Horizontal transmission of parthenogenesis-inducing microbes in *Trichogramma* wasps

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SUMMARY

Complete parthenogenesis (thelytoky) in species of the parasitic wasp *Trichogramma* is usually caused by the cytoplasmically inherited bacterium *Wolbachia*. This symbiont induces gamete duplication, which, in these haplodiploid organisms, results in all-female broods. Antibiotic treatment 'cures' this condition, restoring normal sexual reproduction. Phylogenetic analysis of *Wolbachia* has shown that, in contrast with the strains in other host organisms (where the symbiont also induces different reproductive alterations), those in *Trichogramma* form a monophyletic group. This might be an indication of symbiont–host cocladogenesis. To test this, we performed comparative molecular phylogenetics on 20 parthenogenetic *Trichogramma* cultures and their *Wolbachiae*. We conclude that there is, in fact, little evidence for cocladogenesis. Instead, the phylogenetic distribution of the symbionts appears to result from occasional horizontal transmission, which probably takes place inside the hosts of *Trichogramma* parasitoids (usually lepidopteran eggs). This study therefore suggests that parthenogenesis is not only curable, it can sometimes be contagious also.

1. INTRODUCTION

The endosymbiotic bacterium *Wolbachia* (alpha-Proteobacteria, Rickettsiales) is estimated to be present in as many as 17% of all insect species (Werren *et al.* 1995*a*). It is also known from mites and isopods (see below). Its cytoplasmic inheritance has forced the evolution of various types of host manipulation by the symbiont. These manipulations include: (1) cytoplasmic incompatibility between infected males and uninfected females in insects (e.g. Barr 1980; Breeuwer & Werren 1990), isopods (Legrand & Juchault 1986; Rousset *et al.* 1992) and mites (Johanowicz & Hoy 1996; Tsagarakou *et al.* 1996); (2) feminization of isopod males (Martin *et al.* 1973, 1994; Rousset *et al.* 1992; Juchault *et al.* 1994); and (3) the induction of complete parthenogenesis (thelytoky) in parasitic wasps (Stouthamer *et al.* 1993). This latter effect has been best described for *Trichogramma* (Hymenoptera, Trichogrammatidae), a genus of minute (*ca.* 0.5 mm) parasitoids of lepidopteran eggs (Stouthamer *et al.* 1990, 1993; Stouthamer & Kazmer 1994). *Wolbachia* is known to induce parthenogenesis in more than ten out of the 160 species or so of *Trichogramma* (Pinto & Stouthamer 1994), a condition that can be 'cured' by antibiotic or heat treatment (Stouthamer *et al.* 1990).

Although vertically inherited microorganisms are expected, and sometimes observed to cospeciate with their hosts (Moran & Baumann 1994), phylogenetic analyses have demonstrated that this is not generally the case for *Wolbachia* (O'Neill *et al.* 1992; Rousset *et al.* 1992; Stouthamer *et al.* 1993; Werren *et al.* 1995*b*). Most *Wolbachia* strains appear to have reached their

host species via colonization, rather than by descent (Hurst *et al.* 1992). However, the monophyly of *Wolbachia* strains in *Trichogramma* suggests an exceptionally tight linkage between host and symbiont (Rousset *et al.* 1992; Werren *et al.* 1995*b*) and might indicate cocladogenesis.

In an attempt to reveal patterns of cocladogenesis, we selected 20 parthenogenetic cultures of *Trichogramma*, belonging to nine identified and three unidentified species. All were known to be 'curable' with antibiotics (Stouthamer *et al.* 1990; Silva & Stouthamer 1996; R. Stouthamer, unpublished data). We amplified and sequenced the second internal transcribed spacer (ITS-2), which separates the eukaryotic 5.8S and 28S rDNA genes, and is commonly used for intrageneric phylogenetics in insects (Wesson *et al.* 1992; Campbell *et al.* 1993; Sappal *et al.* 1995; Van Kan *et al.* 1996). DNA sequences for the *Wolbachiae* were gathered using *Wolbachia*-specific PCR primers for *ftsZ*, a prokaryotic cell cycle gene, and supplemented with data from GenBank.

2. METHODS

(a) Molecular techniques

Total DNA was extracted from *ca.* 20 wasps. The ITS-2 (plus portions of the flanking regions of the 5.8S and 28S genes) was amplified with the following trichogrammatid-specific PCR primers: 5'TGTCAACTGCAGGACACAT-G3' (forward) and 5'GTCTTGCCCTGCTCTGAG3' (reverse), which gave products between 376 and 469 bp in length. PCR products for *ftsZ* were obtained with the primers from Holden *et al.* (1993) and Sinkins *et al.* (1995), yielding a

Figure 1. Phylogenetic relationships among the *Wolbachia* strains from the *Trichogramma* cultures in figure 2, based on their *ftsZ* sequences. The *Wolbachia* from *Protocalliphora* was used as an outgroup based on previous phylogenetic work (Werren *et al.* 1995*b*). The numbers on the nodes indicate percentages of 100 bootstrap replicates, the *P*-values refer to the results of the T-PTP tests, while the dots define the clades that are referred to in figure 3.

product of 716 bp, and primers *ftsZBf/r* (Werren *et al.* 1995*b*), which gave a product of 955 bp. All PCR products were purified, cloned into a T-tailed vector and sequenced (in the case of *ftsZ*, in both directions) on an ABI automated sequencer. Five *ftsZ* sequences (including the outgroup) were taken from GenBank (accession numbers U28198–U28202). Our ITS-2 sequences have been deposited in GenBank under accession numbers U74599–U74609, U74673–U74682, the *ftsZ* sequences under accession numbers U59696, U74471–U74485.

(b) Phylogenetic analysis

ITS-2 sequences were aligned manually in the ESEE 3.0s sequence editor (Cabot 1995), with reference to a previous alignment (Van Kan *et al.* 1996). The outgroup sequence from *Uscana semifumipennis*, a member of an unrelated trichogrammatid genus (Doutt & Viggiani 1968) was quite divergent, and was only included where alignment was unambiguous. The data were analysed with the computer program PAUP 3.1 (Swofford 1993). Gaps were treated as 'fifth base' and were weighted as the inverse of the length of the deletion. Ten heuristic searches with various search parameter settings all produced the same nine most parsimonious trees of length 915.332, and a consistency index (CI; Kluge & Farris 1969) of 0.68. (The nine trees differed in the intraspecific topology of *T. deion* only.)

FtsZ sequences were aligned manually in ESEE 3.0s, leaving end-unaligned regions untrimmed. The data were

analysed with PAUP 3.1. The tree in figure 1 is the one identical to the majority rule consensus over 2000 trees (length = 87, CI = 0.95), resulting from a single heuristic search.

Tree length distributions were obtained by generating 10000 random trees in PAUP 3.1. The statistical significance of the skewness measures was determined with the tables in Hillis and Huelsenbeck (1992). Bootstrapping was also done with PAUP 3.1, while T-PTP testing (Faith 1991) was performed with a test version of PAUP* 4.0 (the results are included here with permission from the author).

Trees were manipulated in MacClade 3.04 (Maddison & Maddison 1993). Reconciliations and randomizations were performed with the program TREEMAP (Page 1994).

(c) Provenances of cultures

T. oleae: Tunisia; *T. cf. pretiosum*: Mt Shasta, California; *T. pretiosum* HAW: Kauai, Hawaii; *T. pretiosum* NL: Nuevo Leon, Mexico; *T. cf. deion*: Mojave Desert, California; *T. deion* Men: Menifee Valley, California; *T. deion* MC: Mountain Center, California; *T. deion* PM: Pinyon Mt, California; *T. deion* SW: Mojave Desert, California; *T. deion* Irv: Irvine, California; *T. deion* TX: Sanderson, Texas; 'New Brunswick': New Brunswick, Canada; *T. platneri* NC: Newcastle, California; *T. platneri* RI: Riverside, California; *T. cordubensis*: Spain; *T. sibericum* 231: Richmond, Canada; *T. sibericum* SIB: Canada; *T. chilonis*: Kauai, Hawaii; *T. nubilale*: Nova Scotia; *T. brevicapillum*: Mojave River Forest, California; *Uscana semifumipennis*: New River, Arizona.

Voucher specimens have been placed in the collection of the Department of Entomology, University of California, Riverside, USA.

3. RESULTS AND DISCUSSION

Mean molecular distances (Swofford 1993) for the ingroup were 0.002–0.435 and 0.000–0.021 for the ITS-2 and *ftsZ* sequences, respectively. Both data sets contained strong phylogenetic signals, as evidenced by tree-length distribution skewness ($g_1 = -0.81$, and -1.00 , respectively; $p \ll 0.01$). Searches for the most parsimonious *Trichogramma* tree yielded nine practically identical trees, from which one was arbitrarily selected (figure 1). The tree confirms morphological classifications of the genus (J. D. Pinto, personal communication).

Analysis of the *Wolbachia* data, in addition to confirming the monophyly of the strains in *Trichogramma* (results not shown), produced many optimal trees, due to the presence of a number of very similar sequences. We calculated a majority-rule consensus and selected the optimal tree identical to it (figure 2). A cursory examination reveals two striking features. First, *Wolbachia* strains within a species are generally very similar (with the exception of *T. deion* TX). This corresponds to the finding in a previous study, where we found virtual sequence identity in 45 strains from

T. cf. deion (M. Schilthuizen, J. Honda and R. Stouthamer, in preparation). Second, in contrast with the concordance at the species level, no apparent concordance is present above the species level.

Viewing terminal polytomy or insignificant T-PTP as identity, we interpreted the *Wolbachia* tree as being composed of seven distinct lineages, with some infecting more than one *Trichogramma* lineage. A projection of the *Wolbachia* phylogeny onto the *Trichogramma* tree (figure 3) revealed concordance only at a single node. Randomization tests showed that this could easily be due to chance. Consequently, we conclude that cocoladogenesis is not the predominant mode by which *Wolbachia* has been distributed among *Trichogramma*. The remaining explanations would be sorting of ancestral diversity, horizontal transmission or a combination of both. Sorting of ancestral diversity (the presence of multiple *Wolbachia* strains in the ancestral *Trichogramma*, followed by fixation of alternative strains in the daughter lineages) cannot be ruled out. However, this scenario would predict a *Wolbachia* phylogeny with deep splits (corresponding with the origin of ancestral diversity) and long terminal branches, which does not appear to be the case. Thus, the major factor contributing to the discordance between the two trees is probably horizontal transmission.

To a certain extent, however, this reconstruction

Figure 2. Phylogenetic relationships among 20 *Wolbachia*-infected and parthenogenetic cultures of *Trichogramma*, based on their ITS-2 sequences plus portions of the flanking regions of the 5.8S and 28S genes. The tree was rooted with a member of a related genus, *Uscana semifumipennis*. The numbers on the nodes indicate percentages of 100 bootstrap replicates (values below 50% not shown).

Figure 3. The phylogeny of the seven distinct *Wolbachia*-strains projected onto the *Trichogramma*-phylogeny. The dot indicates the single concordant node. Ten thousand randomizations of the *Wolbachia* tree with the computer program TREEMAP (Page 1994), showed that this concordance can easily be explained by chance.

Figure 4. Tree length distribution, based on 10000 randomizations of the *Wolbachia* tree. The bold arrow indicates the length of the tree that is fully reconciled with the *Trichogramma* tree.

depends upon the chosen *Wolbachia* tree. Other, equally parsimonious trees might be more compatible with the *Trichogramma* tree. To investigate this possibility, we identified the most parsimonious tree that fitted best with the *Trichogramma* tree. A reconciliation of the two trees required at least six horizontal transmissions, which can be regarded as a lower bound on the

horizontal transmission frequency. A full reconciliation of the two trees (i.e. no horizontal transmission) required a *Wolbachia* tree that is very unparsimonious, 30% longer than the most parsimonious ones (figure 4).

Even though horizontal transmission must have happened several times, it is probably a rare event, as

there is no strong geographic component in the *Wolbachia* tree. *T. deion* SW and *T. cf. deion*, for example, which were collected at the same locality, differ in their *Wolbachia*. Similarly, *T. brevicapillum* (largely sympatric with *T. deion*) also carries a different *Wolbachia*. There is, however, one instance where geography might hint at horizontal transmission: *T. chilonis* and *T. pretiosum* HAW, both from Hawaii, have almost identical *Wolbachia* sequences.

Even if horizontal transmission is rare, it is surprising that the *Wolbachia* strains from *Trichogramma* are not found in any other insects. Apparently, after their first colonization of a *Trichogramma*, these *Wolbachias* have only shifted from one member of the genus to another. This suggests that horizontal transmission takes place in an environment that is common to all *Trichogrammas*. Lepidopteran eggs, the typical *Trichogramma* hosts, are the most likely candidates for such an environment. Horizontal transmission of *Wolbachia* between *Trichogrammas* may take place here in cases of superparasitization by more than one wasp species.

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