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Developmental Relationships Between *Drosophila* Larvae and Their Endoparasitoid *Leptopilina* (Hymenoptera: Cynipidae) as Affected by Crowding

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Crowding of *Drosophila* larvae modifies their suitability to the cynipid endoparasitoid *Leptopilina boulardi*. The success of parasitic development rises from 40% in uncrowded host larvae to 90% in crowded ones. Crowding reduces the imaginal size of both wasps and uninfested hosts, but it has opposite effects on their development time: That of flies is increased, whereas that of wasps is reduced.

Key words: parasitic hymenoptera, host suitability, larval crowding, developmental relationships

INTRODUCTION

Developmental relationships between parasitoids and their hosts are governed by the physiological features of both partners. Full development of parasitoids is only possible if host's and parasite's physiologies are properly suited ("host suitability [1] and if the modifications induced in the host's physiology are not deleterious and are even more profitable to the parasitoid ("host regulation" [2]). Analysis of the components of these close physiological relationships is generally difficult, since both partners interact so strongly that physiological variations in the host also reflect in the physiology of the parasitoid.

The cynipid *Leptopilina boulardi* (Eucoilidae) and its host *Drosophila* sp. provides a good model system for such an analysis. The parasitoid is rather specific to *D. melanogaster* and to a few related species, and larvae develop solitarily in host larvae. Moreover, in some strains, like the Tunisian one used here, the parasitoid is never encapsulated, which makes the analysis more simple.

The developmental success of *L. boulardi* within *D. melanogaster* larvae strongly

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depends on the genetic makeup of the host: Genetic variations in hosts (within populations, between populations, or caused by artificial selection) generally result in wide differences in the success of the parasitoid, which ranges from less than 10% to more than 90% [3–7]. There is also a twofold increase in the success of this parasitoid in crowded (underfed) hosts [5,7–9]. Here using morphological criteria along with development times to compare the effects of crowding on uninfested and infested hosts, we estimate the effects of the host on parasitoids, and we try to provide some basis for hypothesized physiological causes of the increased suitability of crowded hosts to the parasitoid.

MATERIALS AND METHODS

Infestation

One hundred twenty batches of 100 *Drosophila* eggs (0–6 h old) were collected. Each batch of eggs was spread upon a dish (18 mm diameter, 4 mm depth) filled with 1 g of medium [10] diluted by 50% with water plus 1.5% agar. After hatching and first molting, 90 batches of larvae out of these 120 batches were each exposed to one mated *L. boulardi* female (12–24 h old, honey-fed) over a 24 h period. Larvae from 30 other batches were kept free of parasitism (controls).

Development

Half of the batches (infested or not) were transferred to standard rearing vials (2 cm diameter, 10 cm depth) containing 20 g of rich, undiluted medium (uncrowded series); the others were kept on the original dish of diluted medium (crowded series). Subsequent development took place at 25°C under 12 h light/12 h dark and 70% relative humidity, the small dishes being prevented from dessication by keeping them individually in 5-cm petri dishes.

Measurements and Calculations

The following parameters were measured or estimated: the success of parasitoid development, which needs an estimate of the actual degree of infestation in each batch; the development times of hosts and parasitoids; the sizes of adult flies; and the weights of parasitoid adults under both uncrowded and crowded conditions.

Degree of Infestation and Superparasitism

Flies and wasps emerging from each batch were counted and sexed. Because no parasitoid rejection occurred through encapsulation, the difference between the numbers of flies in experimental batches and in uninfested controls gives an estimate of the number of hosts that were infested (and destroyed) by the parasitoid in each batch. Preliminary dissection of 200 *Drosophila* larvae exposed to one female parasitoid under the above conditions showed that 93.6% of infested larvae contained only one parasitoid larva, 5.3% two, and 1.1% three, thus indicating a rather low frequency of superparasitism.

The success of parasitoid development (SPD) was measured in each batch as the percentage of the number of adult wasps over the estimated number of infested host larvae, according to Boulétreau and Fouillet [3]. This parameter expressed the probability of an infested host larva giving rise to an adult wasp.

Given the low frequency of superparasitism, it gives also a rough estimate of the egg-to-adult viability of parasitoids.

The developmental times of hosts and parasitoids were measured for males and females between the middle of the oviposition period and emergence, which was recorded twice a day. The precision of this simple method is consistent with the wide range of variations caused by the factors tested (sex and crowding).

The size of adult flies that emerged from uninfested batches, either uncrowded or crowded, was estimated by the wing length and thorax length of a random sample of 30 males. In both the crowded and uncrowded series, dry weights of adult wasps (males and females) were measured on 30 randomly chosen individuals after dehydration.

RESULTS

Crowding does not affect egg-to-adult viability of uninfested flies in control batches (Table 1), but the size of adult flies is reduced (Table 1) and their developmental time increased (Table 1, Fig. 1). These results are typical of the scramble competition that occurs among *Drosophila* larvae and that has been well documented by a number of authors [11–14].

The effects of crowding on parasitized hosts are quite different. The success of parasitoid development increases twofold in crowded cultures over uncrowded ones (Table 1). The weight of adult parasitoids emerging from crowded cultures is reduced (Table 1, Fig. 2), but, surprisingly, their developmental time is significantly reduced (Table 1, Fig. 1). Unfortunately, we did not record the time of pupation for infested hosts, which would have allowed us to assess which part of the development is to be credited with the overall acceleration.

Thus the more striking results are the opposite effects of crowding on hosts and on parasitoids. Viability of hosts is not affected, whereas that of parasitoids is doubled. Developmental time increases in hosts (+ 30 h for averaged sexes), whereas it decreases in parasitoids (− 38 h, Table 1). As a consequence, in crowded cultures wasps will emerge on average 8.5 days later than flies that escaped infestation, whereas in uncrowded control cultures this delay reaches 11.3 days.

A similar negative correlation between suitability of hosts and development time of parasitoids was found in other experiments in which interfamily genetic differences within the *Drosophila* population were responsible for variations in developmental success of *L. boulardi* (unpublished data).

DISCUSSION

A striking positive relationship appears between the success of parasitoid development, as measured by the ratio of emerged wasps to infested host larvae, and the crowding of host larvae. Since crowding in hosts occurred only after they were infested and crowding is known to affect insect physiology [12–14], this effect clearly reflects the influence of variations in the hosts' developmental physiology on their suitability for the development of the parasitoid larva. The question of development time is probably a key point for explain-

TABLE 1. Effects of Crowding on Overall Viability, Size and Developmental Time of Uninfested *D. melanogaster* and of the Parasitoid *L. boulardi

	Viability ± SD		Size ± SD: Unparasitized (control) (mm)		Size ± SD: Wasps	Dev. time (hours ± SD)	
	Unparasitized (control)	Wasps	Wing	Thorax	Dry weight (μg)	Unparasitized (control)	Wasps
Uncrowded	85.9 ± 1.8 (N = 15)	40.2 ± 2.4 (N = 42)	2.19 ± 0.02 (N = 30 ♂)	0.92 ± 0.03 (N = 30 ♂)	92.4 ± 1.6 (a) (N = 30 ♂) 133.3 ± 2.9 (b) (N = 30 ♀)	231.8 ± 0.4 (a) (N = 421 ♂) 224.6 ± 0.4 (b) (N = 470 ♀)	481.8 ± 1.6 (a) (n = 359 ♂) 519.2 ± 1.5 (b) (N = 453 ♀)
Crowded	81.5 ± 2.2 (N = 15)	89.4 ± 1.4 (N = 45)	2.01 ± 0.01 (N = 30 ♂)	0.81 ± 0.06 (N = 30 ♂)	55.8 ± 1.5 (a) (N = 30 ♂) 80.4 ± 2.0 (b) (N = 30 ♀)	259.2 ± 0.7 (a) (N = 403 ♂) 258.1 ± 0.8 (b) (N = 419 ♀)	438.9 ± 1.7 (a) (N = 334 ♂) 486.9 ± 0.9 (b) (N = 580 ♀)
F Value	NS	***	***	***	*** (a) *** (b)	*** (a) *** (b)	*** (b) *** (b)
Variation	–	+122%	−8%	−11.9%	−40% (a) −40% (b)	+11.8% (a) +14.9% (b)	−9.1% (a) −6.2% (b)

Viability of flies is the ratio (%) of the number of adult flies over the initial number of eggs. Viability of wasps is the ratio (%) of emerged adult wasps over the number of infested hosts. Numbers in brackets indicate the numbers of measurements that were done either on batches of 100 individuals (viability: first column) or on adult individuals (sizes and dev. times). Statistical analysis by analysis of variance. Calculations on viability after arcsin $\sqrt{p}$ transformation.

*** $P < 0.001$.

a = Data for males.

b = Data for females.

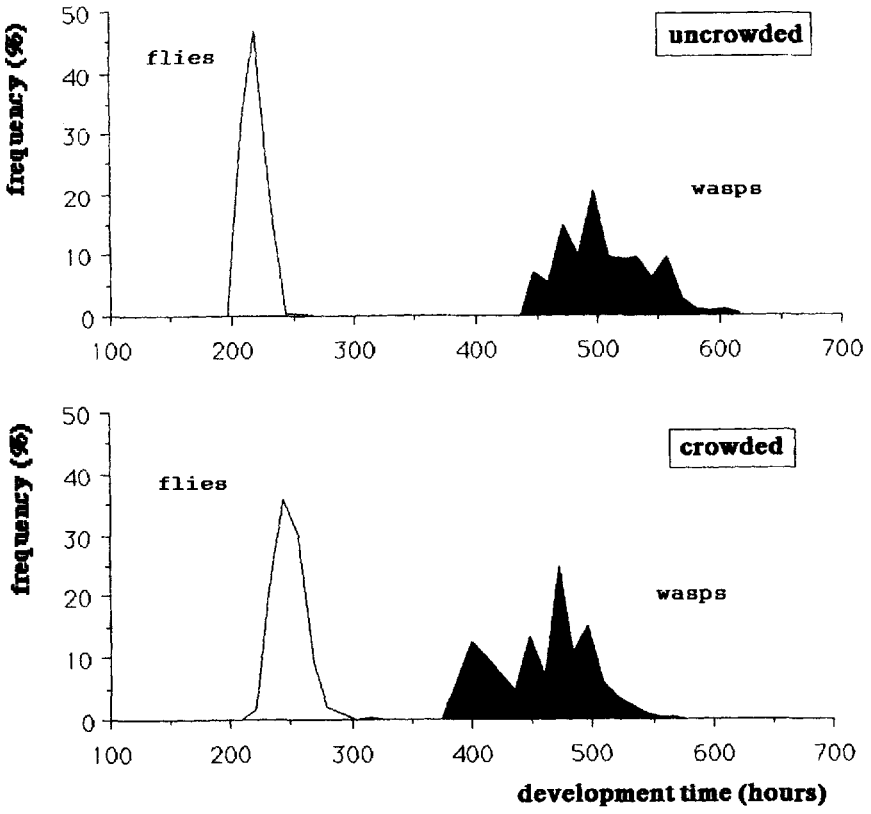

Fig. 1. Development time of *D. melanogaster* from crowded and uncrowded uninfested batches and of *L. boulardi* having developed within either crowded or uncrowded *Drosophila* larvae.

ing the variations in suitability of *Drosophila* larvae to *L. boulardi*, and the effects of crowding on both traits need to be discussed together.

A first hypothesis could be that suboptimal hosts (either genetically or nutritionally, caused high mortality among parasitoids, thereby selecting for the more slowly developing ones, thus truncating the distribution of development time of parasitoids and leading to the higher mean value. However, the distributions of developmental time of parasitoids having developed either in uncrowded or in crowded hosts overlap only partly (Fig. 1). Hence, variations in mean values are unlikely to be accounted for by truncation of the distributions, but rather by a general drop in individual values.

In a number of cases, it has been demonstrated that features of the hosts may influence the size and development time of parasitoids: Smaller hosts, or

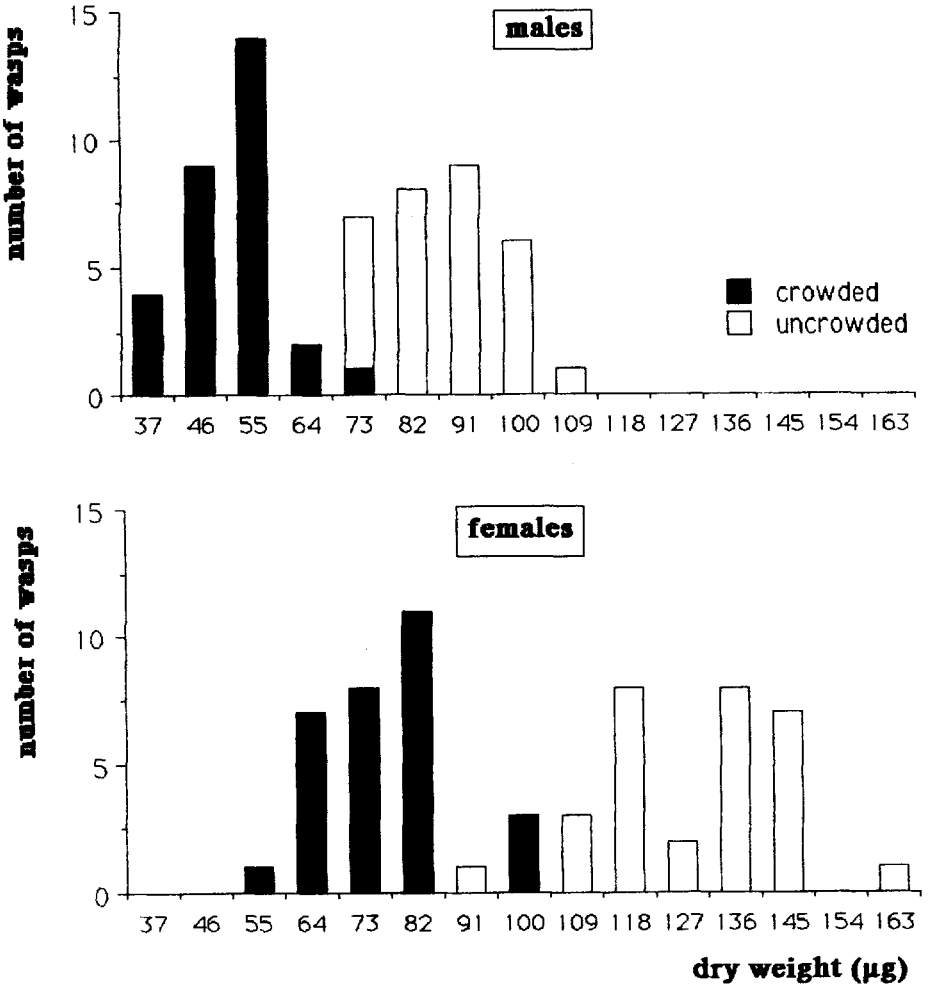

Fig. 2. Dry weights of adult male and female *L. boulardi*, developed within either uncrowded or crowded hosts.

lower availability of food for the hosts, reduce the parasitoid's size and developmental time when they develop either solitarily [15–18] or gregariously [19–21]. The same phenomenon could occur in the underfed *Drosophila* larvae: Food deprivation results in a poor weight gain of the host, which in turn could, through some quantitative nutritional mechanism, induce the precocious molting of the parasitoid larva, thus allowing it to enter its own active feeding and growth period earlier.

However, this simple explanation does not account for the opposite variations in flies' and parasitoids' developmental times. In the parasitoid species studied here, ecdysis to the third stage strictly coincides with the onset of host pupariation [22], and it is likely that this synchrony is a key factor in the parasitoid's development. Since crowding has long been known to prolong the larval period in *Drosophila* [11], induction of a parasitoid's molting by food deficiency (because of poor weight gain of the host) would break down this tuning. This is not consistent with the high success of parasitoids' development in crowded hosts. As a hypothesis, we suggest that tuning between hosts' and parasitoids' development could be actively induced by the parasitoid larva itself, which is consistent with data of Kopelman and Chabora [22].

LITERATURE CITED

1. Vinson SB, Iwantsch GF: Host suitability for insect parasitoids. Annu Rev Entomol *25*, 397 (1980).
2. Vinson SB, Iwantsch GF: Host regulation by insect parasitoids. Q Rev Biol *55*, 143 (1980).
3. Boulétreau M, Fouillet P: Variabilité génétique intrapopulation de l'aptitude de *Drosophila melanogaster* à permettre le développement d'un hyménoptère parasite. C R Acad Sci (Paris) *295*, 775 (1982).
4. Carton Y: Analyse expérimentale de trois niveaux d'interaction entre *Drosophila melanogaster* et le parasite *Leptopilina boulardi*. Gén Sél Evol *16*, 417 (1984).
5. Wajnberg E, Prévost G, Boulétreau M: Genetic and epigenetic variation in *Drosophila* larvae suitability to a hymenopterous endoparasitoid. Entomophaga *30*, 186 (1985).
6. Boulétreau M: The genetic and coevolutionary interactions between parasitoids and their hosts. In: Insect Parasitoids, Waage J, Greathead D, eds. Academic Press, London, pp 169–200 (1986).
7. Boulétreau M, Wajnberg E: Comparative responses of two sympatric parasitoid Cynipids to the genetic and epigenetic variations of their host, *Drosophila melanogaster* larvae. Entomol Exp Appl *41*, 107 (1986).
8. Prévost G: Etude Expérimentale des Interactions entre Parasitisme et Compétition Larvaire chez *Drosophila melanogaster*. Thesis, Lyon, 119 pp (1985).
9. Wajnberg E: Interactions Démographiques et Génétiques entre la Drosophile (*D. melanogaster*) et ses parasites Larvaires Hyménoptères. Thesis, Lyon, 118 pp. (1986).
10. David J, Clavel MF: Interaction entre le génotype et le milieu d'élevage: conséquences sur les caractéristiques du développement de la Drosophile. Bull Biol Fr Belg *99*, 369 (1965).
11. Sang JH: The ecological determinants of population growth in a *Drosophila* culture. Physiol Zool *22*, 183 (1949).
12. Bakker K: Analysis of factors which determine success in competition for food among larvae of *Drosophila melanogaster*. Arch Neerl Zool *2*, 200 (1961).
13. David J, Fouillet P, van Herrewege J: Sous-alimentation quantitative chez la Drosophile. I. Action sur le développement larvaire et sur la taille des adultes. Ann Soc Entomol Fr *6*, 367 (1971).
14. Scheiring JF, Davis DG, Ranasinghe A, Teare CA: Effects of larval crowding on life history parameters in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae). Ann Entomol Soc Am *77*, 329 (1984).
15. Salt G: The effects of hosts upon their insect parasites. Biol Rev *16*, 239 (1941).
16. Arthur AP, Wylie HG: Effects of host size on sex ratio, development time and size of *Pimpla turionellae* L. Entomophaga *4*, 297 (1959).
17. Legner F: Adult emergence interval and reproduction in parasitic Hymenoptera influenced by host size and density. Ann Entomol Soc Am *62*, 220 (1969).
18. van den Assem J: Some experiments on sex ratio and sex regulation in the pteromalid *Lariophagus distinguendus*. Neth J Zool *21*, 373 (1971).
19. Pramanik LM, Choudhury MK: Effect of superparasitism on the development, sex-ratio and progeny of *Bracon greeni* Ash. Entomophaga *8*, 83 (1963).
20. Boulétreau M: Nutrition Larvaire et Exploitation de l'Hôte chez un Hyménoptère Endoparasite, *Pteromalus puparum L.* Influence de la Densité de Population Préimaginale et Conséquences sur les Adultes. Thesis, Lyon, 232 pp (1977).
21. Boulétreau M: Comment les insectes parasites se partagent leur hôte: exemple d'un Hyménoptère endoparasite grégaire des chrysalides de *Pieris brassicae* L. Bull Ecol *16*, 77 (1985).
22. Kopelman AH, Chabora PC: Immature stages of *Leptopilina boulardi* (Hymenoptera: Eucoilidae), a protelean parasite of *Drosophila* sp. (Diptera: Drosophilidae). Ann Entomol Soc Am *77*, 264 (1984).