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Wolbachia infections are distributed throughout insect somatic and germ line tissues

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Abstract

Wolbachia are intracellular microorganisms that form maternally-inherited infections within numerous arthropod species. These bacteria have drawn much attention, due 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*.

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; Rousset 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.

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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 a *Wolbachia* 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 *w*Ri was produced via five generations of backcrossing *D. simulans* Hawaii females with *D. simulans* Riverside males. The *D. simulans* Watsonville strains that were infected with *w*Ri and *w*Ma 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* (=*Ephestia*) *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 1000***g*** 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 80***g*** 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 1
Insect host and *Wolbachia* strains

Host	*Wolbachia*[a]	Reference
D. simulans Riverside	*w*Ri	(Hoffman et al., 1986)
D. simulans Hawaii	*w*Ri	–
D. simulans Hawaii	*w*Ha	(O'Neill and Karr, 1990)
D. simulans Watsonville	*w*Ri	(Giordano et al., 1995)
D. simulans Watsonville	*w*Ma	(Giordano et al., 1995)
D. simulans Coffs Harbour	*w*Cof	(Hoffmann et al., 1996)
D. melanogaster Canton S	*w*MelCS	(Holden et al., 1993b)
D. melanogaster yw^{67c23}	*w*Mel	(Bourtzis et al., 1996)
D. simulans R3A	*w*No	(Merçot et al., 1995)
D. mauritiana	*w*Ma	Bloomington Stock Center #31
Ae. albopictus Houston	*w*AlbA+*w*AlbB	(Sinkins et al., 1995b)
Ae. albopictus Koh Samui	*w*AlbA	(Sinkins et al., 1995b)
Ae. albopictus Mauritius	*w*AlbA	(Sinkins et al., 1995b)
Cx. pipiens Barriol	*w*Pip	(Guillemaud et al., 1997)
C. cautella	*w*CauA	R. T. Carde; U. Mass, Amherst
G. morsitans morsitans	*w*Mor	(O'Neill et al., 1993)

[a]*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 $MgCl_2$, 0.25 mM dNTPs, 0.5 uM *fts*Z 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 *dnaA* gene from *Wolbachia* was random labeled (Boehringer Mannheim) with ^{32}P 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 *w*Ri and *w*Ma of *D. simulans* Riverside and *D. mauritiana* using the protocol described above. The forward primers used were 5′-CGG AAT TCG ATC CTG TTG GTC CAA TAA G-3′ for *w*Ri and 5′-CCA TGG ATC CTG TTG TTC CAA TAA G-3′ for *w*Ma; 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 *w*Ri 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 *w*Ma 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 *w*Ri 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[a]

Wolbachia Strain	Host	Testes	s.e.m.	Male carcass	s.e.m.	Ovaries	s.e.m.	Female carcass	s.e.m.
mod$^+$resc$^+$									
*w*Ri	Ds Riverside	79.3	5.6	90.5	1.1	95.5	1.0	88.1	1.0
*w*No	Ds R3A	63.9	0.8	68.1	1.0	67.8	0.8	66.6	2.4
*w*Ha	Ds Hawaii	30.4	2.1	55.2	5.5	77.8	2.1	58.7	4.0
*w*Mel	Dm yw^{67c23}	25.4	5.7	44.8	4.0	66.6	3.6	34.7	5.8
mod$^-$resc$^+$									
*w*Cof	Ds Coffs Harbour	66.4	3.7	82.1	2.1	83.3	3.5	79.0	4.4
*w*MelCS	Dm Canton S	40.9	4.3	64.9	3.6	79.3	2.4	64.9	4.0

[a]For each tissue, the average optical density units and standard errors represent five replicates. Levels in gonadal tissues are also reported in Bourtzis 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 subesophagial 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).

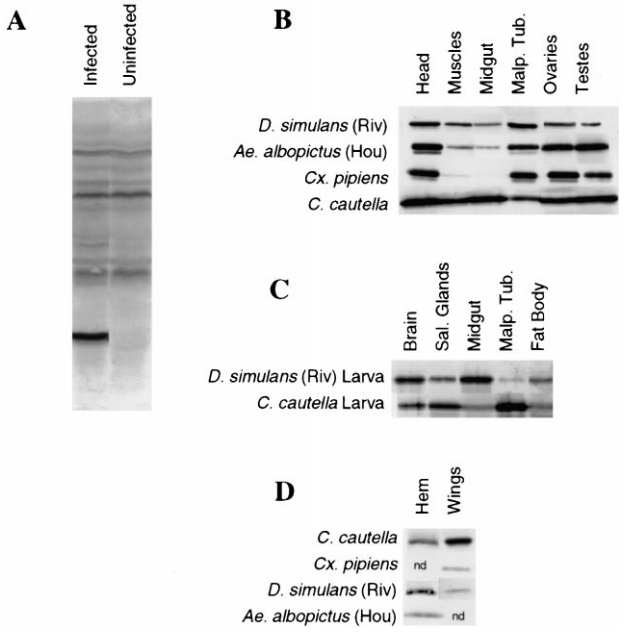

Fig. 1. Representative composite western blot for the detection of WSP protein in tissues from (A) *Wolbachia* infected and uninfected *D. simulans* Riverside whole adult, (B) adults, (C) larvae and (D) wings and hemolymph. Abbreviations include: Malpighian tubules ("Malp. tub."), salivary glands ("Sal. glands"), hemolymph ("Hem."), and not done ("nd"). For B, C, and D, only the WSP band portions of the western blots are shown.

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

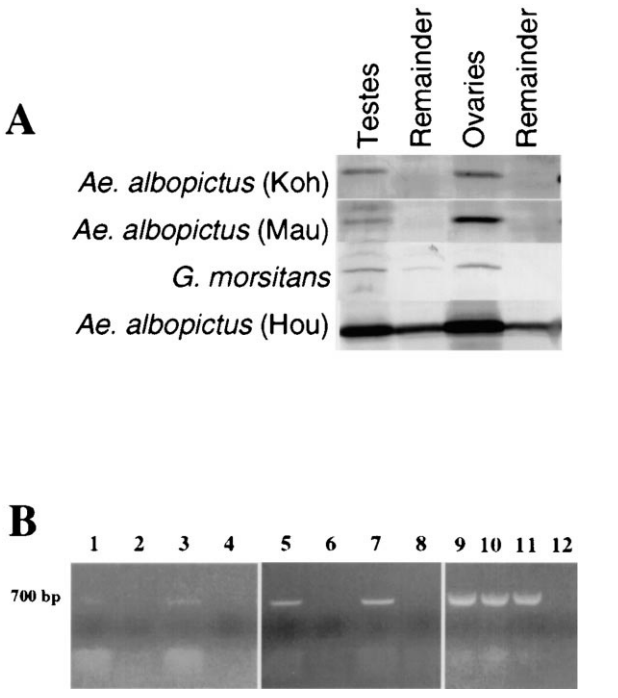

Fig. 2. (A) Representative composite western blot of infections of *Ae. albopictus* Koh Samui, *Ae. albopictus* Mauritius and *G. morsitans*. For comparison, a western blot of *Ae. albopictus* Houston (showing somatic infection) is also shown. (B) Typical results obtained from PCR amplifications of *Ae. albopictus* Koh Samui (1–4), *Ae. albopictus* Mauritius (5–8) and *G. morsitans* (9–12). Lanes 1, 5 and 9 are abdomens of males. Lanes 2, 6 and 10 are combined head and thorax of males. Lanes 3, 7 and 11 are abdomens of females. Lanes 4, 8 and 12 are combined head and thorax of females.

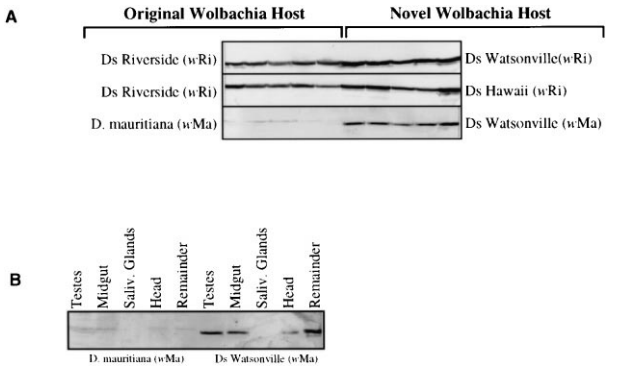

Fig. 3. (A) Western blot results comparing infection levels between three pairs of different *D. simulans* hosts infected with the same *Wolbachia* type. Each western blot consists of ten lanes; each lane represents a whole male. The first five lanes are of the host in which the *Wolbachia* infection originated. The last five lanes are of the host that has been recently infected. (B) Western blot of *w*Ma infected *D. mauritiana* (first five lanes) and *D. simulans* Watsonville (last five lanes) males that have been dissected into testes, midgut, salivary glands ("Saliv. glands"), head, and the remainder. "Ds" indicates *D. simulans*.

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 *w*Ma 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 *w*Ri symbiont in the *D. simulans* Watsonville background (149.6±9.8) relative to *D. simulans* Riverside (108.5.1±16.8). Only a slight increase ($P<0.02$) was found in the introgressed *w*Ri 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 Stordeur, 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/*w*Ri (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 (Stolk 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).

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