis of Rhône valley: Lyon (45.5°N), Valence (44.6°N, 100 km south of Lyon), and on the Mediterranean coast: Hyères (43.1°N, 280 km south of Valence). All three orchards are planted with various fruit trees: cherry, plum, peach, and apple trees, and thus make resources available to *Drosophila* over the whole season. In June, August and October 2001, we placed in each orchard 12–15 open traps baited with split bananas, where both *Drosophila* and parasitoids were free to come in and out. They were exposed to natural colonization for 10–15 days depending on season, so that no fly or wasp could emerge within

them. They were then brought back to the laboratory, and after incubation all emerging *Drosophila* and parasitoids were daily collected and kept in alcohol for further identification. Counts allowed to estimate the relative abundance of each species, and to calculate the apparent parasitization rate (wasps/(wasps + flies)), which probably underestimates the actual prevalence of parasitism since it does not consider pre-imaginal mortality among parasitoids. Quantitative analyses were restricted to *D. melanogaster* and *D. simulans*, the two species that parasitoids use the most, while all other unparasitized *Drosophila* species were pooled together.

**Laboratory experiments.** The possible role of parasitoids in mediating coexistence of *D. melanogaster* and *D. simulans* (Tunisian strains) was tested using experimental population cages and *L. boucardi* as parasite (see Boulétreau, Fouillet & Allemand, 1991 for precision on the protocol). Experiments were performed at three temperatures: 22, 25 and 28°C. At each temperature, three control cages without parasites were compared to three cages with parasitoids. All cages were started with 50% of each *Drosophila* species, and adult parasitoids were introduced weekly in each infested cages (200 females/cage/week). The turn-over of food cups within cages was organized such that flies issuing from unparasitized larvae could emerge within cages, while parasitoids could not due to their longer development time (about twofold). Thus, only fly numbers could fluctuate freely, while parasitoid numbers were kept constant. Periodical sampling has shown that such procedure provides high infestation levels within cages (85–95%) as long as wasp introduction goes on. Proportions of *D. simulans* among flies were weekly checked by sampling eggs and subsequent incubation in large vials for development out of cages.

## Results

### *Field survey of Drosophila–parasitoid communities in south-east of France*

Strong climatic and ecological contrast between Mediterranean and northern regions results in clear-cut variation in community structure and functioning (Allemand et al., 1999). Seasonal survey conducted in 2001 shows that in the South, *Drosophila* communities are clearly dominated by *D. simulans*, whereas

in the North, *D. melanogaster* overtakes all other *Drosophila* species (Figure 1(a)). However, the composition of communities changes in time, with a shift in relative abundance of *D. melanogaster* and *D. simulans* occurring at different times according to latitude. Figure 1(a) shows that *D. simulans* appears early in the season in the southern site (Hyères), later in the intermediate site (Valence), and is only present in autumn with very low abundance in the north (Lyon). *D. melanogaster* predominates in all sites in June, then decreases drastically in south (Hyères and Valence), whereas it remains dominant in north (Lyon). *D. simulans* shows symmetrical variation and progressively replaces almost completely *D. melanogaster* in southern and intermediate sites. What ecological factors account for scarcity of *D. simulans* in north and for seasonal decrease in abundance of *D. melanogaster* in south remains doubtful, but competitive displacement, thermal conditions and parasitism are all possible hypotheses. However, field data do not completely fit thermal requirements of species which are very close (22 and 20.5°C for *D. melanogaster* and *D. simulans*, respectively, David et al., 1983), thus suggesting other factors than temperature being involved in species range and abundance.

Interestingly, parasitoid species display parallel spatial and seasonal variations (Figure 1(b)). *L. heterotoma* appears before *L. boucardi* and like *D. melanogaster*, predominates all over the season in the north, whereas in summer it falls in southern sites (Valence and Hyères), sometimes down to 1%, but without getting extinct (Figure 1(b)). The relative abundance of *L. boucardi* parallels that of *D. simulans*, except that it never occurs beyond 45.4°N in Rhône valley (Allemand et al., 1999). Contrasting with *D. simulans*, the range and seasonal fluctuation of *L. boucardi* fits well its thermal requirements: this Mediterranean species needs higher thermal thresholds than its host to complete development and can enter winter diapause (Carton & Claret, 1982; Hertlein, 1986). We can wonder if weak abundance of *D. simulans* in north could be due to the local absence of *L. boucardi* and to the competitive superiority of *D. melanogaster* under such climatic conditions. Indeed, *L. boucardi* is responsible for high parasitization rates in the south, and if this species does prefer *D. melanogaster* as suggested above, then parasitism could invert the issue of competition between *Drosophila* species and contribute to local supremacy of *D. simulans*. An argument could be that the local population explosion of *L. boucardi* always precedes that of *D. simulans*.

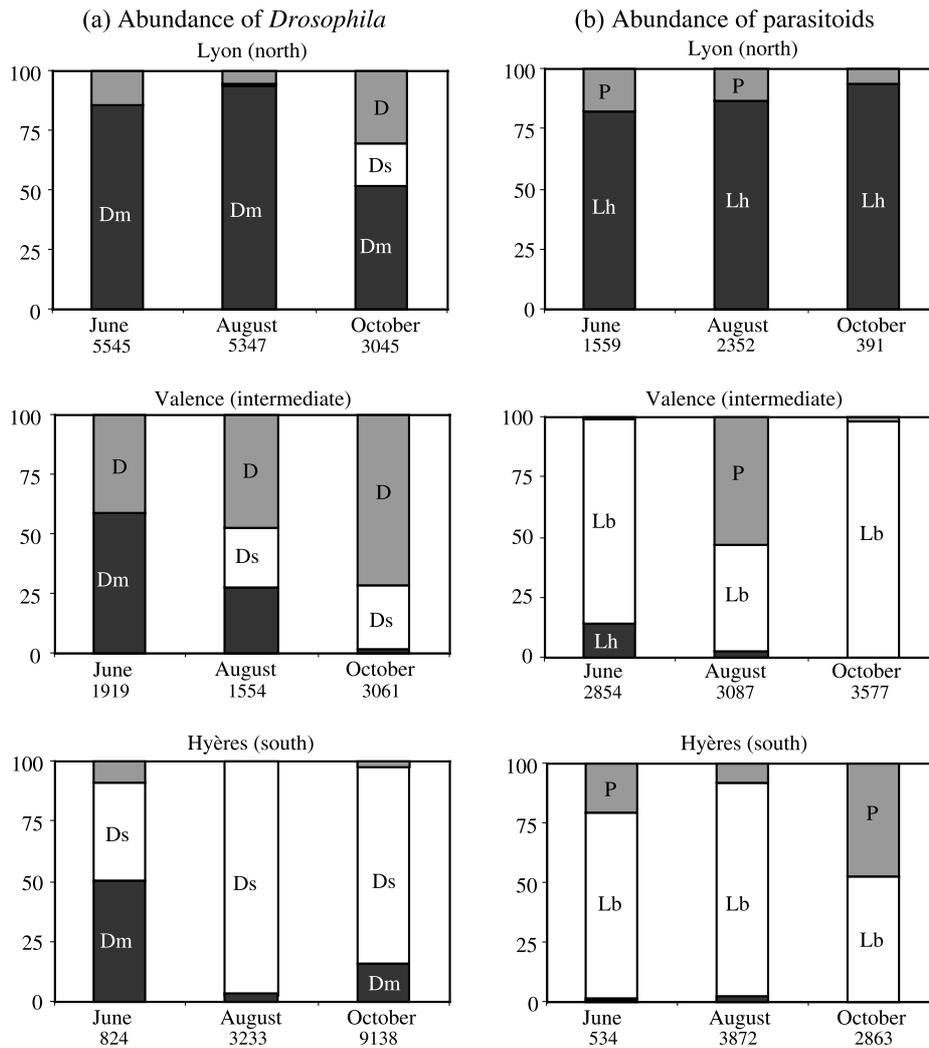


Figure 1. Seasonal variation of the relative abundance of *Drosophila* species (a) and their parasitoids (b) in three sites distributed along a north–south axis of the Rhône valley (France). For *Drosophila* (a), black, white and grey indicate abundance of *D. melanogaster* (Dm), *D. simulans* (Ds) and other *Drosophila* species (D), respectively. For parasitoids (b), black, white and grey indicate abundance of *Leptopilina heterotoma* (Lh), *L. boulandi* (Lb) and other parasitoid species (P), respectively. Numbers of identified insects are given under each sampling date (June, August and October).

Field *Drosophila* populations suffer heavy mortality from parasitoids (Figure 2). Levels of infestation exceed 90% in some fruits, and mean rate of parasitization can reach 76% in Valence (Figure 2). Despite high variability among fruits, parasitization rate seems higher in southern sites (Valence and Hyères), probably due to the very efficient *L. boulandi*. These results demonstrate that parasitism is an important mortality factor which probably exerts strong selective pressure on *Drosophila*, consistently with other previous works

(Janssen et al., 1988; Allemand et al., 1999). In contrast to what was observed in Tunisia (Boulétreau, Fouillet & Allemand, 1991), there is no evidence of any relationship between the rate of parasitization and the relative abundance of *D. melanogaster* and *D. simulans* (Figure 2). A possible explanation could be the higher richness of communities and the presence of several other *Drosophila* and parasitoid species. High parasitization rates makes likely strong competitive interactions to occur among parasitoids. This

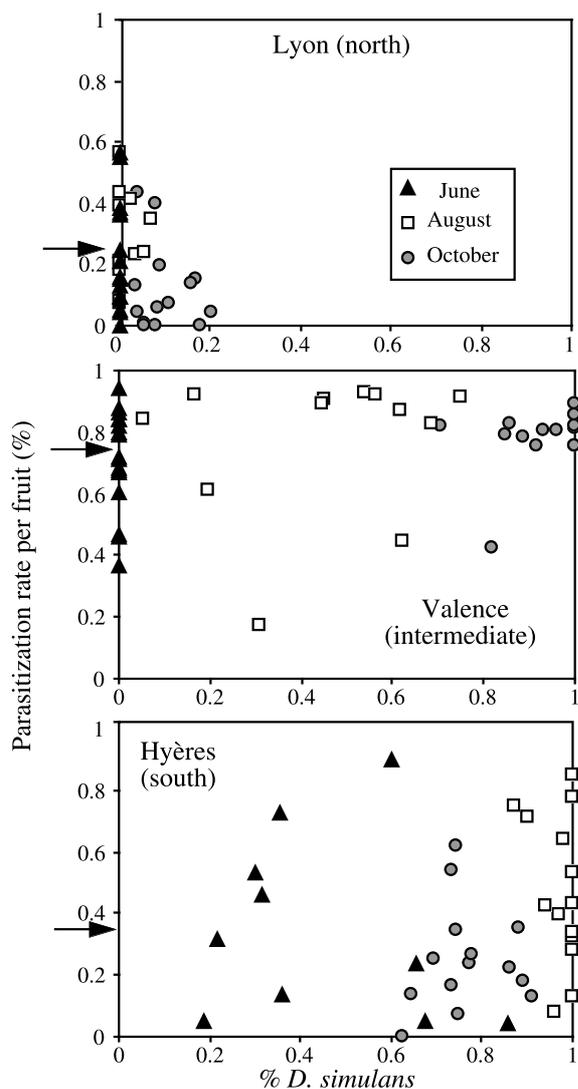


Figure 2. Rate of parasitism according to relative abundance of *D. simulans* (percentage of *D. simulans* among *D. simulans* + *D. melanogaster*). Each graph represents the seasonal survey (June, August and October) of three sites distributed along the Rhône valley (France). Arrow indicates mean rate of parasitization averaged over the three sampling dates.

was evidenced by dissection of field collected second instar *Drosophila* larvae (site of Valence). Among 201 larvae, 181 proved parasitized, of which 44.1% by more than one parasite larva, and some by 5–7 (Figure 3). Since only one parasitoid can develop within each host, strong competition does occur among parasitoids in the field.

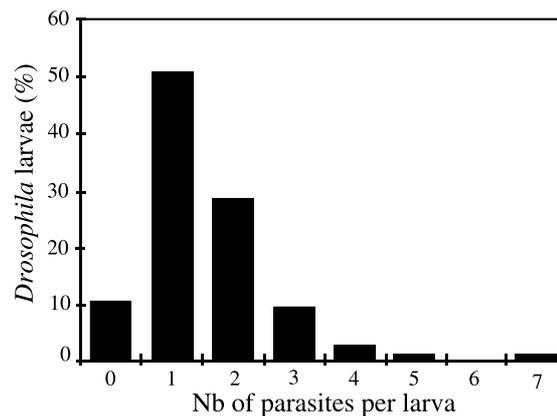


Figure 3. Number of parasitoids within individual *Drosophila* larvae collected in the field and dissected in the lab. 90% of *Drosophila* prove parasitized, of which more than 40% bear more than one parasite larva.

#### Parasitoid-mediated competition between *Drosophila* species in the laboratory

Conforming to previous results on *Drosophila* (Montchamp-Moreau, 1983; Joshi & Thompson, 1995; Hedrick & King, 1996; Davis et al., 1998), the two species did not coexist in the lab, and *D. simulans* went to or near extinction within 5–10 weeks in all control (unparasitized) cages (Figure 4). This confirms the better competitiveness of *D. melanogaster* under this range of temperature (22–29°C). Evolution was quite different in parasitized cages, and outcome of competition varied according to temperature. At 28°C *D. simulans* declined slowly, down to a few percent on week 15, close to extinction. At 25°C both species coexisted, and when the experiment ceased (on week 14) adult populations consisted in 80% *D. melanogaster* and 20% *D. simulans*, a proportion which had established around weeks 5 or 6. At 22°C, presence of the parasite inverted the issue of competition and frequency of *D. simulans* increased until near extinction of *D. melanogaster* (Figure 4). Interrupting introduction of wasps reversed the process and led in fast decline of *D. simulans*, while re-introducing wasps put again *D. simulans* at an advantage (data not shown but see Boulétreau, Fouillet & Allemand, 1991). Continuous presence of parasitoids ended in fixation of *D. simulans* in all three cages (Figure 4). Clearly, the relative abundance of flies directly depends on the presence of wasps, and thus parasitoids can promote coexistence of *Drosophila* species under laboratory

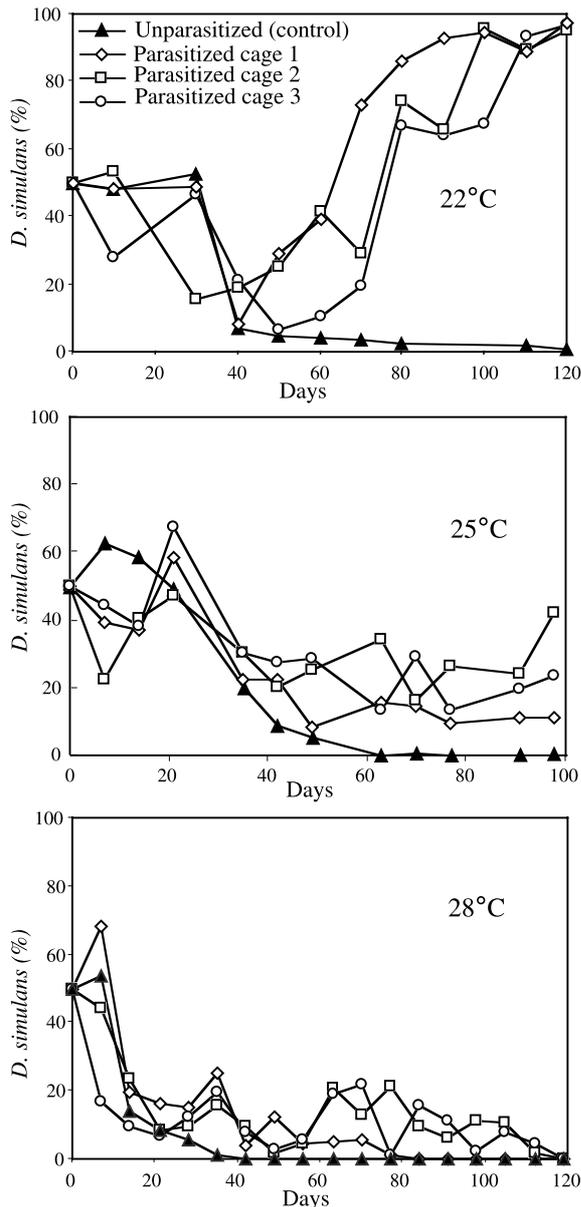


Figure 4. Competition between *D. simulans* and *D. melanogaster*. Evolution of *Drosophila simulans* frequency in infested and uninfested (control) cages at three temperatures (22, 25 and 28°C). At each temperature, three cages with parasites are compared to the average evolution of three uninfested control cages. Results show that parasites mediate competition between *D. melanogaster* and *D. simulans*, resulting in their coexistence at 25°C.

conditions. That is consistent with other results by Davis et al. (1998), which have established the role of parasitoids in coexistence of three *Drosophila* species in a complex experimental set-up.

## Comparative suitability of *Drosophila* species and interactions with temperature

### Materials and methods

We investigated effects of developmental host species and their interaction with temperature on *L. heterotoma* and *L. bouleari*. Twenty females of each wasp species were isolated and provided with 100 host larvae for 24 h, either *D. melanogaster* or *D. simulans* foraging on 15 g of axenic medium. After development at 22 or 25°C, *Drosophila* having escaped parasitism and adult parasitoids were counted in each vial. Wasps were then stored at 22°C for 5 days with honey as food. Two fitness components were measured. Larval survivorship (rate of successful parasite development) was calculated as the percentage of adult wasps emerged from actually parasitized *Drosophila* larvae (details in Boulétreau & Fouillet, 1982). Fecundity was estimated by dissection of 30 females at least, and counting eggs in their ovaries under microscope after coloration with neutral red solution. Egg load provides a good estimate of the reproductive potential of these wasps, since egg production is achieved during preimaginal life (pro-ovigenic species). In *L. bouleari*, a fecundity index was estimated by the mean number of *Drosophila* larvae (300 larvae/female) that females parasitized during a 3-day period (see Boulétreau, Fleury & Fouillet, 1994 for details). Since southern and northern *L. heterotoma* populations display high genetic divergence (Fleury et al., 1995; Allemand et al., 1999), the whole experiment was repeated using two strains originating from Antibes (43°N latitude: southern genotype) and Sainte-Foy-lès-Lyon (45.5°N latitude: northern genotype), in order to check for possible genotype-by-environment interaction.

### Results

Comparison of egg-to-adult survival and adult fecundity of *L. bouleari* and *L. heterotoma* after development under different combinations of host species (*D. melanogaster* or *D. simulans*) and temperature (22 or 25°C) evidenced strong effects of both host species and temperature on the fitness of wasps. At 25°C, survival and fecundity of the two *Leptopilina* species were low on *D. simulans*, especially that of *L. bouleari*. In contrast, at 22°C, host species had little influence. *L. heterotoma* equally thrived on both species (Figure 5(a)). Fecundity of *L. bouleari* was even slightly higher after development on *D. simulans*

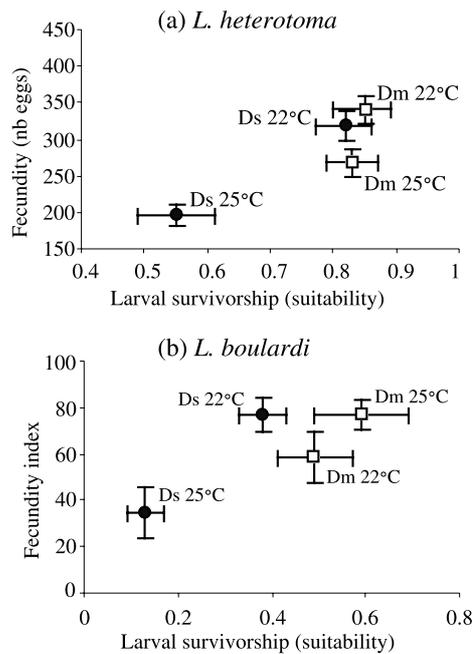


Figure 5. Joint effect of host species (*D. melanogaster* or *D. simulans*) and temperature (22 or 25°C) on larval survival and adult reproductive potential of *L. heterotoma* (a) and *L. boulandi* (b). Mean and confidence intervals are given for each modality.

at 22°C (Figure 5(b)). Clearly, quality of *Drosophila* species as host varies according to temperature, *D. melanogaster* being far better host than *D. simulans* at 25°C while nearly equivalent at 22°C. The deleterious effect that *L. boulandi* suffered when developing in *D. simulans* at 25°C could result from smaller size of *D. simulans* larvae, providing insufficient resources to ensure parasitoid development at high temperature. It is surprising, since this wasp most often encounters this host species in its usual warm southern habitats (south of France). A possible hypothesis could be that *L. boulandi* develops preferentially on *D. melanogaster*.

Geographic populations of *L. heterotoma* species behaved differently. The southern *L. heterotoma* genotype (Antibes) had always higher larval survival and higher fecundity than the northern one (Figure 6(a) and (b)), but responses of the two genotypes to host and temperature combinations were quite different. At 22°C, both genotypes made no difference when developing on either host species (Figure 6(a)), while at 25°C the northern strain proved far more affected by *D. simulans* than the southern one (Figure 6(b)). Differences between genotypes fit in with their local conditions of host abun-

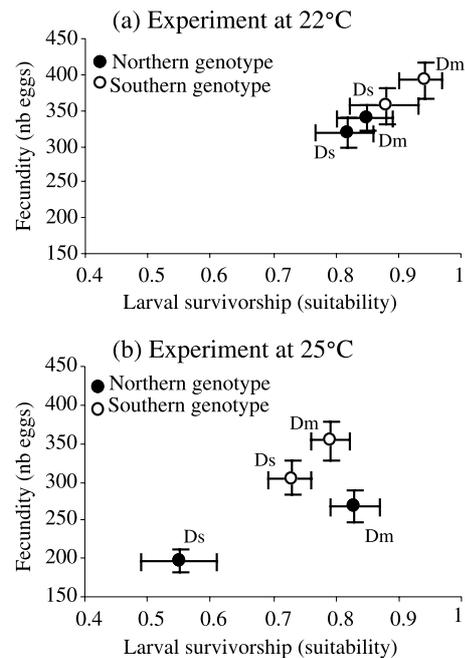


Figure 6. Genotype-by-environment interaction in *L. heterotoma*. Effect of host species (*D. melanogaster* or *D. simulans*) at two temperatures (22 or 25°C) on larval survival and reproductive potential of southern (Antibes 43°N, France) and northern (Ste Foy-les-Lyon 45.5°N France) genotypes of *L. heterotoma*. Mean and confidence intervals are given for each modality.

dance and temperature, the better performance of southern genotypes on *D. simulans* at 25°C compared to northern ones being considered as an adaptation to their native local environment. Other unpublished experiments have compared fecundity of *L. heterotoma* from six clinal populations of the Rhône valley after development in *D. melanogaster* or *D. simulans* at more extreme temperatures (14 and 26°C), and fully reinforce the above interpretation.

## Discussion

### Competition among *Drosophila* species

The role of interspecific competition in structuring insect communities and its importance in evolution and speciation have been hotly debated (Connell, 1980; Schoener, 1982; Shorrocks et al., 1984; Denno, McClure & Ott, 1995). However, studies performed on a wide range of taxa have suggested that competition does occur in nature (Schoener, 1983; Goldberg & Barton, 1992; Denno, McClure & Ott, 1995).

Conclusive evidence has been obtained from *Drosophila* communities (e.g., Barker, 1983; Grimaldi & Jaenike, 1984; Courtney, Kibota & Singleton, 1990; Nunney, 1990; Joshi & Mueller, 1996; Wertheim et al., 2000). Present results provide further arguments for strong competition between *D. melanogaster* and *D. simulans* in south-east of France. Both species develop in the same resource with temporal variation of relative abundance of species which is an indirect evidence for interspecific competition in nature (Barker, 1983). This shift in species composition, with a decline of *D. melanogaster* and a correlated rise of *D. simulans* during the season, strongly suggests that *D. simulans* replaces *D. melanogaster* by competitive displacement. This was rather unexpected since *D. melanogaster* is known as the better competitor under this range of thermal conditions (Tantawy & Soliman, 1967; Montchamp-Moreau, 1983; Davis et al., 1998 and results of this study). It is unlikely that slight preference of *L. boulandi* for *D. melanogaster* can completely account for such variation in abundance *Drosophila* species, thus suggesting that overall competitive ability of species is involved. It is worth noting that high parasitization rate does not significantly reduce competitive interactions between *Drosophila* for two reasons. Firstly parasitoids kill their hosts after pupation, thus after exploitative competition among larvae. Secondly reduction of adult *Drosophila* number is probably not sufficient to significantly reduce larval density in fermenting fruits because of their high reproductive potential.

Under simple laboratory conditions, *D. melanogaster* and *D. simulans* cannot coexist as previously demonstrated by a number of other studies (Joshi & Thompson, 1995; Hedrick & King, 1996; Davis et al., 1998). It remains however unexplained why *D. melanogaster* eliminates *D. simulans* under a wide range of temperature in laboratory experiments of competition, whereas field observations suggest a superiority of *D. simulans* in the south. Coexistence of the sibling *D. melanogaster* and *D. simulans* has focused lots of works which underlined habitat heterogeneity as a major component of their coexistence (see Barker, 1983; Arthur, 1987 for review; Jaenike & James, 1991; Shorrocks, 1991; Sevenster & Van Alphen, 1996; Krijger & Sevenster, 2001). The outcome of competition is however probably the consequence of complex interplay between genetic and environmental factors (Moore, 1952; Tantawy & Soliman, 1967; Montchamp-Moreau, 1983) among

which parasitoid-induced mortality needs to be added.

#### *Parasitism and coexistence of D. melanogaster and D. simulans*

The role of predation and/or parasitism in mediating coexistence of competing species has been studied in various zoological groups (Freeland, 1983; Hanski, 1983; Holt & Lawton, 1994; Tompkins, Dickson & Hudson, 1999), but it has not been considered extensively in *Drosophila* communities. Our results show how parasitoids can modify outcome of competition and mediate either coexistence, or victory of *D. simulans* depending on temperature. Mechanisms responsible for this striking effect are certainly complex. We can first consider that reduction in fly numbers in parasitized cages resulted in fewer eggs deposited and weaker subsequent competition among larvae. However, this scenario is not consistent with data of Prévost (1985) and Wajnberg (1986), who demonstrated that total number of insects emerging from food cups (flies + wasps) were far higher in parasitized cages than in unparasitized ones (flies alone). Moreover, such hypothetical reduction in larval competition could hardly account for effects of high temperatures. We can also evoke preferential infestation of *D. melanogaster* by *L. boulandi*, capable of balancing its competitiveness towards *D. simulans*. However, the clear advantage of *D. melanogaster* in parasitized cages at 28°C would imply either that wasps change their host choice depending on temperature, or that high temperatures make the superiority of *D. melanogaster* weak enough to vanish in parasitized cages at 22°C. We thus lack fully satisfactory explanation, but Davis et al. (1998) also demonstrated the temperature-dependent role of *L. boulandi* in competitive interactions between host and non-host species in a spatially structured experimental set-up (*D. melanogaster* and *D. simulans* vs. *D. subobscura*). Thus, parasitoids are likely to play a major role not only in the *D. melanogaster*–*D. simulans* interaction, but also in the functioning and stability of more complex *Drosophila* communities. Extending such laboratory data to field situations needs extreme care. Coexistence of *Drosophila* species in the wild obviously involves a set of classical factors such as habitat heterogeneity and resource sharing, dispersal, temperature fluctuations, etc. Parasitoids must now be added, especially in south of Europe where their high prevalence can put them among the main natural *Drosophila* mortality

factors. Moreover, their abundance may vary in space and time at a short scale, and together with rapid change in temperature, they could account for rapid change in local relative abundance of *D. melanogaster* and *D. simulans*.

#### *Competition among parasitoids*

In contrast to *Drosophila* species for which we have only indirect evidence for inter-specific competition, results clearly demonstrate heavy larval competition among *Leptopilina* parasitoids. In Mediterranean area, high rates of parasitization (up to 90%) make a number of *Drosophila* larvae multi-infested (super- or multi-parasitized), and that leads to strong within-host competition among parasitoid larvae, which results in death of all of them but one. However, such high parasitization rates have not been reported in all *Drosophila* communities (Nunney, 1990; Wertheim et al., 2000), suggesting that parasitism could vary among *Drosophila* species and over their geographic area, with higher prevalence in southern Europe. Coexistence of parasitoids probably involves various mechanisms of resource partitioning, mainly difference in their host range: the generalist *L. heterotoma* can exploit alternative host species that the specialist *L. boulardi* cannot. Temporal segregation of activity on a daily basis, which results from difference in circadian rhythms of species, can also promote their coexistence (Fleury et al., 2000).

#### *Suitability of D. melanogaster and D. simulans as hosts for parasitoids*

Field studies on natural populations have shown that *Drosophila*–parasitoids have to cope with seasonal change in the relative abundance of *Drosophila* species, whose quality as hosts depends on temperature: *D. melanogaster* has better thermal homeostasis for parasite development, which makes it a better host than *D. simulans*. Change in developmental host (either *D. melanogaster* or *D. simulans*) can modify not only parasitoid survival and fecundity as demonstrated here, but also unexpected behavioral traits, such as activity rhythms (Fleury, 1993). Since patterns of host diversity and abundance vary according to local climatic conditions, it is expected that differential selective pressures that local parasitoid populations suffer could lead to locally adaptive genetic differentiation. Comparison of *L. heterotoma* populations from southeast of France has evidenced marked genetic differences according to their geographic origin (Fleury

et al., 1995). Present results support this conclusion, since southern genotypes show higher fitness traits (survival and fecundity) than northern ones under all environmental conditions used in our experiments. A number of correlated ecological factors, either abiotic or biotic, vary along the latitudinal cline here studied and thus, the specific contribution of host species or abundance to the overall adaptive response of *L. heterotoma* populations is questionable. For instance, we have to consider traits specifically involved in parasitism and perhaps subject to co-evolutionary process, and other traits whose links with parasitism are not so tight, or which only express themselves when interacting with other environmental factors. We can only conclude that clear genetic differentiation of local population of *L. heterotoma* results from differences in environmental selective pressures, to which the relative abundance of *D. melanogaster* and *D. simulans* greatly participates. Of course, we now wonder about the reciprocal effects of this differentiation on selective pressures that *Drosophila* populations suffer from parasitoids.

#### **Conclusions**

Frugivorous *Drosophila* and their parasites offer a good opportunity for studying interacting communities. The two sibling *D. melanogaster* and *D. simulans* are of quite different values as hosts for parasitoids, and high rates of natural infestation that both species suffer will probably deeply affect the biology, ecology and evolution of all partners, flies and wasps. Consequences of high parasitization rate are expected at the within species genetic level, thus shaping a variety of host genome features. Traits could be directly involved in the individual host–parasitoid relationship (e.g., behavioral: Carton & Sokolowski, 1992; immunological: Kraaijeveld & Van Alphen, 1995; Carton & Nappi, 2001), or indirectly affected through pleiotropic effects and/or trade-offs (e.g., competitive ability: Kraaijeveld & Godfray, 1997), or through genetic drift resulting from reduction in effective population size. Geographical variation in diversity and abundance of the parasitoid community may thus contribute to and account for local genetic differentiation of *Drosophila* populations. Conversely, local and seasonal variations in the availability of *Drosophila* species, together with temperature change, proved essential in shaping the phenotype (and also the fitness) of wasps, and they are very likely to participate in local

selective pressures acting on parasitoid differentiation and evolution. Consequences of all these processes at the community level are poorly documented so far, but they promise very exciting. Parasitoids may contribute to understand the puzzling and debated question of species coexistence. Moreover, a few degrees of temperature change can have drastic effects on the *Drosophila*–parasitoids community structure, with two possible consequences. Firstly, interaction of temperature with parasitoids could account for micro-geographic distribution of *Drosophila* species, that climate alone cannot; secondly, we may expect climatic changes, either past, present or future, to affect or have affected species abundance and distribution at a totally unpredicted time scale. Clearly, laboratory studies cannot fully explain complex field situations, but they have open a lot of promising trails that deserve further investigations to better understand the ecological and evolutionary interaction of *D. melanogaster* and *D. simulans*.

### Acknowledgements

We thank F. Vavre for valuable comments on the manuscript. Financial support for field and laboratory experiments was provided by CNRS (UMR 5558, UPR 9034, GDR 2153 and PND BE programs).

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