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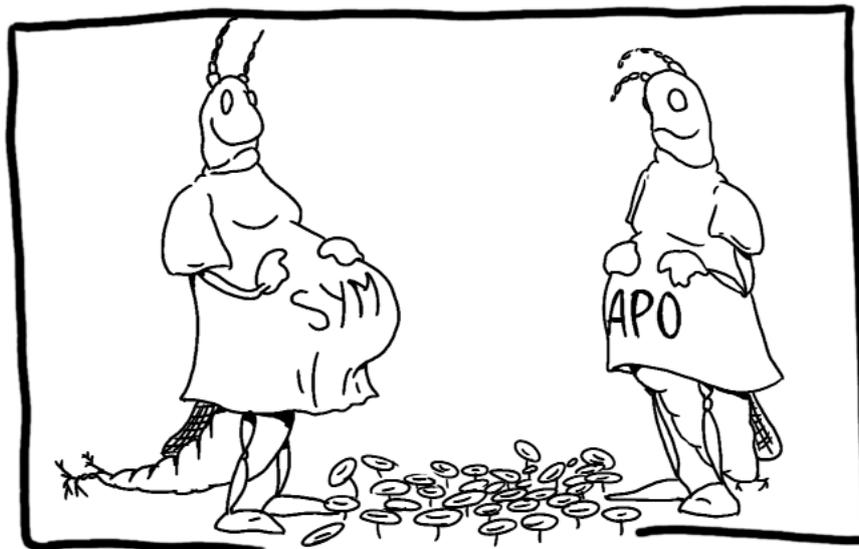
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Diet-dependent effects of gut bacteria on their insect host: the symbiosis of *Erwinia* spec and western flower thrips

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Chapter 4

ABSTRACT – Studies on bacteria in the gut of insect species are numerous, but hardly ever the impact on host performance was elucidated. We showed earlier that *Erwinia* bacteria occur in the gut of western flower thrips, most probably acquired during feeding. Here we investigate whether thrips gain a net benefit or pay a net cost due to these gut bacteria. On a diet of cucumber leaves, time to maturity is shorter and the oviposition rate is higher in thrips with bacteria than in thrips without (aposymbionts). When fed on cucumber leaves and pollen, aposymbionts develop faster and lay more eggs. So, *Erwinia* bacteria benefit or parasitise their thrips hosts depending on the diet, which is in accordance with theoretical predictions for fitness of organisms engaged in symbiotic interactions. Possibly, the transmission of gut bacteria has not become strictly vertical because of this diet-dependent fitness variability.

KEY WORDS – Development time, oviposition, western flower thrips, symbiotic bacteria, *Erwinia* species, gut bacteria, mutualism, biological control

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Symbiotic interactions are defined as close and rather permanent associations between different species (Boucher *et al.*, 1982; Douglas, 1989). The effect of a symbiont on its host can be parasitic, mutualistic, or neutral. Whether the interaction is actually mutualistic or parasitic may vary in space and time, as shown in a considerable number of empirical studies (Bronstein, 1994a,b; Hurst, 1993; Werren and O'Neill, 1997). Mutualism is defined as the reciprocal exploitation that provides a net benefit to each partner (Herre *et al.*, 1999). The outcome of interactions may be stationary under certain conditions, e.g., under vertical transmission of a microbial symbiont from the mother host to her offspring (Ewald, 1994; Law and Dieckmann, 1998; Maynard Smith and Szathmary, 1995; Yamamura, 1993).

Vertical transmission is not a prerequisite for the evolution of mutualistic interactions between symbionts and their hosts. According to Genkai-Kato and Yamamura (1999), evolution of mutualism under conditions of horizontal transmission and a single infection is possible when four criteria are met: (1) vertical transmission involves some cost to the host; (2) the symbiont suffers direct negative effects if it exploits the host too intensively; (3) the host is able to make good use of the excretion products from the symbiont; and (4) the mechanism of vertical transmission is controlled by the host. In fact, mutualism can arise even under strict horizontal transmission as long as increased investments in the symbiont result in increased returns to the host and vice versa (Van Baalen and Jansen, 2001). Such cases are more likely to arise when hosts do not harbour more than a single type of symbiont, because within-host competition among symbiont genotypes may well go to the detriment of the interests of the host. Another condition promoting mutualisms is the ability of the host to take sanctions against non-co-operative symbionts (Sherratt and Roberts, 2002). Whether multiple infection of hosts in the absence of host sanctions (or the presence of counter-sanctions of the symbiont) completely prevents mutualism to evolve, remains an open question. Its answer will critically depend on the precise way in which the symbiont-genotypes and the host play games against each other.

Very few empirical studies have been published on mutualistic interactions in which the main route of transmission is not vertical. Gut bacteria in phytophagous and carnivorous insect species (Baines, 1956; Bignell, 1984; Campbell, 1990; Douglas and Beard, 1997) represent a challenging case. Most of them belong to the gamma group of Proteobacteria, family of Enterobacteriaceae. They are facultative symbiotic bacteria, since they are able to survive and grow outside the host. Whether the interaction is maintained throughout the whole insect life cycle, is still an open question. Some researchers claim that gut bacteria are just accidental passengers (Thibout *et al.*, 1995; Werren and O'Neill, 1997). Others found that the symbiotic interactions between insect hosts and bacteria have a more permanent character. Whereas gut bacteria grow in their host, they can also enhance the fitness of the host (cockroaches, Cruden and Markovetz, 1987; termites, Breznak, 1982; and xylophagous beetles, Bridges, 1981). Thus, there is every reason to suspect a mutualistic interaction. The predominant mode of transmission, however, is horizontal. Only in some cases, they are shown to be transmitted vertically, albeit not transovarial. For example, in cockroaches females smear bacteria on the shell of their eggs or juveniles eat the contaminated faeces of their mother (Cruden and Markovetz, 1987; Koch, 1967).

In this article, we study the interaction between western flower thrips (*Frankliniella occidentalis* [Thysanoptera: Thripidae]), a herbivorous insect, and *Erwinia* spec bacteria inhabiting their gut. There is no evidence for transovarial vertical transmission or any exter-

nal route of egg contamination with bacteria (De Vries *et al.*, 2001b). Instead, the larvae of the thrips acquire the gut bacteria, each generation again, by feeding preferentially on leaves that have been fed upon by other conspecific thrips. Whether this mode of transmission is more close to being vertical or horizontal now depends on the spatial pattern of relatedness in thrips (meta-)populations. We assess whether *F. occidentalis* benefit from the symbionts in their gut system in terms of increased population growth rates, especially in developmental period and egg production rate, being the main determinants (Van Rijn *et al.*, 1995). The results of these studies will be discussed in the light of the possible population structures and the different modes of transmission.

MATERIALS AND METHODS

The model system

Western flower thrips is a small, plant feeding insect that has symbiotic bacteria in its hindgut (De Vries *et al.*, 1995; Ullman *et al.*, 1989). Thrips have a permanent association with bacteria from the genus *Erwinia*; the particular strain used in this study was labelled TAC (Thrips Amsterdam Chrysanthemum). This strain was shown to be present in all second instar larvae over a large number of generations on various host plant species (De Vries *et al.*, 2001a). Thrips larvae are free of bacteria directly after hatching, but they acquire gut bacteria during feeding on the plant. The bacteria are deposited by other thrips on the leaf surface, either via the faeces or possibly via regurgitation. In thrips larvae, TAC bacteria proliferate exponentially, presumably only controlled by the thrips' food uptake (De Vries *et al.*, 2001b). Bacteria-free thrips can be cultured on leaves and the *Erwinia* spec bacteria can be grown on artificial media. Thus, the symbiont-host interaction is facultative. The function of the gut bacteria for their host has not yet been studied and we will test their impact on food conversion in the thrips. Thrips feed primarily on leaf parenchyma, leaf petals, and pollen, of a large number of plant species (Bryan and Smith, 1956; Kirk, 1985; Yudin *et al.*, 1986).

Thrips populations

Thrips were obtained from a culture on chrysanthemum (*Dendranthema grandiflora*), started in 1994 with a batch of *F. occidentalis* provided by the Centre for Plant Breeding and Reproduction Research in Wageningen, the Netherlands. They were reared on intact, small (ca. 40 cm long) chrysanthemum plants, obtained from local garden centres. The culture was maintained in a climate box at 25 °C, 60% relative humidity, and 16 h light per day. Thrips identity was checked once per year in samples submitted to G. Vierbergen at the Plant Protection Service of The Netherlands; contamination with other thrips species was not found.

Plant material

Thrips only thrive on intact chrysanthemum plants, not on detached leaves. Therefore the experiments were done on cucumber (*Cucumis sativa*) or bean (*Phaseolus vulgaris*) leaves instead. The leaves were taken from plants grown in separate, insect-free climate rooms.

Unless stated otherwise, thrips was supplied with mixture of bee-collected pollen provided commercially (Koppert Inc., Berkel en Rodenrijs, the Netherlands).

Synchronised thrips rearing

To obtain batches of freshly hatched thrips larvae, a random sample of adult females was transferred to leaves where they were allowed to oviposit for 24 h. After removal of the females, these leaves were placed on an agar layer in large Petri dishes (200 mm diameter). The dishes were made insect-proof by sealing them with parafilm. The eggs hatched after approx. 3 days. The thrips larvae were transferred to fresh leaves at the end of the second larval stage and, ultimately, the emerging adults were used in our experiments. Another way of creating batches of young thrips is to rear them from isolated eggs. Normally, thrips eggs are positioned inside the leaf tissue where they are difficult to detect. Therefore, the leaf was avoided altogether and the thrips were made to oviposit in a water layer between two sheets of parafilm in a Murai cage (Murai and Ishii, 1982). They were fed with pollen. After removing the water, eggs were taken from the parafilm and incubated on wet filter paper for 2 days. Then, the eggs were transferred to fresh leaves in a Petri dish on an agar layer and they developed normally.

Detection of symbiotic bacteria

The presence of symbiotic *Erwinia* bacteria was determined by incubating thrips homogenate on Luria Bertoni agar medium. Thrips were homogenised after external sterilisation, according to a method described in De Vries *et al.* (2001a). We incubated bacteria for 24 h at 25 °C, after which the number of bacteria and colony morphology were scored. Following De Vries *et al.* (2001a), *Erwinia* species was identified as small white opaque round colonies with small motile rods. Plates containing bacteria that were morphologically similar to the type strain of *Erwinia* species were classified as positive, and plates containing no bacteria or only different types of bacteria were considered to be negative. Plates containing less than 30 colonies were scored negative since such a low number may result from accidental contamination during the isolation procedure. The presence of bacteria was checked by plating homogenates of at least 10 randomly collected thrips at the end of each experiment.

Aposymbiotic thrips

In order to study fitness effects of gut bacteria, it is necessary to create symbiont-free hosts, called aposymbionts (Douglas, 1989; Koch, 1967). They can be obtained by treating hosts with antibiotics (Breeuwer and Werren, 1993; Houk and Griffiths, 1980; Sasaki, 1991), or heat (Chang, 1974; Nardon, 1973; Van Opijnen and Breeuwer, 1999). In one set of experiments we used antibiotics to create aposymbiotic thrips. Adult female thrips were put in a Murai cage (Murai and Ishii, 1982) and fed with pollen. The antibiotic tetracycline (1 mg ml⁻¹) was added to their drinking water (De Vries *et al.*, 2001b). The control group of thrips was kept in another Murai cage without antibiotics. After 2 days of treatment, the individual thrips were transferred to small leaf discs of ca. 10 mm diameter. Each disc was kept in an isolated cell of a macro well plate, positioned on an agar layer to prevent dehydration.

Fitness studies solely based on antibiotic treatment to eliminate symbionts have met criticism because the antibiotics may also influence the insect itself and may be passed on to the offspring (Douglas, 1989; Houk and Griffiths, 1980). To reduce side-effects on the insect we decided to use the offspring of thrips treated with tetracycline for a second set of experiments. These individuals are still aposymbiotic, but have never been in contact with the antibiotic, assuming it is not passed on from mother to offspring. Another solution is to avoid the use of antibiotics altogether. For the case of thrips, this is simply done by rearing them individually from isolated eggs on *Erwinia*-free (= non-thrips-grazed) leaves (as described above). Such thrips can be compared with a symbiotic control group, reared in the same way except that the leaf discs were grazed by other thrips larvae during 24 h prior to the actual experiment. The latter treatment has proven to lead to reliable horizontal transmission of bacteria (De Vries *et al.*, 2001b).

Oviposition rate

We measured oviposition per day for a period of 3-7 days, using females that had moulted 2 days before. This oviposition period coincides with peak oviposition in *F. occidentalis* (Gaum *et al.*, 1994; Lublinkhof and Foster, 1977; Van Rijn *et al.*, 1995). Adult females were placed individually on leaf discs in ca. 10 mm diameter compartments of a macro wells plate. The compartments were sealed insect-proof with parafilm and were incubated under standard conditions (25 °C, 60% relative humidity, and 16 h light per day). The females were transferred to a new compartment with a fresh leaf disc every day. Because the eggs hidden in the leaf cannot be directly counted, the old leaf disc was incubated under the same conditions to score the number of larvae after 4 days. We assumed that hatching success is similar between the two treatments (see below). This experiment was carried out with symbiotic and aposymbiotic thrips females on two diets (leaf and leaf with pollen) with at least 20 individuals. The results were analysed by a three-level nested ANOVA with unequal sample sizes.

Unfortunately, it is not feasible to assess oviposition on pollen alone, because the thrips need water and an appropriate substrate for egg insertion. For this reason, the experiment on a diet of pollen alone had to be modified and therefore was not included in the above ANOVA. The modifications involved the use of Murai cages (with the water film between parafilm layers as an oviposition substrate) and the release of 20 females per Murai cage (instead of a single female per unit), necessitated by the fact that only eight of such cages were at our disposal. Moreover, the number of eggs could be assessed directly. These experiments were done with symbiotic and aposymbiotic thrips females and analysed by means of a t-test.

Hatching rate

In another set of experiments, we examined to what extent the number of emerged larvae corresponds to the number of eggs deposited in leaves. To enable direct egg counts, leaves with eggs were heated in a microwave, which causes thrips eggs to turn whitish and thus visible (De Kogel, 1997). Adult female thrips, either symbiotic or aposymbiotic, were allowed to lay eggs in leaves on a layer of wet cotton in a large Petri dish. In one group of 25 thrips females, the eggs were directly counted and in another the number of larvae emerg-

ing after 4 days was determined. The difference between the results of these two experiments provides an estimate of egg mortality.

Larval development time

Thrips development occurs in five stages: egg, first instar larva, second instar larva, prepupa, and pupa. Feeding occurs only in the larval stages. The larval development time was defined as the period between egg hatching and the onset of the prepupal stage (development of short antennae and wings). This period was chosen because preliminary experiments with groups of symbiotic and aposymbiotic thrips showed no sign of a symbiont effect on the pupal stage, but an 18 h difference during larval development (ca. 192-210 h).

The experiment was initiated with individual eggs obtained from Murai cages. The eggs were kept for 2 days on wet filter paper. By the time the larval eyes become visible as red spots through the egg chorion, the eggs were transferred to undamaged, clean leaf discs (aposymbiotic thrips larvae) or to leaf discs that had been fed upon previously by second instar larvae. In the latter case the freshly emerged larvae are known to acquire *Erwinia* species bacteria from the grazed leaf discs (De Vries *et al.*, 2001b). The hatching of larvae and the entering of the prepupal stage was monitored every 8 h. During the last day of development, the larvae were transferred to a new leaf disc. The aposymbiotic and symbiotic thrips were offered either of two diets: leaves or leaves with pollen. The effect of the symbionts (treatment) and the thrips diet was tested with a three-level nested ANOVA with unequal sample sizes.

RESULTS

How gut symbionts and diet (leaf discs, leaf discs with pollen, or pollen alone) affect thrips fitness, was investigated by experimentally assessing larval development time and oviposition rate in aposymbiotic and symbiotic thrips.

Larval development time

Aposymbiotic thrips had a significantly longer larval development time on bean leaf discs than symbiotic thrips. To reach the prepupal stage, they required 21.5 h more time in one replicate experiment and 13.8 h in the other (Table 4.1). However, when the bean leaves were supplemented with pollen, the effect was reversed. Aposymbiotic larvae had a shorter larval development time than symbiotic larvae. To reach the prepupal stage, they required 16.8 h less in one replicate experiment and 14.9 h in the other (Table 4.1). Strikingly, larval developmental time of symbiotic thrips did not differ between the dietary treatments and between the replicates: the maximum difference between treatment means was only 5 h. For aposymbiotic thrips the differences between replicates were small but those between dietary treatments were significant and pronounced (more than 40 h). Post-larval checks on the presence of symbionts confirmed their symbiont-free or symbiont-rich status.

TABLE 4.1 – Mean (\pm SD) larval development time of western flower thrips in the presence (Sym = symbiotic) and absence of gut bacteria (Apo = aposymbiotic). Thrips were tested on two different food sources: (bean) leaves and leaves with additional pollen. Larval development time is the time between hatching and entering the prepupal stage. The experiment was repeated (replicate I and II). n = number of thrips larvae in the sample.

Replicate	Food source	Bacteria	Larval development time (h)	n
I	leaf	Apo	148.4 \pm 11.8	33
		Sym	126.9 \pm 13.4***	38
	leaf + pollen	Apo	105.1 \pm 13.8	39
		Sym	121.9 \pm 23.6***	30
II	leaf	Apo	138.9 \pm 17.5	28
		Sym	125.1 \pm 13.0**	49
	leaf + pollen	Apo	108.7 \pm 18.4	41
		Sym	123.6 \pm 27.1**	29

Two treatments differ at $P < 0.01$ (**) or $P < 0.001$ (***) level (t-test)

Oviposition rate

First, we measured the oviposition rate in a group of adult female thrips that were either treated with tetracycline or not, and fed on cucumber leaves, with or without pollen. The two independent replicate experiments yielded very similar results (Table 4.2A,C). On cucumber leaves, oviposition was significantly lower in aposymbiotic thrips (0.58-0.21 eggs per day) compared to symbiotic females (0.79-1.33 eggs per day). On a diet of leaves with pollen there was no difference in oviposition between aposymbiotic and symbiotic thrips (Table 4.2A,C). Thrips fecundity was higher on leaves with pollen. Very similar results were obtained using bean instead of cucumber leaves (data not shown). We checked whether egg mortality was higher in aposymbiotic thrips, but no difference was found in egg mortality between aposymbiotic and symbiotic females (both had 5-10% mortality). On pollen as the only food source (i.e., in Murai cages), symbionts did not matter for oviposition: aposymbiotic thrips laid 0.85-1.03 eggs per day, whereas symbiotic thrips laid 0.73-1.07 eggs per day (Table 4.2B).

To check for interference of tetracycline with the thrips (instead of only with its gut bacteria), an experiment was done where aposymbiotic offspring were obtained from females that had been treated with the antibiotic. The aposymbiotic offspring had a significantly lower oviposition rate on cucumber leaves than the control group (Table 4.3), a result that was much in agreement with the experiments in which aposymbiotic thrips had been in direct contact with tetracycline. Hence, we have no reason to suspect an effect of this antibiotic on the thrips.

Finally, we avoided tetracycline treatment altogether in an experiment where aposymbiotic thrips were obtained by rearing thrips from isolated eggs on clean leaf discs with pollen. The results corroborated the results obtained with the two other experiments. Aposymbiotic thrips produced 0.4-0.9 fewer eggs per day on bean leaf discs than symbiotic thrips, but this difference vanished on leaves with pollen (Table 4.4). In all oviposition experiments, checks on the presence of symbionts confirmed the symbiont-free or symbiont-rich status of the thrips under test.

Effect of gut bacteria on fitness of thrips

TABLE 4.2 – Mean (\pm SE) oviposition rate of western flower thrips of variable age. Rate is expressed as average number of eggs produced per female per day. Thrips with (Sym = symbiotic) and without bacteria (Apo = aposymbiotic) are compared. Different food sources were used: cucumber leaves, pollen (tested in Murai cages), and leaf + pollen (pollen is added to the leaves). The experiment was carried out for three or four consecutive days. The leaf disc experiments were done with individuals; the pollen experiment was done with Murai cages of more than 25 individuals per cage. Feeding females with tetracycline 2 days prior to the experiment created bacteria-free thrips, whereas the control group only received sterilised water. n = number of females included in the daily average, nd. = not determined.

Food source	Repl.	Infec.	n	day 1	day 2	day 3	day 4	Total day 1-3
A: Cucum-ber leaf	I	Sym	29	1.28 \pm 0.40	1.16 \pm 0.34	0.85 \pm 0.23	0.79 \pm 0.26	4.08
		Apo	40	0.54 \pm 0.12**	0.45 \pm 0.09**	0.46 \pm 0.17*	0.38 \pm 0.15*	1.83 **
	II	Sym	23	1.33 \pm 0.29	1.22 \pm 0.41	1.06 \pm 0.32	0.83 \pm 0.27	4.44
		Apo	26	0.58 \pm 0.13**	0.71 \pm 0.19**	0.46 \pm 0.17*	0.21 \pm 0.15*	1.96 **
B: Pollen (Murai)		Sym	4	0.88	1.07	0.73	nd.	2.68
		Apo	4	0.90 ns	1.03 ns	0.85 ns	nd.	2.78 ns
C: Leaf + pollen	I	Sym	23	1.67 \pm 0.35	1.72 \pm 0.52	1.75 \pm 0.41	1.39 \pm 0.51	6.53
		Apo	26	0.83 \pm 0.29*	1.67 \pm 0.50 ns	1.54 \pm 0.46 ns	0.58 \pm 0.18*	4.62*
	II	Sym	45	2.95 \pm 0.45	2.83 \pm 0.39	2.65 \pm 0.44	3.04 \pm 0.49	11.47
		Apo	38	2.80 \pm 0.40 ns	3.53 \pm 0.48 ns	2.81 \pm 0.47 ns	3.39 \pm 0.59 ns	12.53 ns

Two treatments differ at P<0.01 (**), P<0.05 (*), or P>0.05 (ns) level (t-test)

TABLE 4.3 – Mean (\pm SE) reproductive performance of western flower thrips with and without gut bacteria, on a cucumber leaf disc. The oviposition rate, expressed as average number of eggs produced per female per day, was measured. The aposymbiotic thrips (Apo) were offspring of mothers treated with antibiotics; the symbiotic thrips (Sym) were offspring from untreated mothers.

Food source	n	Infection	day 1	day 2	day 3	Total day 1-3
cucumber	25	Sym	1.00 \pm 0.22	0.79 \pm 0.23	0.81 \pm 0.21	1.95
	26	Apo	0.61 \pm 0.24*	0.88 \pm 0.27 ns	0.46 \pm 0.19*	2.60 ns

Two treatments differ at P<0.05 (*), or P>0.05 (ns) level (ANOVA)

TABLE 4.4 – Diet related effect of gut bacteria on the thrips oviposition rate, expressed as the mean (\pm SE) number of eggs produced per female per day. The females are all from roughly the same age, and the experiment started 2 days after emergence from the pupa (day 2). Sym = thrips with gut bacteria, Apo = bacteria-free thrips, n = number of thrips tested in the experiment.

Food source	Bacteria	n	day 2	day 3	day 4	day 5	day 6	day 7	day 8	Total
Bean leaf	Sym	54	1.4 \pm 0.2	2.6 \pm 0.3	2.9 \pm 0.4	1.7 \pm 0.2	1.1 \pm 0.2	1.1 \pm 0.2	1.1 \pm 0.2	11.9
	Apo	45	0.8 \pm 0.2	2.3 \pm 0.3	1.2 \pm 0.2	1.3 \pm 0.2	0.6 \pm 0.1	0.6 \pm 0.1	0.2 \pm 0.1	7.0
Leaf + pollen	Sym	68	1.0 \pm 0.2	2.4 \pm 0.3	2.6 \pm 0.4	1.9 \pm 0.3	1.2 \pm 0.2	1.2 \pm 0.2	1.0 \pm 0.2	11.2
	Apo	112	1.5 \pm 0.2	2.8 \pm 0.4	3.2 \pm 0.4	1.9 \pm 0.3	1.3 \pm 0.2	1.1 \pm 0.2	0.9 \pm 0.2	12.7

DISCUSSION

Erwinia spec are bacteria that inhabit the gut of western flower thrips. This permanent association with bacterial symbionts has fitness consequences for the host. On a diet of plant leaves and pollen, thrips with gut bacteria take longer to mature than aposymbiotic thrips. On a diet of plant leaves alone, however, the effects are reversed: thrips with gut bacteria develop faster and lay more eggs than thrips without. Thus, the effects of gut bacteria on development time and oviposition rate are diet-dependent; on leaves alone, thrips benefit from gut bacteria, whereas they incur a cost on leaves and with pollen. Assuming the *Erwinia* spec bacteria profit from inhabiting the thrips gut (but see Douglas and Smith, 1989, for a critical evaluation), this would represent a diet-dependent switch from mutualism to parasitism. Such environment-dependent outcomes may be more common than formerly thought, as argued by Bronstein (1994a,b) in her review of the empirical (often conflicting) evidence for mutualistic interactions.

According to the literature published to date, insect hosts usually suffer from elimination of the symbionts. When deprived of mycetomic symbionts known to provide amino acids, vitamins, and / or sterols, aphids need more time to develop, reach a smaller adult size, and are unable to produce offspring (Baumann *et al.*, 1995; Douglas, 1992; Sasaki *et al.*, 1991). Freed of hindgut symbionts, cockroaches develop slower and have smaller hindguts, and, without fat body mycetomes, they build up higher concentrations of uric acid, indicating disruption of the insects' nitrogen recycling (Cochran, 1985; Cruden and Markovetz, 1987). Tsetse flies without gut epithelium mycetomes lay fewer eggs (Nogge and Gerresheim, 1982). Aposymbiotic termites die prematurely (Eutick *et al.*, 1978) and aposymbiotic weevils develop slower and lay fewer eggs, and may even lose their ability to fly (Nardon and Grenier, 1988).

None of these studies investigated the consequences of variation in diets, but there is one other study reporting diet-dependent effects quite similar to those shown in this article. Crickets freed of hindgut bacteria performed worse on one of 3 diets that was poorest in vitamins, fatty acids and nitrogen (Kaufman *et al.*, 1991; Ulrich *et al.*, 1981). Also, the aposymbiotic thrips studied by us performed worse on the poorest of 2 diets (leaves alone vs. leaves with pollen). Thus, the symbionts seem to benefit their host especially in poor environments. This is exactly what is predicted from theoretical models of the evolution of mutualism and parasitism on a gradient of habitat qualities (Hochberg *et al.*, 2000; Hochberg and Holt, 2002).

To understand the impact on their thrips host, it is important to assess the role of gut symbionts in more detail. We do not yet know how the bacteria are affected by nutrient composition of the thrips diet. The pollen used in this study is bee-collected, originates from a number of different plant species, and was produced in the Mediterranean area (Koppert Inc., pers. comm.). It contains many necessary amino acids, vitamins, and sterols, and this may render any contribution of bacteria to thrips nutrition superfluous. On leaves however, some food components will be lacking, either because the pollen are absent or because they stem from one or a few plant species. In that case, gut bacteria may provide scarce amino acids as is the situation in aphids (Baumann *et al.*, 1995; Douglas, 1989), degrade carbohydrates (like in cockroaches [Cruden and Markovetz, 1987], crickets [Kaufman and Klug, 1991], firebrats [Treves and Martin, 1994], and beetles [Bauchop and Clark, 1975; Bayon, 1980]), fix nitrogen (as for *Pantoea agglomerans* strains in termites and xylophagous bee-

bles; Bridges, 1981; Potrikus and Breznak, 1977), protect the host against disease agents (*Pantoea agglomerans* in the desert locust *Schistocerca gregaria*; Dillon and Charnley, 1995; Dillon *et al.*, 2000), or detoxify secondary plant compounds and insecticides (Boush and Matsumura, 1967).

The list of possible roles of the symbiont is not exhaustive and it remains a challenge to identify new functions and effects on the host. One aspect to be considered is how the role of gut bacteria changes in the course of thrips life history. Thrips larvae, more than the adults, require gut symbionts because they are born on leaves and inhabit the leaves during much of their developmental period. On leaves they have no access to pollen or nectar. The adult thrips are much more mobile and can reach a larger variety of plant foods (parenchymous tissue, pollen, and nectar) and plant parts (petals, leaves, and flowers). This may be why the numbers of gut bacteria decrease during the non-feeding stages (prepupa and pupa) preceding maturation and finally reach lower levels in mature thrips.

The interaction between gut symbionts and thrips represents an example of what Van Baalen and Jansen (2001) referred to as a “dangerous liaison”. It seems likely that the interests of the two interacting species are aligned on a diet of leaves alone, but on a diet of leaves and pollen the interests of the host conflict with those of the gut bacteria. Such variation in interest alignment and conflict is more likely to occur, when vertical transmission is costly, horizontal transmission takes place, within-host competition varies depending on single vs multiple infections and there are (negative) effects of too intensive exploitation of the host (Genkai and Yamamura, 1999; Van Baalen and Jansen, 2001; Van Baalen and Sabelis, 1995). Clearly, thrips do not transmit the gut bacteria transovarially, nor externally via the egg (De Vries *et al.*, 2001b). However, this does not necessarily imply loss of control of the host. This is because thrips larvae may selectively pick up *Erwinia* spec bacteria, they may select the leaf area where to pick them up, they may selectively influence their growth by altering conditions in the gut, and they may encounter bacteria left by their mothers or relatives, if they fed earlier on the same leaf. Thus, whether the bacteria are transmitted among relatives or not, really depends on spatial patterns of relatedness among thrips individuals, and this will in turn determine the intensity of within-host competition among gut symbionts. Even though vertical transmission *sensu stricto* does not take place, the route of transmission may, in effect, still be close to it, depending on the behaviour and population structure of the host. Unravelling the consequences of such modes of transmission for the evolution of mutualism and parasitism is an important task for the future.

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