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Lack of evidence for western flower thrips biotypes based on intra and inter-strain variation in gut bacteria

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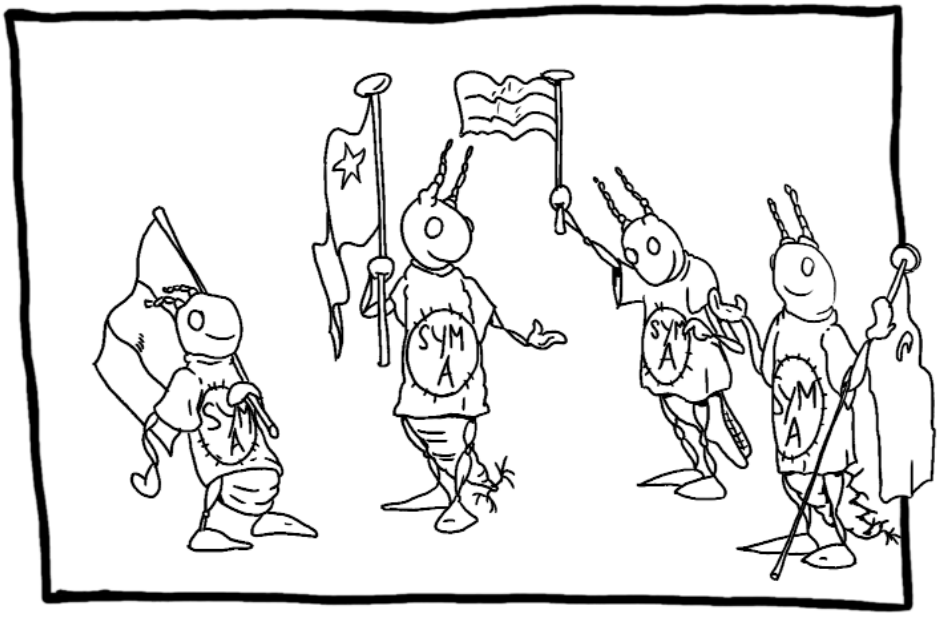

ABSTRACT – Western flower thrips is a polyphagous insect, which during the last 30 years has become a world wide pest. It was found earlier that these thrips are associated with a type of *Erwinia* species gut bacteria. In this study we examine the variation of bacteria within and between thrips individuals and try to find evidence for biotypes in western flower thrips regarding the type of gut bacteria. The existence of biotypes in this thrips species has been suggested by different authors. For example, thrips populations have been found that differ in resistance against pesticides and in their ability to transmit plant viruses. With biotypes we mean groups of individuals (strains, populations, lines) of a species which differ in one or more traits with other groups of that species. The gut bacteria of thrips are acquired by young thrips larvae via the host plant and have a beneficial effect on thrips development and oviposition. We studied thrips strains from different countries and host plants, and the isofemale lines that were created from them, on bean plant leaves. All thrips lines that we studied contained *Erwinia* species gut bacteria. Morphological and biochemical characteristics of gut bacteria from the thrips isofemale lines were similar to the *Erwinia* type strain from the reference, a thrips strain cultured on chrysanthemum in Amsterdam (TAC 93.XII.8). Per isofemale line we studied five thrips individuals and per thrips we studied four bacterial colonies, with RAPD markers. The genetic variation between bacteria isolated from thrips was as large among isofemale lines as within isofemale lines. No evidence for thrips biotypes was found. Bacteria within one thrips individual show a stronger degree of similarity than bacteria from different thrips individuals within a single rearing. This is probably due to a bottleneck caused by the limited number of successful infections of bacteria into the gut of the thrips.

KEY WORDS – Biotype, symbiosis, gut bacteria, *Erwinia* species, RAPD, genetic variation, Thysanoptera, Thripidae

The biotype concept was introduced to describe variation in host plant use between populations of a generalist insect species (Claridge and Den Hollander, 1983; Diehl and Bush, 1984; Saxena and Barrion, 1987). Biotypes are different populations of the same species that show variation in host plant use, particularly in terms of preference for, or in performance on, certain host plants (Gould, 1979). Only in a few cases of biotypes, the trade-offs were found that explain the variation in host plant use (Douglas, 1997; McKenzie, 1996). In a limited number of cases, the genetic differences between the biotypes were studied as well (Jaenike, 1990; Thompson, 1996). Biotypes are called host races when they differ genetically (Diehl and Bush, 1984). Some authors have the opinion that it is not possible to speak of biotypes without genetic differentiation, to discriminate the biotype concept from situations of phenotypic plasticity (Rossiter, 1987; Via, 1990). Moreover, adaptation to host plants will lead to a decrease in genetic exchange between biotypes and this may ultimately result in speciation (Bernays and Graham, 1996; Bush and Smith, 1998; Menken, 1996).

The biotype concept has broadened to cover all situations where groups of individuals of a generalist insect species vary in traits other than just host plant use. Examples are plant resistance against insects and insect sensitivity to insecticides (Dreyer and Campbell, 1987; Eisenach and Mittler, 1987). The presence and nature of symbiosis with gut bacteria is another trait in which insect strains may vary and hence form biotypes. Many phytophagous insect species have symbiotic bacteria in their gut system or elsewhere in their body (Buchner, 1965; Campbell, 1990; Koch, 1967). The symbiont influences the dietary requirements of the insect host (Baumann *et al.*, 1995; Cruden and Markovetz, 1987; Douglas, 1989) or plays a role in the insect defence system against pathogens (Boush and Matsumura, 1967; Dillon *et al*., 2000). The relation between feeding and the presence of symbionts has lead to the hypothesis that bacteria play a role in insect adaptation to host plants and that this may result in host plant specialisation (Berenbaum, 1988; Thompson, 1987). Moran *et al.* (1994) for example, have found that the phylogenies of aphids and their mycetomic bacteria are congruent, suggesting co-evolution between symbionts and hosts. In another study it was found that aposymbiotic (bacteria-free) aphids perform differently on various host plants than aphids with symbiotic bacteria (Adams and Douglas, 1997). Next to aphids, there are other polyphagous insects that possess close associations with bacteria. Following earlier findings of gut bacteria by Ullman *et al.* (1989), we have found that western flower thrips has a symbiosis with *Erwinia* species gut bacteria (De Vries *et al.,* 1995 and 2001a).

Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), has been known as a polyphagous insect herbivore for a long time (Bryan and Smith, 1956). Since the 1980s, it has been a pest of commercially important plants worldwide. It has been found in Europe (Mantel and De Vrie, 1986; Zur Strassen, 1986), in South Africa (Giliomee, 1989), in Israel, in Japan, and in New Zealand (Martin and Workman, 1994). The opportunistic behaviour of this phytophagous generalist is enhanced by the fact that the insect is able to use pollen and nectar as food source, and it even can be a predator of other small insects and mites. Pollen in the diet increases thrips reproduction (Kirk, 1985a; Van Rijn *et al.*, 1995). However, to explain the spread of thrips across the world, we have to assume that it is able to survive on plants without pollen or nectar, because seedlings of plants have been carrying thrips to other regions of the world (Frey, 1993). Pollen is not indispensable since thrips can thrive on male sterile plants, such as cucumber (De Kogel *et al.*, 1997b).

Based on its wide distribution, polyphagy and pest status, the existence of biotypes of Western flower thrips has been suggested several times in the literature. Indeed, thrips

strains were found to vary in insecticide resistance (Brødsgaard, 1994; Immaraju *et al.*, 1992), transmission efficiency of tomato spotted wilt virus (Van de Wetering, 1999), and response to plant resistance (De Kogel *et al.*, 1997a,b; Soria and Mollema, 1994). It is not known whether variation exists in the gut bacteria of thrips. Earlier, no variation in thrips gut microflora was found, but this was based on only a few populations of thrips and only thrips from two different host plants, cucumber and chrysanthemum (De Vries *et al.,* 1995, 2001a). A larger and more variable group of thrips needs to be studied before any conclusion can be drawn.

Thrips acquire bacteria when feeding on their host plants (De Vries *et al.*, 2001b). Transmission is probably done via the leaves, because other thrips, also present on the host plant, deposit bacteria via their faeces. Under laboratory conditions the prevalence of bacteria in larval thrips increases to 100% in the population, and up to 10^5 bacteria can be found per thrips in the second larval stage. The effect of bacterial presence on the performance of thrips is diet dependent: symbionts have a positive effect on the thrips only in the absence of pollen (De Vries *et al.*, 2004).

In this article, we study variation among and within different strains of thrips regarding their symbiosis with gut bacteria. The possibility of thrips biotypes, in terms of the gut bacteria they possess, is discussed. We used the same thrips lines as those which had been studied before to describe biotypes of thrips regarding host plant resistance and virus transmission efficiency (De Kogel *et al.*, 1997a; Van de Wetering *et al.*, 1999). This enables us to compare the following thrips related factors: sensitivity to plant resistance, ability to transmit plant virus and symbiosis with gut bacteria.

MATERIAL AND METHODS

Thrips strains

Two groups of thrips strains were studied. The first group consisted of thrips strains collected from commercial greenhouses in the Netherlands in 1988 and 1991. These were given names that reflected the host plant and the laboratory where they were cultured (Faculty of Biology of the University of Amsterdam or Centre for Plant Breeding and Reproduction Research [CPRO] in Wageningen) (Table 8.1). The second group of thrips strains were obtained in 1993 and 1994 from research laboratories in different countries (Table 8.1). These strains were cultured in small closed jars at CPRO on bean pods with additional, bee-collected pollen. Culture conditions and precautions taken to reduce the risk of cross-contamination between cultures were described elsewhere (De Kogel *et al.*, 1997a). During the sampling experiments in 1995 and 1997, thrips were collected from rose (*Rosa* spec.) and gerbera (*Gerbera* spec.) in several commercial greenhouses in the Netherlands and directly used for experiments without prior culturing.

Isofemale lines

Isofemale lines were created from seven thrips strains that all had been cultured at CPRO. Western flower thrips is haplo-diploid and virgin females produce only male offspring. The isofemale lines were created with single virgin females. Their eggs were reared to adulthood

TABLE 8.1 – The strains of western flower thrips that were used in this study. Country (origin) and original host plant (host plant), as well as host plant on which we have been culturing them (cultured on) and start of our cultures (year) are mentioned. The column 'isofemale line' gives the letter codes of the isofemale lines that were created and used in this study.

Code	Origin	Host plant	Cultured on	Year	Isofemale line
NL1	Netherlands	Cucumber	Cucumber	1988	C,D
NL2	Netherlands	Chrysanthemum	Chrysanthemum	1991	B,C,D,E,G,I,J
AMC	NL1	Chrysanthemum	Chrysanthemum	1993	
NL3	Netherlands	Cucumber	Bean pods	1990	
NKB	NL1	Cucumber	Bean pods	1994	
NCK	NL2	Chrysanthemum	Cucumber fruit	1994	
NCB	NL2	Chrysanthemum	Bean pods	1994	
HoBo	Hungary	Bean	Bean pods	1993	
ItBo	Italy	Bean	bean pods	1993	
SpGo	Spain	Cotton	Bean pods	1993	
DuGe	Germany	Gerbera	Bean pods	1993	
ZeBr	Sweden	Rape seed	Bean pods	1994	
NzAu	New Zealand	Aubergine	Bean pods	1994	A,B
GBCh	Great Britain	Chrysanthemum	Chrysanthemum	1994	
UCD	Denmark	bean pods	Bean pods	1995	A
JaCh	Japan	Chrysanthemum	Bean pods	1995	A,C,D,E
USA1	USA	Gloxina	Bean pods	1995	A,B,C,E
IsMa	Israel	Mango	Bean pods	1995	A,B

and mother - son pairings were done. Subsequent offspring was allowed to interbreed. After ten generations, the isofemale lines had grown to population sizes large enough to do experiments with (De Kogel, 1997). Twenty-one isofemale lines were created (De Kogel, 1997), which we all used in the present study (Table 8.1). When more than one isofemale line was created from one thrips strain, we used alphabetic characters to designate the various isofemale lines (A-E).

Host plants

The plants used for culturing thrips, namely cucumber cultivar G6 (*Cucumis sativa*), chrysanthemum cultivar 'Sunny Casa' (*Dendranthema grandiflora*) and bean cultivar Prelude (*Phaseolus vulgaris*) were grown in confined, arthropod-free greenhouses at CPRO. For the thrips strains in Amsterdam we used chrysanthemum plants from local, commercial garden centres. Bean leaves were used as food source for thrips during the experiments.

Isolation of gut bacteria

All thrips strains were examined for the presence of gut bacteria. Samples of 20 to 30 thrips were taken from the cultures and tested individually. The thrips were externally sterilised and homogenised with small glass rods in a buffer as described elsewhere (De Vries *et al.*,

2001a). The thrips homogenate was transferred to Luria Bertoni medium and plates were incubated for 24 h at 25 °C. All steps of the isolation procedure were done in a laminar flow hood to reduce the risk of contamination with outside bacteria. After incubation, the number and type(s) of bacteria were recorded. Bacterial colonies were described in size, colour, clarity, and shape, and the size and motility of their constituent bacteria. The number of bacteria was assessed as well. Only plates with more than 30 bacterial colonies were taken into account to avoid including individuals with only some bacterial contaminants but no symbiotic gut bacteria. We compared colony morphology of the isolates from each thrips strain with the type strain *Erwinia* species TAC, the gut bacterium found in the Dutch reference thrips strain (De Vries *et al.*, 2001a).

Rearing of synchronised larvae

Synchronised larvae were used in one set of the experiments. These larvae were obtained by allowing 25 adult females to feed and oviposit for 24 h on a bean leaf on wet cotton in a large Petri dish. Pollen was added to enhance oviposition (Van Rijn *et al.*, 1995). The Petri dish was sealed with Parafilm to prevent thrips from moving in or out. After 24 h, adults were removed. Larvae of an average age of 156 h were used to isolate bacteria from. At this age, bacterial numbers and prevalence are highest (De Vries *et al.*, 2001b).

Biochemical test on bacterial identity

Bacterial strains obtained from the various thrips were biochemically characterised using the API 20E method (Biomérieux). For a complete outline of all biochemical characteristics which are tested with this method, see De Vries *et al.* (2001a) and references therein. We tested a few individuals of each thrips strain, and one colony was selected from each individual and grown to a pure culture with two times re-inoculation on fresh LB plates. Only colonies morphologically resembling *Erwinia* species TAC bacteria were selected. A single colony was taken from each of the pure bacteria cultures and transferred to physiological salt medium (0.9% w/v NaCl), and subsequently tested with a standard API 20E test strip. Test scores were compared with the results of the gut bacterial type strains TAC 93.XII.8 and 94.III.1 (De Vries *et al.*, 2001a).

RAPD reaction set up

Since morphological and biochemical methods do not suffice to measure variation between bacterial strains, molecular methods were used in addition. We used the RAPD method to further characterise the gut bacterial strains (Odinot, 1995; Oakey *et al.*, 1996). DNA was isolated from pure cultures of gut bacterial strains obtained from individual thrips. A 5% chelex emulsion in sterile tissue culture water (both from Sigma Inc.) was used to suspend bacteria, and proteinase K (20 µg per sample) was added for DNA isolation (Walsh *et al.*, 1991). DNA isolates were incubated for 2 h at 37 °C, after which proteinase K was denatured by heating the samples at 95 °C for 15 min. The isolates were diluted 100-fold before use in PCR. RAPD reactions were done in 25 µl, according to PCR reaction conditions and with reaction agents as described earlier (De Vries *et al.*, 2001a). We used six RAPD primers (De Vries *et al.*, 2001a).

Cluster analysis of RAPD data

RAPD results were recorded by electrophoresis of the complete PCR sample on 1.5% agarose gels, at 4 V/cm, for at least 4 h. Visualisation of ethidium bromide dyed marker bands was done at 320 nm light and recorded with an IS1000 digital imaging system (Alpha Innotech). A 100 bp DNA size marker (Gibco BRL) was used to assess the size of the marker bands, with RFLPscan software (Scanalytics, CSPI). The bands were entered in an Excel database as 1 (present) and 0 (not present) for each PCR sample and marker. With Paup 3.1.1 (Swofford, 1991), a distance matrix was made according to the Nei and Li distance algorithm (Nei, 1987). Average distances between and among bacteria from various isofemale lines were examined. The distance matrix was used for cluster analysis according to the neighbour joining method (Phylip 3.4). Clusters were displayed in an unrooted phenogram.

AMOVA

AMOVA was done using the method described earlier by Excoffier *et al.* (1992). A database was created containing the RAPD data from 87 bacteria samples and was structured in populations (bacteria samples from one thrips) and groups of populations (bacteria samples from different thrips all belonging to one culture of thrips). The database was entered into the Arlequin programme, version 3.1 (Excoffier *et al.,* 2005). The Arlequin programme is able to compute AMOVA from RAPD data and present results in percentages of the variation belonging to the three existing categories; within population, among populations and within groups, and among groups.

RESULTS

Western flower thrips strains have been examined for the presence of gut bacteria in adults since 1994 (Table 8.2). In 1996, it was found that second instar larvae have the highest prevalence of bacteria (De Vries *et al.*, 2001b), so these stages where used from then on. In all thrips strains included in this study, we have found bacteria morphologically similar to the *Erwinia* species TAC bacteria, which were earlier described as type species of thrips gut bacteria (De Vries *et al.*, 2001a). These were small, white, opaque, round colonies containing small, motile rods. The number of bacteria per isolation from a single thrips was high, between 10^4 and 10^5 cfu, comparable to the results obtained with the reference thrips strain AMC. Incidentally, other types of bacteria were found in high numbers, however these other types never occurred in more than one or two thrips individuals and never could be found in the same strain of thrips for more than 1 year (Table 8.2). These other bacteria were not taken into account.

Isofemale lines were created from seven different thrips populations to determine variation in bacterial numbers and composition among thrips from the same population (Table 8.1). There were 21 isofemale lines in 1996, of which 17 survived to 1997. In both years, approximately 156-h-old larvae were randomly taken from a synchronised thrips rearing of each isofemale line and homogenised to study the gut bacteria (Table 8.3). The bacterial colonies we isolated from each larva of the isofemale lines had the same morphology as *Erwinia* species TAC bacteria. The number of larvae infected (prevalence of bacteria in thrips larvae) was comparable to data obtained earlier with the AMC strain (De Vries, *et al.*, 2001b)

TABLE 8.2 – The presence of gut bacteria in adults of strains of western flower thrips. %pos = percentage of thrips with gut bacteria (at least 30 colony forming units), %*Erw* = percentage of thrips with a majority of *Erwinia* spec TAC bacteria. n = number of thrips included.

Year	Thrips strain	%pos	%*Erw*	n
1994	AMC	55	55	40
	NL2	50	44	18
	NL1	74	74	19
	NKB	89	56	9
	GBCh	61	56	18
	DuGe	37	37	19
	NzAu	42	42	19
	ZeBr	33	27	15
	ItBo	67	67	9
	HoBo	100	100	9
	SpGo	100	100	9
1995	AMC	70	70	20
	NL2	75	75	12
	NL1	85	77	13
	NKB	75	75	12
	NCB	50	17	12
	NCK	82	82	11
	NzAu	50	50	12
	UCD	73	73	11
	ItBo	67	58	12
	HoBo	75	67	12
1996-I	AMC	58	58	40
	NL1	56	56	27
	NL2	81	81	26
	NzAu	67	67	21
	USA1	80	65	20
	NCB	35	30	20
	UCD	61	61	23
	ItBo	65	65	20
1996-II	NL2	67	52	21
	NL1	80	60	20
	NL3	55	55	22
	JaCh	74	63	19
	USA1	65	65	20
	NzAu	50	50	20
	IsMa	65	50	20

(Table 8.3). Type strains of each isofemale line are coded with a three character combination. The first character identifies the appropriate isofemale line, the second character, 96 or 97, the year of isolation of the bacteria, and the last character indicates the thrips individual.

TABLE 8.3 – Presence of gut bacteria in different isofemale lines of western flower thrips. Isofemale lines were created from seven different thrips strains (Table 8.1). The bacteria that are recorded in this table are *Erwinia* spec TAC. Only thrips with more than 30 colonies of bacteria were considered to be positive (%pos = percentage of thrips with gut bacteria). n = number of thrips included in the sample. %sim = percentage of similarity between the type strain of the isofemale line and the type strain of TAC, in biochemical characteristics (API 20E). All thrips tested were larvae of approximately 156 h old.

	1996		1997			
Isofemale line	%pos	n	%pos	n	Type strain	%sim
IsMa-A	90	30	92	24	ISA.97.7	100
IsMa-B	100	30	74	23	ISB.96.1	100
JaCh-A	97	30	100	25		
JaCh-C	86	29	88	24	JAC.97.1	95
JaCh-D	100	15	100	24	JAD.96.8	100
NL1-C	87	15	-	-		
NL1-D	100	15	-	-	N1D.96.11	100
NL2-B	83	30	-	-		
NL2-C	85	27	-	-		
NL2-D	77	31	83	24	N2D.97.4	95
NL2-E	97	30	56	16		
NL2-G	93	15	91	23	N2G.96.13	100
NL2-I	93	15	94	18	N2I.96.2	100
NL2-J	100	30	-	-		
NzAu-A	100	30	100	24	NZA.96.23	95
					NZA.97.5	100
NzAu-B	93	15	92	24	NZB.96.13	100
UCD-A	96	24	86	21	UCA.96.2	100
USA1-A	59	29	-	-	U1A.96.3	90
USA1-B	100	15	96	24	U1B.97.4	100
USA1-C	93	30	92	24		
USA1-E	100	30	-	-		

The bacteria isolated from the thrips strains, as well as those from the isofemale lines, resembled *Erwinia* species TAC morphologically. To verify this tentative identification, random bacterial colonies from different isolations were subjected to biochemical tests (API 20E). The tests confirmed our idea that the strains were *Erwiniae* spec. In fact, most strains had exactly the same biochemical characteristics as type strains *Erwinia* species TAC 93.XII.8 and 94.III.1: positive in beta-galactosidase, acetoin production, glucose utilisation (ut), mannitol ut, rhamnose ut, sucrose ut, amygdalin ut, and arabinose ut. Negative in argenine dehydrolase (dh), lysine dh, ornithine dh, citrate ut, H_2S production, urease, tryptophane deaminase, indole production, gelatinase, inositol ut, sorbitol ut, and cytochrome oxidase (De Vries *et al.*, 2001a). The only variation was in the utilisation of inositol and/or utilisation of melibiose but this has not lead to any identification other than *Erwiniae* spec. In conclusion, all gut bacteria in thrips strains from different host plants and geographical ori-

TABLE 8.4 – Gut bacteria of western flower thrips, collected in commercial greenhouses in the area of Aalsmeer (The Netherlands). Thrips with more than 30 colonies were considered to be positive. %pos = percentage of thrips with gut bacteria. All bacteria found were similar to *Erwinia* species TAC. n = number of thrips in the sample. Life stage indicates whether thrips were collected as adults or larvae (of a random age). Pure bacterial strains from three thrips of this populations were compared biochemically (API 20E) with the type strain of western flower thrips gut bacteria, TAC.93.XII.8. %sim = percentage of biochemical similarity.

Date	Thrips collected in	Host plant	Life stage	%pos.	n	%sim.
1995	Kudelstaart	Rose	adult	33	18	
	Amstelveen	Rose	adult	5.5	18	
	Aalsmeer	Rose	adult	25	16	100
1997	Aalsmeer	Rose	larva	79	24	95
	Kudelstaart	Rose	larva	50	12	
	Aalsmeer-2	Rose	larva	92	24	
	Amstelveen	Rose	larva	76	21	100
	Aalsmeer-3	Gerbera	larva	48	21	

gin, contained *Erwinia* spec bacteria, possibly *Erwinia herbicola,* recently renamed *Pantoea agglomerans* (γ-Proteobacteriaceae; Enterobacteriaceae) (Gavini *et al*., 1989).

All thrips populations and isofemale lines used in this study had been reared in the laboratory of CPRO in Wageningen for at least 25 generations before the bacterial isolations were done. Host plants, both for the thrips cultures and for the experiments, originated from CPRO greenhouses. The same researchers were involved in all isolation experiments but they used different climate rooms and laboratories. Although we took all possible precautions to prevent cross-contamination of thrips, bacteria, or both, it can never be completely excluded. The constant presence of gut bacteria in thrips may have been a laboratory effect. To exclude this possibility it was deemed necessary to collect thrips directly from the 'field', which in the Dutch situation means commercial greenhouses.

In 1995, we sampled thrips from several greenhouses with ornamentals and bacteria were isolated directly from adults. Sampling was done in the summer when thrips abundance in crops and ornamentals is highest. Very few thrips contained bacteria, but the bacteria found were morphological similar to *Erwinia* spec TAC (Table 8.4). In 1997, this experiment was repeated but now we collected mainly larvae. Again, bacteria were directly isolated from these larvae, without any pre-culturing of thrips, to test for the presence of gut bacteria in the field. This time, we found a high prevalence of gut bacteria. These bacteria were similar to *Erwinia* spec TAC that are normally found in thrips, according to our morphological observations on the colonies and to the biochemical tests (Table 8.4).

The fact that all gut bacteria from western flower thrips are *Erwinia* spec does not exclude the possibility of small differences between gut bacteria from different strains of thrips. Since these small differences cannot be studied using bacterium or colony morphology or biochemistry, we applied molecular genetic techniques. The RAPD method, used before on *Erwinia* spec TAC, TWC, and TWK (De Vries *et al.,* 2001b), was selected. The sensitivity of this method was discussed in earlier papers (De Vries *et al.*, 2001b). Variation in gut bacteria within and among thrips was tested in the following five isofemale lines, each

TABLE 8.5 – Relationship between gut bacteria from thrips, measured at the level of a single thrips, at the level of thrips cultured on the same leaf, and at the level of all thrips from all leaves. The relationship is measured using 73 RAPD markers and pair-wise comparisons. For explanation of the thrips cultures (isofemale lines) see Table 8.2. Average differences in markers and standard deviation is given. n = number of pair-wise comparisons included.

Group	Thrips culture	Thrips number	Genetic distance gut bacteria	n
Per culture	Israel A	2	8.2 ± 2.8	6
		3	12.3 ± 1.8	6
		4	9.3 ± 4.4	6
		5	14.5 ± 5.7	6
		7	14.5 ± 5.4	6
		Between thrips	20.7 ± 8.4	190
	Japan C	1	8.0 ± 3.8	6
		3	13.5 ± 8.1	6
		4	7.8 ± 2.6	6
		5	12.2 ± 6.8	6
		6	14.3 ± 10.0	6
		Between thrips	15.2 ± 6.3	190
	New Zealand A	1	4.3 ± 2.2	6
		2	8.0 ± 4.9	6
		4	8.3 ± 3.3	6
		5	16.8 ± 5.2	6
		6	14.2 ± 4.8	6
		Between thrips	17.3 ± 6.9	190
	USA B	1	17.7 ± 8.3	6
		3	18.2 ± 7.6	6
		4	15.5 ± 9.0	6
		5	16.8 ± 4.8	6
		6	14.2 ± 4.7	6
		Between thrips	22.1 ± 7.0	190
	Netherlands2 D	3	15.5 ± 7.3	6
		4	17.2 ± 16.0	6
		5	18.5 ± 15.2	6
		6	9.8 ± 8.7	6
		Between thrips	17.1 ± 9.7	120
All individual thrips, within thrips			13.0 ± 8.0	144
All thrips cultures, within culture			18.6 ± 8.0	880
All thrips, within and among cultures			23.0 ± 7.3	4560

from a different geographic origin and different host plant: NZA-A, JPC-C, ISM-A, USG-B, and NL2-D (Table 8.3). For each isofemale line we studied the bacteria of five individual thrips, and from each thrips we created pure cultures from four randomly selected bacterial colonies. The morphology of all colonies was comparable to that of *Erwinia* spec TAC, our reference strain.

TABLE 8.6 – AMOVA (Analysis of Molecular Variation) for bacteria present in western flower thrips populations. The results indicate the relative proportion of variation within the total variation present that is caused by variation between bacteria from one thrips (within populations), bacteria of thrips from one culture (among populations and within groups) and bacteria from thrips of different cultures (among groups). d.f. = degrees of freedom, SS = sum of squares. Nine samples from the original 96 were deleted from this analysis because too many RAPD data were missing.

Source of variation	d.f.	SS	Variance components	% variation
Among groups	4	263	2.54	22
Among populations and within groups	19	418	4.99	44
Within populations	63	244	3.88	34
Total	86	925	11.4	

The five isofemale lines, five thrips per line (except for NL2 D), and four bacterial colonies per thrips lead to a total of 96 RAPD samples. Six RAPD primers were applied on these samples. We coded 73 polymorphic markers. To make sure that we were coding markers properly, we applied the rules for marker coding of Hadrys *et al.* (1992). After scoring, a database was created from which a distance matrix was made. The neighbour-joining algorithm cluster analysis method was used and clusters were represented in an un-rooted phenogram (Figure 8.1). Overall, variation between gut bacteria of one thrips is lower than between gut bacteria from different thrips (13.0 vs. 23.0). The relatedness among bacteria from thrips from one isofemale line was only somewhat smaller as the one between all bacteria of all thrips of different lines (18.6 and 23.0, respectively, Table 8.5).

To assess the bacterial variation within and among cultures of thrips in a statistical manner, AMOVA was performed. The AMOVA results confirm our findings in the phenogram and with the pair-wise comparisons. The variation between bacteria from different thrips from different cultures is only explaining 22% of the total variation found, so 78% of all variation found is due to the variation between bacteria from different thrips from the same culture (Table 8.6). This does not give any indication for biotype formation in thrips when it comes to specific types of *Erwinia* spec gut bacteria for the cultures of thrips that we studied. The variation between bacteria from one thrips is only a half as much as the variation between bacteria from different thrips (34 vs. 66%, see Table 8.6). This confirms the earlier findings of relatively low variation of bacteria from one thrips.

DISCUSSION

All thrips strains included in this study contain gut bacteria. The permanent association between western flower thrips and *Erwinia* spec TAC gut bacteria (De Vries *et al.*, 2001a), is present in all thrips populations examined here, even though they originate from different countries and host plants. Twenty-one Isofemale lines, created from seven different thrips strains, had an association with similar types of bacteria (according to morphological and biochemical data). Occasionally we have found additional gut bacteria that were morphologically different. These gut bacteria may occur in high numbers (more than 10^5 in some of the thrips). But they would only occur in one thrips of the population. The bacterial types

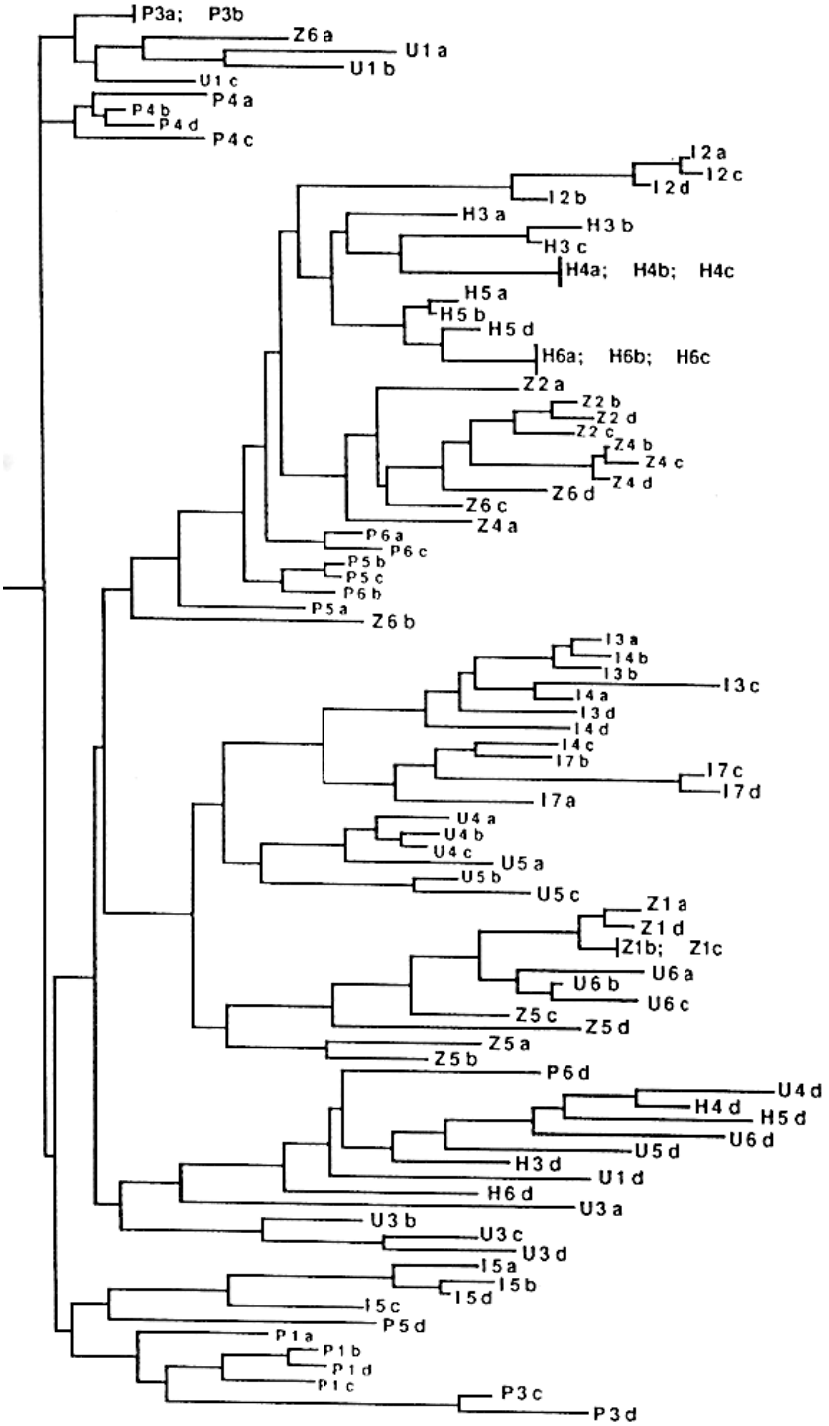

Figure 8.1 – Cluster analysis of gut bacterial strains of the *Erwinia* species TAC type from different isofemale lines of western flower thrips. Analysis was done using the neighbour joining method with Nei and Li's distance algorithm on basis of 73 polymorphic RAPD markers. The strains are projected as an un-rooted phenogram tree. In total 96 strains are presented as three character abbreviations. The first character refers to the iso-female line (H = the Netherlands, NL2-D; I = Israel, IsMa-A; P = Japan, JaCh-C; U = USA, USA1-B; and Z = New Zealand, NzAu-A). The second character is the number of the thrips individual, and the third character is the bacterial strain, because four bacterial colonies were tested per thrips (a-d).

similar to *Erwina* spec TAC were the only type consistently present in each strain, and indeed in many thrips the only gut bacterium occurring.

Obviously, bacterial colony morphology alone is insufficient to draw conclusions on the variation in thrips gut microbiology. Additional methods, such as biochemical tests and DNA variation analysis were employed and confirmed our conclusions. All thrips gut bacteria in this study were identified as *Erwinia* with API 20E, possibly being *P. agglomerans*. The strains were biochemically identical to the type strain of thrips bacteria *Erwinia* spec TAC 93.XII.8 (Tables 8.3 and 8.4). As far as there was biochemical variation, this did not hint at another bacterial species, because these were characteristics that are normally variable among the *Erwinia* species and among strains of the species *P. agglomerans*, namely inositol and melibiose utilisation. Because of the difficulties with species identification in Erwiniae using API 20E (Mergaert *et al.*, 1993), we have decided to just use the genus name *Erwinia* and the name of the type strain TAC to refer to the thrips gut bacteria.

The fact that all thrips strains tested were laboratory strains may have resulted in an overestimation of the dominance of TAC gut bacteria. Earlier experiments where attempts were done to create aposymbiotic populations of thrips have shown that the spread of gut bacteria can be very quick (De Vries *et al.*, 2001b). The transmission route used for thrips gut bacteria, via the leaf as food source, enhances the rapid spread of certain bacteria. To find out whether such a bias exists, we have looked at the presence of bacteria in thrips directly collected from the field, in the thrips' case commercial greenhouses. In thrips from commercial greenhouses we have only found gut bacteria that were morphologically and biochemically similar to *Erwinia* spec TAC. Apparently, this type of bacteria is the only one constantly present in thrips.

The highest prevalence of bacteria is found in larvae of about 156 h old (De Vries *et al.,* 2001b). In the survey of different isofemale lines of thrips, we could confirm this high prevalence for larvae of an average age of 156 h. However, there were a few exceptions. Isofemale line USA-A had less than 60% prevalence of bacteria, and isofemale line NL2-E also had a low prevalence (56%). IsMa-B and NL2-D had only 75% prevalence in one of the replicates of the experiment. Deviation from the expected 100% prevalence values in 156-h-old larvae may be due to the fact that some isofemale lines develop more slowly than the average thrips strain, and hence will reach the 100% prevalence of bacteria later. This hypothesis was tested in the USA-A strain. We did the isolation of bacteria at an average age of larvae of 180 h, and found that considerably more larvae (more than 80%) were infected with gut bacteria than at 156-h-old larvae (data not shown). Apparently, this thrips strain takes more time to mature.

From the results on morphological, biochemical, and molecular genetic variation between gut bacteria from different thrips strains and different isofemale lines of these thrips strains, it can be concluded that we have found no evidence for gut bacterial biotypes in western flower thrips. Similar results were found with the same isofemale lines regarding the use of various host plants: variation within strains is as high as among strains (De Kogel *et al.*, 1997b).

The bacterial strains have been randomly taken from the high number of bacteria isolated from one individual thrips, but they still show more genetic similarity than bacteria from different thrips (Tables 8.6). Apparently, gut bacteria from one particular thrips are genetically more similar. Variation between bacteria from one thrips is sometimes so small that mutation within one clone of a bacterium seems to be enough to explain the genetic varia-

tion observed. On the other hand, occasionally, more genetic difference is observed in bacteria from one thrips compared to bacteria of other thrips of the culture (Tables 8.6). These are likely to be thrips that had been eating from the same leaf disc, where the same type of bacteria would have been available for all thrips.

Absence of variation among bacterial strains from one thrips must be related to the uptake of bacteria. Presumably, successful uptake of bacteria is difficult and bacteria that make it to the hindgut will experience a serious bottleneck. Sometimes it seems as though the bacterial flora all come from one single infection and grow to high numbers in the thrips hindgut. However, in other individuals of the 25 thrips included in our bacterial RAPD study, the observed thrips microbiology can only be explained with multiple founding bacteria.

There is no evidence for biotypes in western flower thrips regarding the symbiosis with gut bacteria. Neither on the presence or absence of gut bacteria (all thrips strains have gut bacteria), nor on the association of particular gut bacterial strains with particular strains of thrips hosts (random genetic variation among all thrips observed, see also Table 8.6). The question remains whether other kinds of thrips biotypes exist. Biotypes were reported earlier for the thysanopterans *Thrips tabaci* (Zawirska, 1976) and *Apterothrips secticornis* (Karban, 1989), but whether they are present in *F. occidentalis* remains unclear. De Kogel *et al.* (1997a) have found variation between thrips strains in the ability to use susceptible and resistant cucumber strains as food source. However, De Kogel *et al.* (1997b) contradicted their own results when they studied iso-female lines, stating that variation in the use of these host plants as food source within populations was even bigger than among populations. Van de Wetering *et al.* (1999a) reported variation between thrips populations in transmission efficiency of tomato spotted wilt virus.

Several researchers have studied genetic variation among western flower thrips strains from Europe, Asia, and other regions over the last decades. No clear genetically defined biotypes have been reported in any of these studies (J. Frey, pers. comm.; Gillings *et al.*, 1995; Mound *et al.,* 1997; E. de Vries, unpubl.). Even though thrips were collected in different geographical regions and from various host plants, the reported level of genetic difference is apparently very low. This can be explained by a high gene flow, very recent establishment of populations (both were made possible by international trade), and the polyphagy of the insect. One other possible explanation is that, in spite of precautions taken, bacteria may have been exchanged between the iso-female lines during their period of culturing on bean pods. Or the bacteria may have been spread primarily via the bean pods and have overtaken bacteria originally present in the thrips. However, thrips taken directly from the field, from other host plants, also have the same type of gut bacteria, which renders a host plant effect from bean pods rather improbable (Table 8.4). It seems quite unlikely that all iso-female lines have cross-infected each other in the same manner.

In our study we have used thrips strains collected by various researchers. In future studies we should analyse and describe variation in host plant use between thrips strains. When on this basis biotypes are found in the thrips, it should be studied whether bacterial composition of the gut, or other ecological or physiological factors, have influenced this variation between thrips strains.

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