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

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Studies on bacteria in the gut of insect species are numerous, but their focus is hardly ever on the impact on host performance. 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 because of these gut bacteria. On a diet of cucumber leaves, the 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 parasitize 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.

Keywords: development time; oviposition; western flower thrips; symbiotic bacteria; *Erwinia* species; gut bacteria

1. INTRODUCTION

Symbiotic interactions are defined as close and rather persistent 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 (Hurst 1993; Bronstein 1994*a*,*b*; Werren & O'Neill 1997). Mutualism is defined as a reciprocal exploitation that provides a net benefit to each partner (Herre *et al.* 1999). The common understanding is that under certain conditions, e.g. vertical transmission of a microbial symbiont from the maternal host to her offspring, the mutualistic interaction may become stationary (Yamamura 1993; Ewald 1994; Maynard Smith & Szathmary 1995; Law & Dieckmann 1998).

Vertical transmission is however not a prerequisite for the evolution of mutualistic interactions between symbionts and their hosts. According to Genkai-Kato & Yamamura (1999), evolution of mutualism under conditions of horizontal transmission and a single infection is possible when four criteria are met: (i) vertical transmission involves some cost to the host; (ii) the symbiont suffers direct negative effects if it exploits the host too intensively; (iii) the host is able to make good use of the excretion products from the symbiont; and (iv) 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 & 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 be to the detriment of the interests of the host. Another condition promoting mutualism is the ability of the host to take sanctions against non-cooperative symbionts (Sherratt & Roberts 2002). Whether multiple infection by symbionts of hosts—without a clear intersymbiont competition leading to one prevailing type or in the absence of host sanctions (or the presence of countersanctions of the symbiont), completely prevents mutualism evolving—remains an open question. The 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 between insects and bacteria in which the main route of transmission is not vertical. Non-vertical transmission leading to mutualistic interactions has often been demonstrated in plant–microbe interactions, such as mycorrhizas (Lewis 1991), but not so much in insect–microbe symbiosis. Gut bacteria in phytophagous and carnivorous insect species (Baines 1956; Buchner 1965; Bignell 1984; Campbell 1990; Douglas & Beard 1997) represent a challenging case. Most of them belong to the gamma group of Proteobacteria, a family of Enterobacteriaceae. They are facultative symbiotic bacteria, because 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 & O'Neill 1997). Others found that the symbiotic interactions between insect hosts and bacteria have a more permanent character. Where gut bacteria grow in their host, they can also enhance the fitness of the host (for example, in cockroaches (Cruden & Markovetz 1987); termites (Breznak 1982); and xylophagous beetles (Bridges 1981)). Thus, there is

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every reason to suspect a mutualistic interaction. The predominant mode of transmission, however, is horizontal. Only in some cases are they shown to be transmitted vertically, albeit not transovarian. For example, in cockroaches, females smear bacteria onto the shell of their eggs or juveniles eat the contaminated faeces of their mother (Koch 1967; Cruden & Markovetz 1987).

In this paper, we study the interaction between western flower thrips (*Frankliniella occidentalis* (Thysanoptera: Thripidae)), a herbivorous insect, and *Erwinia* bacteria inhabiting their gut. To our knowledge, there is no evidence for transovarial vertical transmission or any external route of egg contam-ination with bacteria (De Vries *et al.* 2001*b*). Instead, each generation of larvae of the thrips acquires the gut bacteria anew by feeding preferentially on leaves that have been fed upon by other conspecific thrips. One type of *Erwinia* bacteria quickly outcompetes all other gut bacterial types (De Vries *et al.* 2001*b*). Whether this mode of transmission is closer 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 a shorter developmental period and increased egg production rate, these being the main determinants (Van Rijn *et al.* 1995). The results of these studies are discussed in the light of the possible population structures and the different modes of transmission.

2. MATERIAL AND METHODS

(a) *The model system*

Western flower thrips is a small, plant-feeding insect that has symbiotic bacteria in its hindgut (Ullman *et al.* 1989; De Vries *et al.* 1995). 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.* 2001*a*). 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.* 2001*b*). Bacteria-free thrips can be cultured on leaves and the *Erwinia* 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 test their impact on food conversion in the thrips. Thrips feed primarily on leaf parenchyma, leaf petals and pollen of a large number of species (Kirk 1985; Yudin *et al.* 1986).

(b) *Thrips populations*

Thrips were obtained from a culture on chrysanthemum (*Dendranthema grandiflora*) that was 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 of light per day. The thrips identity was checked once per year in samples submitted to G. Vierbergen at the Netherlands Plant Protection Service; contamination with other thrips species was not found.

(c) *Plant material*

Thrips thrive only on intact chrysanthemum plants, not on detached leaves. Therefore the experiments were carried out on cucumber (*Cucumis sativa*) or bean (*Phaseolus vulgaris*) leaves. 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., Naaldwijk, The Netherlands).

(d) *Synchronized 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 (diameter of 200 mm). The dishes were made insect-proof by sealing them with parafilm. The eggs hatched after *ca*. 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 & 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. The eggs were then transferred to fresh leaves in a Petri dish on an agar layer, where they developed normally.

(e) *Detection of symbiotic bacteria*

The presence of symbiotic *Erwinia* bacteria was determined by incubating thrips homogenate on Luria Bertoni agar medium. Thrips were homogenized after external sterilization, according to a method described in De Vries *et al.* (2001*a*). 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.* (2001*a*), *Erwinia* species TAC 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 TAC were classified as positive, and plates containing no bacteria or only different types of bacteria were considered to be negative. Plates containing fewer than 30 colonies were scored as negative because 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.

(f) *Aposymbiotic thrips*

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

Table 1. Larval development time of western flower thrips in the presence (symbiotic) and absence of gut bacteria (aposymbiotic).
(Thrips were tested on two different food sources: (bean) leaf discs and leaf discs 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* is the number of thrips larvae in the Sample. ANOVA gives a test of whether the two groups, symbionts and aposymbionts, have a different larval development time. sym, thrips with gut bacteria; apo, bacteria-free thrips; ** two treatments differ at the $p < 0.01$ level; *** two treatments differ at the $p < 0.001$ level.)

replicate	food source	bacteria	larval development time (h), mean ± s.d.	ANOVA	*n*
I	leaf disc	apo	148.4 ± 11.8	***	33
		sym	126, 9 ± 13.4		38
	leaf disc + pollen	apo	105.1 ± 13.8	***	39
		sym	121.9 ± 23.6		30
II	leaf disc	apo	138.9 ± 17.5	**	28
		sym	125.1 ± 13.0		49
	leaf disc + pollen	apo	108.7 ± 18.4	**	41
		sym	123.6 ± 27.1		29

Fitness studies based solely 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 (Houk & Griffiths 1980; Douglas 1989). 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 in § 2d). 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 been proved to lead to reliable horizontal transmission of bacteria (De Vries *et al.* 2001*b*).

(g) *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 & 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 rendered insect-proof with a parafilm seal and were incubated under standard conditions (25 °C, 60% relative humidity, and 16 h of light per day). Each female was 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 § 2h 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 only had to be modified and therefore were 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.

(h) *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 emerging after 4 days was determined. The difference between the results of these two experiments provides an estimate of egg mortality.

(i) *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* bacteria from the grazed leaf discs (De Vries *et al.* 2001*b*). 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 one of two diets: leaves or leaves with pollen. The effect of the symbionts (treatment) and the thrips diet was

Table 2. ANOVA on larval development, using a three-level nested ANOVA with unequal sample sizes.
(The three effects are: replicates (I and II), food source (leaf disc or leaf disc with pollen) and presence of symbionts (aposymbionts and symbionts). The data from which this table is prepared are summarized in table 1. n.s., not significant; *** significant at the $p < 0.001$ level.)

source of variation	d.f.	SS	MS	*F*	
replicates	1	160	160	00 105	n.s.
food source	2	30 285	15 142	11 635	n.s.
symbionts	4	52 056	13 014	4 47 279	***
error	277	80 596	291		
total	284	1 63 097			

tested with a three-level nested ANOVA with unequal sample sizes.

3. 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.

(a) *Larval development time*

Aposymbiotic thrips, i.e. thrips that did not have the possibility to pick up gut bacteria, had a significantly longer larval development time on bean leaf discs than symbiotic thrips (that lived on leaf discs where gut bacteria were present). To reach the prepupal stage, they required 21.5 h more time in one replicate experiment and 13.8 h in the other (tables 1 and 2). 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 less in the other (tables 1 and 2). Strikingly, the 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.

(b) *Oviposition rate*

First, we measured the oviposition rate in a group of adult female thrips that were either treated with tetracycline or not treated, and fed on cucumber leaves, with or without pollen. The two independent replicate experiments yielded very similar results (tables 3–5). On cucumber leaves, oviposition was significantly lower in aposymbiotic thrips (0.58–0.21 eggs per day) compared with 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 (tables 3–5). 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

Table 3. Oviposition rate of western flower thrips of variable age.
(The rate is expressed as average number of eggs produced per female per day. Thrips with (symbiotic) and without bacteria (aposymbiotic) are compared. Different food sources were used: cucumber leaf discs and leaf discs with pollen (pollen is added to the disc). The experiment was carried out for 3 or 4 consecutive days. The leaf disc experiments were carried out with individual females (standard error of the mean is given). Sym, thrips with gut bacteria; apo, bacteria-free thrips; repl, replicate number; infect, thrips with or without gut bacteria; *n*, number of females included. Statistical tests were carried out with a *t*-test. ** Two treatments differ at $p < 0.01$; * two treatments differ at $p < 0.05$; n.s., two treatments differ at $p > 0.05$.)

food source	repl	infect	*n*	day 1		day 2		day 3		day 4		mean day 1–3	
cucumber leaf disc	I	sym	31	1.27 ± 0.40	**	1.18 ± 0.34	**	0.88 ± 0.23	*	0.81 ± 0.26	*	1.03	**
		apo	26	0.54 ± 0.12		0.46 ± 0.09		0.46 ± 0.17		0.38 ± 0.15		0.46	
	II	sym	18	1.33 ± 0.29	**	1.22 ± 0.41	**	1.06 ± 0.32	*	0.83 ± 0.27	*	1.11	**
		apo	24	0.54 ± 0.13		0.71 ± 0.19		0.46 ± 0.17		0.21 ± 0.15		0.48	
leaf disc with pollen	I	sym	18	1.67 ± 0.35	*	1.72 ± 0.52	n.s.	1.78 ± 0.41	n.s.	1.39 ± 0.51	*	1.64	*
		apo	24	0.83 ± 0.29		1.67 ± 0.50		1.54 ± 0.46		0.58 ± 0.18		1.16	
	II	sym	35	1.46 ± 0.18	n.s.	1.34 ± 0.26	n.s.	1.31 ± 0.17	n.s.	0.46 ± 0.11	n.s.	1.14	n.s.
		apo	35	0.89 ± 0.24		1.31 ± 0.30		1.26 ± 0.23		0.43 ± 0.15		0.97	

Table 4. Oviposition rate of western flower thrips of variable age.
(The rate is expressed as average number of eggs produced per female per day. Thrips with (symbiotic) and without bacteria (aposymbiotic) are compared. The experiment was carried out with pollen as the food source and took place in Murai cages of at least 25 individuals per cage. Feeding females with tetracycline 2 days prior to the experiment created bacteria-free thrips, whereas the control group received only sterilized water. sym, thrips with gut bacteria; apo, bacteria-free thrips; n.d., not determined; repl, replicate number; infect, thrips with bacteria or without; *n*, number of females included. Statistical tests were carried out with a *t*-test.)

food source	infect	*n*	day 1	day 2	day 3	day 4	total day 1–3
pollen (Murai)	sym	4	0.88 n.s.	1.07 n.s.	0.73 n.s.	n.d.	2.68 n.s.
	apo	4	0.90	1.03	0.85	n.d.	2.78

Table 5. ANOVA on oviposition of thrips, using a three-level nested ANOVA with unequal sample sizes.
(The three effects are: replicates (I and II), food source (leaf disc or leaf disc with pollen) and presence of symbionts (aposymbionts and symbionts). The data from which this table is prepared are summarized in table 3. n.s., not significant; ** significant at the $p < 0.01$ level.)

source of variation	d.f.	SS	MS	*F*
replicates	1	2,70	2,70	0,536 n.s.
food source	2	10,08	5,04	1,694 n.s.
symbionts	4	11,90	2,98	4,123 **
error	203	146,47	0,72	
total	210	17,115		

difference was found in egg mortality between aposymbiotic and symbiotic females (both had 5–10% mortality). With pollen as the only food source (i.e. in Murai cages), the presence of symbionts did not affect oviposition: aposymbiotic thrips laid 0.85–1.03 eggs per day, whereas symbiotic thrips laid 0.73–1.07 eggs per day (table 4).

To check for interference of tetracycline with the thrips (instead of only with its gut bacteria), an experiment was carried out 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 6), 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 did symbiotic thrips, but this difference vanished on leaves with pollen (tables 7 and 8). In all oviposition experiments, checks on the presence of symbionts confirmed the symbiont-free or symbiont-rich status of the thrips under test.

4. DISCUSSION

Erwinia species TAC 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 and lay fewer eggs per day 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 with pollen. Assuming that the *Erwinia* TAC bacteria profit from inhabiting the thrips gut (but see Douglas & 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 (1994*a*,*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 (Sasaki *et al.* 1991; Douglas 1992; Baumann *et al.* 1995). Freed of hindgut symbionts, cockroaches develop more slowly and have smaller hindguts, and, without fat body mycetomes, they build up higher concentrations of uric acid, indicating disruption of their nitrogen recycling (Cochran 1985; Cruden & Markovetz 1987). Tsetse flies (*Glossina morsitans*) without gut epithelium mycetomes lay fewer eggs (Nogge & Gerresheim 1982). Aposymbiotic termites die prematurely (Eutick *et al.* 1978) and aposymbiotic weevils develop more slowly and lay fewer eggs, and may even lose their ability to fly (Nardon & 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 worst on one of three diets that was poorest in vitamins, fatty acids and nitrogen (Ulrich *et al.* 1981; Kaufman *et al.* 1989). Also, the aposymbiotic thrips that we studied performed worst on the poorest diet (leaves alone versus leaves with pollen). Thus, the symbionts seem to benefit their host most 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 & Holt 2002).

To understand their impact on the thrips host, it is important to assess the role of gut symbionts in more detail.

Table 6. Reproductive performance of western flower thrips with and without gut bacteria on a cucumber leaf disc.
(The oviposition rate was measured as the average number of eggs produced per female per day. The aposymbiotic thrips (apo) were offspring of mothers treated with antibiotic, the symbiotic thrips (sym) were offspring from untreated mothers. infect, presence of gut bacteria in the thrips; *n,* number of thrips included in the test. Standard error of the mean is given and the difference between the two groups is tested with a *t*-test. * Two treatments differ at $p < 0.05$; n.s. two treatments differ at $p > 0.05$.)

food source	*n*	infect	day 1	day 2	day 3	total day 1–3
cucumber leaf disc	25	sym	1.00 ± 0.22 *	0.79 ± 0.23 n.s.	0.81 ± 0.21 *	1.95 n.s.
	26	apo	0.61 ± 0.24	0.88 ± 0.27	0.46 ± 0.19	2.60

Table 7. Diet-related effect of gut bacteria on the thrips oviposition rate, expressed as the number of eggs produced per female per day, over a period of 7 days.
(The females were all of 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. Standard error of the mean is given.)

food source	bacteria	*n*	day 2	day 3	day 4	day 5	day 6	day 7	day 8	total
bean leaf	sym	37	1.4 ± 0.2	2.6 ± 0.3	2.9 ± 0.4	1.7 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	11.9
	apo	48	0.8 ± 0.2	2.3 ± 0.3	1.2 ± 0.2	1.3 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.2 ± 0.1	7.0
leaf + pollen	sym	42	1.0 ± 0.2	2.4 ± 0.3	2.6 ± 0.4	1.9 ± 0.3	1.2 ± 0.2	1.2 ± 0.2	1.0 ± 0.2	11.2
	apo	44	1.5 ± 0.2	2.8 ± 0.4	3.2 ± 0.4	1.9 ± 0.3	1.3 ± 0.2	1.1 ± 0.2	0.9 ± 0.2	12.7

Table 8. ANOVA on oviposition of thrips, using a two-level nested ANOVA with unequal sample sizes.
(The two effects are: food source (leaf disc or leaf disc with pollen) and presence of symbionts (aposymbionts and symbionts). The data from which this table is prepared are the total number of offspring presented in table 7. n. s., not significant; ** significant at the $p < 0.01$ level.)

source of variation	d.f.	SS	MS	*F*
food source	1	400	400	1,07 n.s.
symbionts	2	746	373	4,6 **
error	167	13 554	81,2	
total	170	14 700		

We do not yet know how the bacteria are affected by the nutrient composition of the thrips diet. The pollen used in this study is bee-collected, originates from several different plant species and was produced in the Mediterranean area (Koppert Inc., personal communication). 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 is absent or because they stem from one or a few plant species. In that case, gut bacteria may degrade carbohydrates (as in cockroaches (Cruden & Markovetz 1987), crickets (Kaufman & Klug 1991), firebrats (Treves & Martin 1994) and beetles (Bauchop & Clarke 1975; Bayon 1980)), fix nitrogen (as for *Pantoea agglomerans* strains in termites and xylophagous beetles (Potrikus & Breznak 1977; Bridges 1981)), protect the host against disease agents (*Pantoea agglomerans* in the desert locust *Schistocerca gregaria* (Dillon & Charnley 1995; Dillon *et al.* 2000)), or detoxify secondary plant compounds and insecticides (Boush & 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, but 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 & 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 are more likely to occur: when vertical transmission is costly, horizontal transmission takes place, within-host competition varies depending on single versus multiple infections, and there are (negative) effects of excessively intensive exploitation of the host (Van Baalen & Sabelis 1995; Genkai-Kato & Yamamura 1999; Van Baalen & Jansen 2001). Clearly, thrips do not transmit the gut bacteria transovarially, nor externally via the egg (De Vries *et al.* 2001*b*). However, this does not necessarily imply a loss of control of the host. This is because thrips larvae may selectively pick up *Erwinia* 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 or not the bacteria are transmitted among relatives 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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