

## COMMENTARY

# Horizontal transmission of *Wolbachia* by strepsipteran endoparasites? A response to Noda *et al.*, 2001

D. P. HUGHES\*, P. PAMILO\* and J. KATHIRITHAMBY†

\*Department of Biology, PO Box 3000, FIN-90014, University of Oulu, Finland; †Department of Zoology, University of Oxford, Oxford, UK

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The noncongruence between molecular phylogenies of the endosymbiont bacteria, *Wolbachia* and their hosts imply frequent horizontal transfer between species (Werren *et al.* 1995). A suggested mechanism for this is infection by macro-parasites, notably parasitoids. A recent report in *Molecular Ecology* (Noda *et al.* 2001) noted that two planthopper species shared *Wolbachia* strains with those recovered from their shared strepsipteran endoparasites, and concluded that Strepsiptera were agents for horizontal transmission of *Wolbachia* in these planthopper species. There are a number of problems with this *prima facie* interpretation given certain aspects of the strepsipteran life history, which we outline here. The presented arguments have applicability to other studies, which speculate upon the role that parasitoids play in the transmission of *Wolbachia*, and underscore the need for a fuller consideration of parasitoid life history during hypothesis generating. We discuss the following main points, which are based upon published findings.

1) Shared strains of *Wolbachia* between two hosts and their common parasitoid do not necessarily support the hypothesis that the parasitoid is the agent of transfer. An equally plausible hypothesis is that Strepsiptera simply share the same *Wolbachia* strains as their hosts because of their biology and intimate association with the host. The majority of their lifecycle is spent in contact with the host haemolymph, and nutrients are ingested by larval stages through a mouth and by the neotenic adult female via a modified structure, the 'apron' (analogous to the insect gut), which lies on the outer body wall (see Kathirithamby 2000). A gut is present in Strepsiptera only in the larval stages, which becomes redundant in the neotenic adult female stage after the extrusion of the cephalothorax (the cephalothorax is extruded through the host cuticle, whereas the majority of

the body remains within the host). There is no mouth opening in the extruded cephalothorax and the brood canal opening is not analogous to the mouth. The transition from the possession of a gut to nutrient absorption via the apron is in stark contrast to the feeding method of the two other parasites that were screened (a dryinid wasp and mermithid nematode), or the planthoppers (Noda *et al.* 2001), and may promote the occurrence of *Wolbachia* from the planthoppers being found within the strepsipteran. It has already been hypothesized that *Wolbachia* probably do not survive passage through the digestive tract (Johanowicz & Hoy 1996; Cordaux *et al.* 2001), but the apron is unlikely to pose such a barrier considering its position. (The dryinid wasp had different *Wolbachia* strains and the mermithid nematode was uninfected.)

The body of the neotenic female is filled with oocytes (and nurse cells which together form egg chambers), which are free floating in the haemocoel (see Kathirithamby *et al.* 1990 and references therein). Thus the adult female is essentially a 'bag of eggs' (Kathirithamby *et al.* 1990), lying in the host haemolymph. Noda *et al.* (2001) stated (though provided no evidence) that *Wolbachia* were found within the 'ovaries'. This is a misnomer as paired ovaries do not exist after the second instar stage. Kathirithamby (1998), using TEM, described rickettsia-like micro-organisms within the nurse cells adjacent to oocytes, and it is possible that the *Wolbachia* detected by Noda *et al.* were also within the nurse cells. Thus, the reason that the strepsipteran parasites had the same strain of *Wolbachia* as their hosts might be because of their unique and intimate associations with their hosts, and does not imply that they were infected or were agents of transfer between disparate hosts.

2) Assuming that Strepsiptera are infected, can they realistically transfer *Wolbachia* to their host? One notable problem with the role of parasitoids in horizontal transfer is that parasitoids generally kill their hosts as a developmental necessity (Godfray 1994), and this is even before the

Correspondence: D. P. Hughes. Fax: + 358-8-553 1061; E-mail: david.hughes@oulu.fi

host has reproduced and passed on the acquired infection. Strepsiptera do not kill their hosts as a developmental necessity but do castrate them, which has the same effect for this argument. In fact, in the family Delphacidae (Homoptera), to which planthoppers belong, the castration is the most severe seen in any stylopized hosts, i.e. not only are the internal genitalia lost/reduced, but Delphacidae also undergo extreme reduction/loss of the external genitalia when stylopized (see Kathirithamby 1998). Transmission of *Wolbachia* between parasitoid and host supposes parasitoid death prior to castration or death being affected (West *et al.* 1998); hence the claim by Noda *et al.* that the 'successful inoculation ... of the host would be achieved when an infected [strepsipteran] dies within the body of its host'.

Strepsiptera are in the minority of parasitic insect (~ 15%, Eggleton & Belshaw 1992) where entry/parasitization of host is via the free-living first instar larva. Therefore, if death occurs after entry and successful host immune response, then any *Wolbachia* within the strepsipteran would need to travel from strepsipteran gametic tissue through the cuticle of the first instar strepsipteran and the melanized haemocytes (employed in defence) to the host tissue. This is a difficult scenario to imagine in comparison to the methods employed by other parasitoids when infecting hosts. The majority of parasitoids infect hosts through a female placing an egg into the haemocoel, or targeted tissue of the host and these present a more realistic vehicle of transmission. *Wolbachia* in *Drosophila* can migrate to the germ plasma of the egg (Hadfield & Axton 2000), and within the parasitoid, *Asobara tabida*, it has been found in the posterior cytoplasm (Dedeine *et al.* 2001). It is therefore possible that such migration may promote horizontal transfer before the death of the parasitoid egg. Such a mechanism might also explain the 'infectious parthenogenesis' seen among trichogrammatid wasps where transmission between infected and uninfected parasitoid occurred if they shared the same host (in this case an egg, Huigens *et al.* 2000). Further, ovipositing females may themselves be candidates for horizontal transmission of *Wolbachia* via the 'dirty needle' hypothesis (*sensu* Houck *et al.* 1991).

3) Our final point concerns itself with systematics, though is related to strepsipteran biology. Noda *et al.* (2001) claimed that two distant planthoppers were parasitized by the same species of strepsipteran. However the shared species of Strepsiptera might in fact be two distinct species or subspecies of Strepsiptera, which are specialized upon their respective planthopper hosts. Both (sub)species of Strepsiptera could have the same *Wolbachia* strain as the hosts for a number of reasons, besides the one given above, which are beyond the remit of this response. However, our salient point is that Strepsiptera are difficult to separate into species on morphological grounds (Kinzelbach 1978; Kathirithamby 1989). This is particularly true for females, which are neotenic (they lack eyes, antennae, mouthparts,

wings, legs and external genitalia), and stress has been placed upon the efficacy and utility of molecular separation of species (Halbert *et al.* 2001). Future work of this kind should attempt an identification of species on molecular grounds. Furthermore, authors should submit voucher specimens of hosts and parasites (e.g. Wenseleers *et al.* 1998).

Parasitoids (including Strepsiptera) display an enormous range of life histories which present challenges to speculations about mechanisms of horizontal transmission of *Wolbachia*. The ease with which *Wolbachia* can be detected in trophic cascades (e.g. West *et al.* 1998; Kittayapong *et al.* 2003) is likely to result in further possible candidates that account for the noncongruence between *Wolbachia* and host phylogenies. However, given that the details of life histories of many probable candidate taxa (mites, nematodes and parasitoids) are not well known to general audiences these should be investigated in detail before any hypothesis is presented.

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