Wolbachia infections are distributed throughout insect somatic and germ line tissues

Stephen L. Dobson1, a, Kostas Bourtzis a, b, Henk R. Braig 2, a, Brian F. Jones a, Weiguo Zhou3, a, François Rouset a, c, Scott L. O’Neill a,*

a Section of Vector Biology, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06520, USA
b Insect Molecular Genetics Group, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology—Hellas, Heraklion, Crete, Greece
c Laboratoire Génétique et Environnement, Institut des Sciences de l’Evolution, Université de Montpellier II, 34095 Montpellier, France

Received 8 April 1998; received in revised form 2 November 1998; accepted 3 November 1998

Abstract

Wolbachia are intracellular microorganisms that form maternally-inherited infections within numerous arthropod species. These bacteria have drawn much attention, in part to the reproductive alterations that they induce in their hosts including cytoplasmic incompatibility (CI), feminization and parthenogenesis. Although Wolbachia’s presence within insect reproductive tissues has been well described, relatively few studies have examined the extent to which Wolbachia infects other tissues. We have examined Wolbachia tissue tropism in a number of representative insect hosts by western blot, dot blot hybridization and diagnostic PCR. Results from these studies indicate that Wolbachia are much more widely distributed in host tissues than previously appreciated. Furthermore, the distribution of Wolbachia in somatic tissues varied between different Wolbachia/host associations. Some associations showed Wolbachia disseminated throughout most tissues while others appeared to be much more restricted, being predominantly limited to the reproductive tissues. We discuss the relevance of these infection patterns to the evolution of Wolbachia/host symbioses and to potential applied uses of Wolbachia. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Wolbachia pipientis; Drosophila simulans; Drosophila melanogaster; Aedes albopictus; Culex pipiens; Cadra cautella; Glossina morsitans; WSP antibody; dnaA

1. Introduction

The Wolbachia pipientis clade consists of intracellular, maternally-inherited bacteria that occur within numerous arthropod hosts (Bourtzis and O’Neill, 1998; O’Neill et al., 1997; Werren, 1997). Although rare horizontal transmission events have been hypothesized (O’Neill et al., 1992; Rouset et al., 1992; Werren et al., 1995), naturally-occurring infectious transfer of Wolbachia has not been observed. The reliance of Wolbachia upon vertical transmission through maternal lineages has provided strong selective pressure for mechanisms that might increase its own transmission. Indeed, several mechanisms have been described by which Wolbachia alters host reproduction to enhance its vertical transmission to subsequent generations. The best described of these reproductive alterations include cytoplasmic incompatibility, feminization and parthenogenesis (Bourtzis and O’Neill, 1998; O’Neill et al., 1997; Werren, 1997).

The reliance of Wolbachia on transovarial transmission together with its associated reproductive phenotypes has directed much of the research attention on Wolbachia infections to host reproductive tissues. This narrow focus has persisted despite reports demonstrating infection of non-reproductive tissues (Binnington and Hoffmann, 1989; Louis and Nigro, 1989; Rigaud et al., 1991; Yen, 1972). As a result, interactions of Wolbachia with non-reproductive tissues have been largely ignored.
The significance of *Wolbachia* infections in insect non-reproductive tissues has recently reemerged with the description of a *Wolbachia* strain that forms heavy infections in nervous and muscle tissues of *Drosophila* and drastically reduces the life-span of adult flies (Min and Benzer, 1997). The potential significance of *Wolbachia* infections in insect non-reproductive tissues is also supported by hemolymph transfer experiments and observations of heavy *Wolbachia* infections throughout isopod tissues (Juchault et al., 1994; Rigaud et al., 1991). These examples indicate that early assessments of *Wolbachia* tissue distribution in insects may have underestimated the extent and significance of somatic infections.

We report studies that address the tissue distribution of several different *Wolbachia* insect associations. Three indirect assays were employed on isolated insect tissues; PCR and dot blot hybridizations were used to detect *Wolbachia* genomic DNA and a western blot assay was used to detect an outer surface protein (WSP) (Braig et al., 1998; Sasaki et al., 1998; Zhou et al., 1998). Our results demonstrate that somatic infection by *Wolbachia* is a common event. In some hosts however, *Wolbachia* infection can only be detected in the gonads.

### 2. Materials and methods

#### 2.1. Insect handling

The insects used in this study are listed in Table 1. All *Drosophila* strains were reared under similar low-density conditions to minimize size differences between individuals. The introgressed strain of *D. simulans* Hawaii infected with wRi was produced via five generations of backcrossing *D. simulans* Riverside males. The *D. simulans* Watsonville strains that were infected with wRi and wMa had been produced via embryonic microinjection (Giordano et al., 1995) and maintained for three years. Unless otherwise indicated, adult insects used for dissections were 3–7 days post eclosion. However, two month old *Aedes albopictus* females were also examined by western assay. All dissections were done in STE [0.1 M NaCl, 10 mM Tris HCl, and 1 mM EDTA (pH 8.0)]. At least five replicate insects were examined for each assay. Wings from *Drosophila simulans* Riverside, *Culex pipiens* Barriol (common house mosquito) and *Cadra cautella* (almond moth) were cut distally so as to not include any flight muscle. Third instar *Drosophila* larvae and fourth instar *Cadra* larvae were similarly dissected in STE for larval assays. Hemolymph from *D. simulans* Riverside and *Ae. albopictus* Houston (Asian tiger mosquito) was collected by puncturing the thorax of 20 adults using a fine probe. These wounded adults were centrifuged at 1000g for 5 minutes through a sieve made from a microcentrifuge tube (USA/Scientific #1405) punctured with a 27.5 gauge needle. Hemolymph from *C. cautella* was collected by puncturing fourth instar larvae and centrifuging at 80g for 15 minutes through glass wool.

#### 2.2. PCR

For the PCR assay, body segments were tested instead of dissected tissues to reduce artifacts caused by cross-contamination of tissue samples. Body segments were homogenized in STE with 0.4 mg/ml proteinase K. This mixture was incubated at 56°C for one hour and heat inactivated at 95°C for 15 minutes. Samples were extracted with phenol/chloroform/isoamyl alcohol and

<table>
<thead>
<tr>
<th>Host</th>
<th>Wolbachia</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. simulans</em> Riverside</td>
<td>wRi</td>
<td>(Hoffman et al., 1986)</td>
</tr>
<tr>
<td><em>D. simulans</em> Hawaii</td>
<td>wRi</td>
<td>–</td>
</tr>
<tr>
<td><em>D. simulans</em> Hawaii</td>
<td>wHa</td>
<td>(O’Neill and Karr, 1990)</td>
</tr>
<tr>
<td><em>D. simulans</em> Watsonville</td>
<td>wRi</td>
<td>(Giordano et al., 1995)</td>
</tr>
<tr>
<td><em>D. simulans</em> Watsonville</td>
<td>wMa</td>
<td>(Giordano et al., 1995)</td>
</tr>
<tr>
<td><em>D. simulans</em> Coffs Harbour</td>
<td>wCof</td>
<td>(Hoffmann et al., 1996)</td>
</tr>
<tr>
<td><em>D. melanogaster</em> Canton S</td>
<td>wMelCS</td>
<td>(Holden et al., 1993b)</td>
</tr>
<tr>
<td><em>D. melanogaster</em> yw65/25</td>
<td>wMel</td>
<td>(Bourtzis et al., 1996)</td>
</tr>
<tr>
<td><em>D. simulans</em> R3A</td>
<td>wNo</td>
<td>(Mérot et al., 1995)</td>
</tr>
<tr>
<td><em>D. mauritiana</em></td>
<td>wMa</td>
<td>Bloomington Stock Center #31</td>
</tr>
<tr>
<td><em>Ae. albopictus</em> Houston</td>
<td>wAlbA+wAlbB</td>
<td>(Sinkins et al., 1995b)</td>
</tr>
<tr>
<td><em>Ae. albopictus</em> Koh Samui</td>
<td>wAlbA</td>
<td>(Sinkins et al., 1995b)</td>
</tr>
<tr>
<td><em>Ae. albopictus</em> Mauritius</td>
<td>wAlbA</td>
<td>(Sinkins et al., 1995b)</td>
</tr>
<tr>
<td><em>Cx. pipiens</em> Barriol</td>
<td>wPip</td>
<td>(Guillemaud et al., 1997)</td>
</tr>
<tr>
<td><em>C. cautella</em></td>
<td>wCauA</td>
<td>R. T. Carde; U. Mass, Amherst</td>
</tr>
<tr>
<td><em>G. morsitans</em> morsitans</td>
<td>wMor</td>
<td>(O’Neill et al., 1993)</td>
</tr>
</tbody>
</table>

*Wolbachia* strain terminology is based on Zhou et al., 1998.
ethanol precipitated. These DNA preparations were PCR amplified in 50 mM KCl, 10 mM Tris HCl (pH 9.0), 0.1% Triton-X 100, 2.5 mM MgCl₂, 0.25 mM dNTPs, 0.5 uM ftsZ primers (Holden et al., 1993a; Sinkins et al., 1995b) and 0.5 U Taq DNA polymerase (Promega) in a total volume of 20 μl. Samples were denatured for 3 minutes at 94°C, cycled 35 times at 94°C, 55°C and 72°C (1 minute each), followed by a 10 minute extension at 72°C using a PTC-200 Thermal Cycler (MJ Research). 10 μl of each PCR was electrophoresed on 1% agarose gels, stained with ethidium bromide and visualized under ultraviolet illumination.

2.3. Dot blot hybridizations

_Drosophila_ tissues were prepared as described for PCR. Following heat inactivation, 50 μl of 0.8 M NaOH, 20 mM EDTA was added and the samples boiled for 10 minutes. Tissue samples were blotted using a Bio-Dot apparatus (BioRad) onto ZetaProbe membrane (BioRad) following the recommended protocol. Membranes were baked at 80°C for 1 hour, hybridized and washed using standard procedures (Church and Gilbert, 1984; Sambrook et al., 1989). A PCR amplified fragment of the _dnmA_ gene from _Wolbachia_ was random labeled (Boehringer Mannheim) with 32P and used to probe the blots (Bourtzis et al., 1996, 1994). Labeled blots were exposed to Transcreen MS film with an appropriate intensifying screen (Kodak). Autoradiograms were scanned and analyzed using the NIH Image package (version 1.61).

2.4. Antibody generation

The coding sequences of the mature _wsp_ gene product (Braig et al., 1998; Zhou et al., 1998) were separately amplified from wRi and wMa of _D. simulans_ Riverside and _D. mauritiana_ using the protocol described above. The forward primers used were 5’-CGG AAT TCG ATC CTG TTG TTC CAA TAA G-3’ for wRi and 5’-CCA TGG ATC CTG TTG TTC CAA TAA G-3’ for wMa; 5’-TCC GCT CGA GCT AGA TCC CAG TGT CAT G-3’ was used as the reverse primer for both. The amplification products were each cloned into pGEM-T (Promega) and then subcloned into the expression vector pET-32a (Novagen). After induction of the _E. coli_ cultures with IPTG, each recombinant WSP protein was separately harvested from inclusion bodies, solubilized in urea and purified through a nickel chelating resin following the recommended protocols (Novagen). The histidine tag was removed by thrombin digestion, and the recombinant proteins further purified by preparative electrophoresis using the Mini Prep Cell (BioRad) under the manufacturer’s recommended conditions. The recombinant WSP from wRi was used to immunize a rabbit with complete Freund’s adjuvant. To secure a wide spectrum of cross-reactivity with WSP from other _Wolbachia_ strains, the recombinant WSP from wMa was mixed with incomplete Freund’s adjuvant and used to boost the rabbit.

2.5. Western blots

For the western assay, dissected tissues were homogenized in 0.1 mM NaCl, 10 mM Tris HCl, 1 mM EDTA (pH 8.0) and 10% SDS. These samples were boiled for 15 minutes, desalted and concentrated using a previously developed method (Wessel and Flügge, 1984) with a substitution of acetone for methanol in the last step. Proteins were electrophoresed on 12% Laemmli SDS gels and blotted onto PVDF membranes (Immobilon-P; Millipore) under semi-dry conditions using the Transblot SD apparatus (BioRad) and Bjerrum and Schafer-Nielsen buffer (Bjerrum and Schafer-Nielsen, 1986) with 20% methanol and 0.004% SDS. Membranes were blocked with 3% non-fat dried milk, 10 mM Tris HCl (pH 8.0), 135 mM NaCl, 0.1% Tween 20, 0.05% sodium azide for 45 minutes, rinsed with TBS [10 mM Tris HCl (pH 8.0), 135 mM NaCl, 0.1% Tween 20], and incubated with anti-WSP antibody (1:1000 in TBS). Membranes were rinsed in TBS and incubated with anti-rabbit IgG alkaline phosphatase conjugated antibody (Boehringer Mannheim) diluted 1:1000 in TBS. Blots were developed using the standard BCIP/NBT protocol (Sambrook et al., 1989). Densitometry of western blots was conducted as for the dot blot analysis.

3. Results

To initially examine tropism, testes and ovaries were dissected from several _Wolbachia_-infected _Drosophila simulans_ and _Drosophila melanogaster_ strains. Total DNA was extracted from these gonads and the remaining carcasses. Extracted DNA was dot blotted and probed with the _Wolbachia dnaA_ gene. Five replicates were used for each _Drosophila_ strain. As shown in Table 2, the results demonstrate that _Wolbachia_ genomic DNA was detected in both reproductive and non-reproductive tissues of each of the _Drosophila_ strains. Comparison showed that ovaries tended to have the highest levels, followed by male carcasses, female carcasses, and testes. Similar to a previous report demonstrating no significant difference between the infection levels in gonads of mod’resc’ and mod’resc’ strains (Bourtzis et al., 1998), we did not observe any significant differences in infection levels of non-reproductive tissues between these phenotypes.

The _D. simulans_ Riverside strain (infected with the wRi strain of _Wolbachia_) was initially selected for a more detailed examination of _Wolbachia_ tissue tropism. For this analysis, specific tissues from larvae and adults
Table 2
Levels of dnaA in reproductive and non-reproductive (carcass) tissues of D. simulans (Ds) and D. melanogaster (Dm) resulting from dot blot hybridizations

<table>
<thead>
<tr>
<th>Wolbachia Strain</th>
<th>Host</th>
<th>Testes (s.e.m.)</th>
<th>Male carcass (s.e.m.)</th>
<th>Ovaries (s.e.m.)</th>
<th>Female carcass (s.e.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mod 'resc' wRi</td>
<td>Ds Riverside</td>
<td>79.3 (5.6)</td>
<td>90.5 (1.1)</td>
<td>95.5 (1.0)</td>
<td>88.1 (1.0)</td>
</tr>
<tr>
<td>wNo</td>
<td>Ds R3A</td>
<td>63.9 (0.8)</td>
<td>68.1 (1.0)</td>
<td>67.8 (0.8)</td>
<td>66.6 (2.4)</td>
</tr>
<tr>
<td>wHa</td>
<td>Ds Hawaii</td>
<td>30.4 (2.1)</td>
<td>55.2 (5.5)</td>
<td>77.8 (2.1)</td>
<td>58.7 (4.0)</td>
</tr>
<tr>
<td>wMel</td>
<td>Dm ywRiv,1c25</td>
<td>25.4 (5.7)</td>
<td>44.8 (4.0)</td>
<td>66.6 (3.6)</td>
<td>34.7 (5.8)</td>
</tr>
<tr>
<td>mod 'resc' wCof</td>
<td>Ds Coffs Harbour</td>
<td>66.4 (3.7)</td>
<td>82.1 (2.1)</td>
<td>83.3 (3.5)</td>
<td>79.0 (4.4)</td>
</tr>
<tr>
<td>wMelCS</td>
<td>Dm Canton S</td>
<td>40.9 (4.3)</td>
<td>64.9 (3.6)</td>
<td>79.3 (2.4)</td>
<td>64.9 (4.0)</td>
</tr>
</tbody>
</table>

*For each tissue, the average optical density units and standard errors represent five replicates. Levels in gonadal tissues are also reported in Bourzis et al. (1998).

were dissected and western blotted using an anti-WSP antibody (Fig. 1). In adults, high levels of WSP protein were observed in preparations of the head, thoracic muscles, midgut, Malpighian tubules, ovaries and testes. In addition, the super- and subesophageal ganglia were dissected from the heads of adults and assayed. This isolated nervous tissue and the remainder of these head dissections were both observed to contain WSP (data not shown). An analysis of the distal tips of the wings and hemolymph extracted from adults were also found to contain WSP. In larvae, WSP protein was again observed in the brains, salivary glands, midguts and fat bodies (Fig. 1). Of the tissues assayed, the accessory glands of adult males were the only tissues in which WSP could not be detected. This is in agreement with an earlier report that also observed the absence of Wolbachia in the accessory glands (Binnington and Hoffmann, 1989). As a negative control, tissues were examined of a D. simulans Riverside strain that had been treated with tetracycline to remove Wolbachia (O’Neill and Karr, 1990). No WSP bands were observed in these tissues (Fig. 1).

Similar disseminated infection patterns were observed in Ae. albopictus Houston, Cx. pipiens and C. cautella. In adults, WSP protein was observed in adult heads, thoracic muscles, midguts, Malpighian tubules, ovaries and testes (Fig. 1). In Cx. pipiens, detection of WSP in the midguts and thoracic muscles was difficult due to the small amounts of tissue that could be obtained. Although at the lower limits of detection using the western blot assay, faint bands indicating the presence of WSP were observed in these tissues. WSP was also detected in wings from Cx. pipiens and C. cautella and in hemolymph from adult Ae. albopictus Houston. Examination of C. cautella larvae demonstrated WSP in the brain, salivary glands, gut, Malpighian tubules, fat body and hemolymph.

In Ae. albopictus Koh Samui and Ae. albopictus Mauritius adults, WSP protein was detected in the ovaries and testes at levels lower than that observed in the Houston strain (Fig. 2). This is consistent with previous reports indicating lower infection densities in these strains (Sinkins et al., 1995a). WSP could not be detected in the non-reproductive tissues by western blot. Similarly, the PCR assay demonstrated the presence of Wolbachia genomic DNA in the abdomens but failed to detect Wolbachia DNA in the head or thorax. In Glossina m. morsitans females, the western blot and PCR assay also detected WSP and Wolbachia genomic DNA.
only in the ovaries (Fig. 2). This is similar to an earlier report in which Wolbachia infection was only detected in the ovaries of Glossina females and not in midguts (O’Neill et al., 1993). In G. morsitans males however, weak levels of WSP and Wolbachia genomic DNA were detected in the head, thorax and abdomen. To examine the potential for host age effects on Wolbachia tissue tropism, western assays of two month old adults of Ae. albopictus Koh Samui and Mauritius were compared with the results from the young adults. Results obtained for older mosquitoes were similar to the previously described results for young adult mosquitoes. Again, WSP was only detected in the reproductive tissues.

To address questions concerning the potential for host effects on Wolbachia infection levels, we compared pairs of different Drosophila hosts infected with the same Wolbachia strain. In each pair, one host was the origin of the Wolbachia infection and the other host had recently been infected by the same Wolbachia type via injection or introgression. This comparison revealed significant differences in total levels of WSP (Fig. 3). WSP levels of the wMa symbiont were significantly (P<0.0001; t-test) higher in the transinfected D. simulans Watsonville host background (106.1±14.2 optical density units) relative to the original D. mauritiana infection (21.4±4.0). Similarly, significantly (P<0.0006) higher WSP levels were also observed from the wRi symbiont in the D. simulans Watsonville background (149.6±9.8) relative to D. simulans Riverside (108.5±16.8). Only a slight increase (P<0.02) was found in the introgressed wRi infected D. simulans Hawaii (126.2±26.5) relative to the originating D. simulans Riverside infection (94.1±12.9). No differences were observed in the types of tissues in which WSP was expressed. WSP expression was detected throughout host tissues in each Wolbachia/host complex (Fig. 3).

4. Discussion

These results demonstrate that the WSP protein (detected by western blot) occurs in both reproductive and non-reproductive host tissues. This is consistent with the results expected for Wolbachia infections extending throughout host tissues. This broad tissue tropism is also supported by our detection of Wolbachia genomic DNA (detected by both dot blots and PCR) throughout host insects. This demonstrates the somatic infection of Wolbachia in females and males of multiple Drosophila species, Ae. albopictus Houston, Cx. pipiens Barriol, C. cautella and in males of G. morsitans. Infected tissues within these hosts include the brain, muscles, midgut, salivary glands, Malpighian tubules, fat body, wings, hemolymph, testes and ovaries. When comparing different tissues, we did not attempt to quantify the infection levels, since any comparison of Wolbachia levels using the western assay can not exclude the possibility that Wolbachia’s expression of the WSP protein varies between tissues. Thus, the differing levels detected using
the western technique could result from similar numbers of Wolbachia that are differentially expressing WSP.

Examination of Ae. albopictus Koh Samui, Ae. albopictus Mauritius, and G. morsitans females detected Wolbachia genomic DNA and WSP protein only in the gonads. While this suggests the absence of Wolbachia infection in non-reproductive tissues, we cannot exclude the possibility that Wolbachia infection is present at levels below detectable limits using these assays. A restriction of Wolbachia infection to the gonads in some hosts may have contributed towards the previous reports of insignificant infection levels of Wolbachia in the non-reproductive tissues of Cx. pipiens (Yen, 1972; Yen and Barr, 1974). Thus the discrepancy between our observations and these earlier reports may reflect real biological differences between the Wolbachia infection patterns of the Barriol strain examined here and the strain used in the previous study. This variability in Cx. pipiens infection patterns would be similar to our observed differences between different Ae. albopictus strains.

It should be noted that our report has focused on young adults. As shown in previous reports, host age may influence Wolbachia infection levels and tissue tropism (Binnington and Hoffmann, 1989; Bressac and Rousset, 1993; Min and Benzer, 1997). To examine this question, western blots of tissues from two month old adults of Ae. albopictus Koh Samui and Ae. albopictus Mauritius and larvae of D. simulans Riverside and C. cautella were compared with the corresponding results from young adults. In each case, no differences were observed in the infection levels and tissue tropism of these differently aged individuals. However, studies indicate that both infection levels and tropism may change with age in tsetse flies (S. Aksoy, personal communication). This potential variability in the roles to which age-related factors determine Wolbachia tissue tropism indicates that each Wolbachia/host association may have a unique tropism and will need to be examined separately.

Host effects were suggested by the different infection levels observed in our comparison of Drosophila pairs infected with the same Wolbachia type. Interestingly, the comparison of Drosophila pairs showed lower total infection levels in hosts with longer associations with a Wolbachia strain. This is in contrast to earlier reports showing lower infection levels in newly introgressed or microinjected hosts relative to the original infection (Boyle et al., 1993; Clancy and Hoffmann, 1997; Rousset and de Storqueur, 1994). As previously mentioned however, we cannot exclude the possibility that these results reflect variable WSP expression within the different host strains. The presence of host effects is also supported by the observed differences between G. morsitans males and females. While infection was consistently restricted to ovaries in young females, infections in similarly aged males extended throughout somatic tissues.

To address the potential for effects of Wolbachia type on infection levels, a comparison should be made of different Wolbachia types within the same host backgrounds. We did not attempt to make this comparison using the western blot assay due to the potential for differences in the WSP antibody's affinity for different Wolbachia types.

Decreased longevity in D. melanogaster associated with the rapid replication of Wolbachia in somatic tissues of older adults has been recently observed (Min and Benzer, 1997). Our results demonstrate that somatic infection occurs in several Drosophila strains, Ae. albopictus Houson, Cx. pipiens, C. cautella and in G. morsitans males. This demonstrates the need for additional studies to detect possible virulence associated with these other examples of Wolbachia somatic infections. Drosophila infections have been best characterized for fitness costs associated with infection. Although these previous studies did not examine the effect of Wolbachia infection on host longevity, no significant fitness cost associated with infection was detected in field populations (Hoffmann et al., 1998, 1990; Turelli and Hoffmann, 1995). A similar absence of fitness costs were observed in laboratory populations with the exception of D. simulans Riverside/wRi (Clancy and Hoffmann, 1997; Hoffmann et al., 1996, 1994, 1990).

Examination of fitness effects associated with somatic infections may also address the potential adaptive significance of Wolbachia somatic infections. For example, somatic infections may improve the host’s fecundity (Stolc and Stouthammer, 1995) or the vertical transmission rates of Wolbachia. Broad somatic infections may also benefit Wolbachia by increasing opportunities for horizontal transfer. Although cytoplasmic inheritance through females is the most widely recognized mode of Wolbachia transmission, phylogenetic analyses suggest that extensive horizontal transfer between species has occurred (O’Neill et al., 1992; Rousset et al., 1992; Werren et al., 1995). For example, a phylogenetic comparison between an endoparasitic wasp and its fly host show that both are infected with similar Wolbachia types, suggesting an interspecific horizontal transfer (Werren et al., 1995). This type of transfer would be more likely if Wolbachia were capable of somatic infection instead of being solely limited to the reproductive tissues.

The results presented here are significant to future attempts at artificial horizontal transfer of Wolbachia between insect hosts. Although Wolbachia has been successfully transferred in isopods via hemolymph transfers (Rigaud and Juchault, 1995) or injection of homogenized nervous tissue or fat body (Juchault et al., 1994), this approach has not been attempted in insects due in part to a belief that Wolbachia is restricted to the germ tissue. Therefore, most of the previous attempts have focused on the use of embryonic cytoplasm transfers as a means
to move *Wolbachia* (Boyle et al., 1993; Braig et al., 1994; Chang and Wade, 1994; Clancy and Hoffmann, 1997; Sinkins et al., 1995b). Our results suggest that transfer techniques similar to those used in isopods may provide an alternative to cytoplasmic microinjections for some *Wolbachia* infections in insects.

The broad tissue tropism of *Wolbachia* shown in this study suggest its potential usefulness as a gene expression vector. Infection throughout host somatic and germ line tissues demonstrates that *Wolbachia* has the potential to deliver gene products to a variety of host tissues. A *Wolbachia*-based expression system for foreign genes would have the advantage of being generally applicable to a broad range of invertebrate taxa (O’Neill et al., 1997). In addition, the cytoplasmic drive mechanisms of *Wolbachia* might serve to spread desired genes into host insect populations (Beard et al., 1993; Curtis, 1992; Miller, 1992; O’Neill et al., 1997).

Acknowledgements

We would like to thank Serap Aksoy for providing insect material and Rhoeo Dingalasen for his technical assistance. This work was supported by grants from the National Institutes of Health (AI07404-07, AI34355, AI40620), the McKnight foundation, the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, and the Greek Secretariat for Research and Technology (PENED 15774).

References


Bjerrum, O.J., Schaffer-Nielsen, C., 1986. Buffer systems and transfer techniques similar to those used in isopods may provide an alternative to cytoplasmic microinjections for some *Wolbachia* infections in insects.


