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Wolbachia endosymbiont in some insect pests of sericulture

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The polymerase chain reaction method was applied to screen some insect pests of sericulture for the presence of *Wolbachia*, a rickettsial alpha-proteo-bacterium. The results revealed that out of 16 insect species representing six major orders, viz. Lepidoptera, Diptera, Hemiptera, Coleoptera, Hymenoptera and Dictyoptera comprising fifteen families tested, four were positive for *Wolbachia* infection. Two among these are insect pests of *Morus* species, one of *Terminalia* species and another major dipteran endoparasite of tasar silkworm, *Antheraea mylitta* harbours *Wolbachia.* These results indicate that the occurrence of *Wolbachia* is widespread among insect pests of sericulture.

Keywords: Endosymbionts, insect pests, sericulture, *Wolbachia*.

CONVENTIONAL biocontrol involves the establishment of food chain against the target pests through introduction/release/inundation of parasites and parasitoids. Exploitation of microbial pathogenesis against insect pests includes specially designed control agents to suit the particular requirements of insect-to-insect and insect-to-host plant relationship[1]. Keeping this in view, the use of *Wolbachia* has been considered as a mechanism, with the necessity to screen all the insect pests of sericultural importance. Earlier, Puttaraju *et al.*[2–6] have screened different silkworm races and its pest uzifly, *Exorista sorbillans.* They found the presence of *Wolbachia* in *Exorista* species and its absence in silkworm *Bombyx mori*. The present communication further records the presence of *Wolbachia* in certain insect pests of silkworm, *B. mori* and its host plant *Morus* species; so also of tasar silkworm, *Antheraea mylitta* and its food plant *Terminalia* species.

Symbiotic association between microorganisms and higher eukaryotes is extremely common and ranges from mutualistic to commensal and parasitic[7]. Rickettsial member of the genus *Wolbachia* belongs to α-proteobacteria and has been identified as an intracellular, obligate endoparasite in several taxa of arthropods. *Wolbachia* is known to cause a number of reproductive abnormalities in its hosts, including cytoplasmic incompatibility[8–10], parthenogenesis[11] and feminisation[12,13]. It has a wide host range and multiple infection sites within the host and plays a role in the ageing of insects by degrading different tissues[14]. Its presence in the host is detected by PCR-based DNA diagnosis using a set of primers that specifically amplify

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the bacterial surface protein genes[15]. Recently, *Wolbachia* has aroused considerable interest among biologists for its role in reproductive physiology of arthropods[16–18], its implication for evolution and speciation[19–22], mechanism of genetic modification and biological control of pest arthropods[23,24].

Insect pests collected from various parts of Karnataka and Madhya Pradesh were frozen at –80°C. DNA from a minimum of single insect was extracted following the usual stepwise methods of extraction with phenol, phenol : chloroform : isoamylalcohol, chloroform and finally precipitated with two volumes of double-distilled alcohol in the presence of 3M sodium acetate[25]. The precipitated DNA was washed in 70% alcohol, dried and dissolved in TE (Tris-EDTA) buffer; the volume of TE depends on the size of the pellet. DNA was subjected to RNAase-A treatments followed by further re-extraction with phenol : chloroform : isoamyl alcohol and chloroform. RNA-free DNA was precipitated with alcohol in the presence of sodium acetate, the pellet was dried and dissolved in TE buffer. The concentration of 10–12 ng/µl was realized through quantification on 0.8% agarose gel using standard concentration of lambda DNA in Tris–boric acid–EDTA buffer.

A PCR assay based on specific amplification of the *Wolbachia wsp* gene primer pair *wsp81F* 5′-TGG TCC AAT AAG TGA TGA AGA AAC-3′ and *wsp691R* 5′-AAA AAT TAA ACG CTA CTC CA-3′, which was synthesized (Bangalore Genei, India) based on published sequence information for the *Wolbachia* surface protein gene[15] of *Wolbachia pipientis* was used to detect *Wolbachia* in individual insects. It was carried out with PTC 200 of MJ Research Thermocycler, in 25 µl reaction mixture containing 2.5 µl of 10X PCR buffer, 0.5 µl of dNTPs (10 mM each), 2.5 µl of 25 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Bangalore Genei), 1 µl of 26 µM forward primer and 1 µl of 35 µM reverse primer, 30 ng template DNA; distilled, deionized water was added to a final volume of 25 µl. PCR was carried out with a cyclic condition of initial denaturation step at 94°C for 5 min followed by 30 cycles with denaturation step at 92°C for 1 min, primer annealing at 55°C for 2 min and primer extension in the presence of *Taq* DNA polymerase at 72°C for 2 min and final extension at 72°C for 5 min. The amplified PCR products were separated through 1.5% agarose gel electrophoresis run in 1X TBE (89.2 mM Tris-HCl, 88.9 mM boric acid and 2 mM disodium EDTA) buffer for a length of about 5 cm at a constant voltage of 70 V. The gel was stained with 0.5 µg/ml ethidium bromide prior to casting. Documentation was done with gel documentation system.

Sixteen different arthropod species were screened for *Wolbachia* by PCR method using general *Wolbachia wsp* gene primers[15]. Among the sixteen insect species, four (25%) were found to be infected with *Wolbachia* (Figure 1 and Table 1). *Wolbachia* present in *Eurybrachys tomentosa* (family Eurybrachidae) and *Myllocerus discolor* (family Curculionidae) are pests of *Morus* species, a food plant of silkworm, *B. mori* L. The presence of *Wolbachia* was also recorded in the pests of the tasar silkworm, *Antheraea mylitta* and its food plant *Terminalia* species. These pests include *Phyllaplecta hirsuta* (family Psyllidae), a pest of *Terminalia* species and *Blepharipa zebina* (family Tachinidae), an endoparasitoid of tasar silkworm. However, no amplification of *wsp* gene was recorded in the pests of *Morus* species, viz. *Spilosoma obliqua*, *Spodoptera litura*, *Sthenius grisator*, *Amata passalis* and *Margaronia pyloalis* and in the pest of silkworm, *Dermestes* species. The absence was also recorded in the pests of tasar silkworm *Aeolesthes holocericea*, *Xanthopimpla pedator*, *Vespa orientalis*, *Canthecona furcellata*, *Sycanus collaris* and *Hierodulla bipapilla.*

Table 1. *Wolbachia* infection status in some insect pests of sericulture

Insect	Order/family	Host	PCR result
Spilosoma obliqua	Lepidoptera/Arctidae	*Morus* sp.	–
Eurybrachys tomentosa	Hemiptera/Eurybrachidae	*Morus* sp.	+
Spodoptera litura	Lepidoptera/Noctuidae	*Morus* sp.	–
Sthenias grisator	Coleoptera/Cerambycidae	*Morus* sp.	–
Amata passalis	Lepidoptera/Amatidae	*Morus* sp.	–
Margaronia pyloalis	Lepidoptera/Pyrallidae	*Morus* sp.	–
Myllocerus discolor	Coleoptera/Curculionidae	*Morus* sp.	+
Dermestes sp.	Coleoptera/Dermestidae	*Bombyx mori*	–
Phylloplecta hirsuta	Hemiptera/Psyllidae	*Terminalia* sp.	+
Aeolesthes holocericea	Coleoptera/Cerambycidae	*Terminalia* sp.	–
Xanthopimpla pedator	Hymenoptera/Ichneumonidae	*Antheraea mylitta*	–
Vespa orientalis	Hymenoptera/Vespidae	*A. mylitta*	–
Canthecona furcellata	Hemiptera/Pentatomidae	*A. mylitta*	–
Blepharipa zebina	Diptera/Tachinidae	*A. mylitta*	+
Sycanus collaris	Hemiptera/Reduviidae	*A. mylitta*	–
Hierodulla bipapilla	Dictyoptera/Mantidae	*A. mylitta*	–

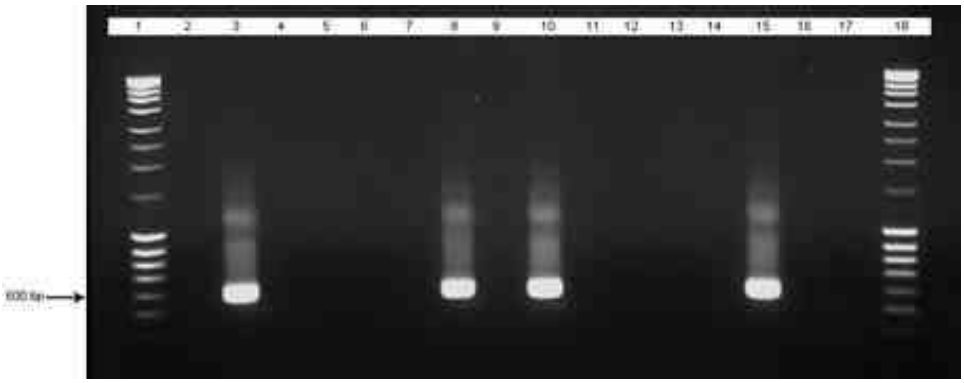

Figure 1. *Wolbachia wsp* gene amplified in some insect pests of sericulture. Lanes 1 and 18, Molecular weight marker (mass ruler); lanes 2–8, Insect pests of *Morus* species including *S. oblique*, *E. tomentosa*, *S. litura*, *S. grisator*, *A. passalis*, *M. pyloalis* and *M. discolour* respectively; lane 9, *Dermestes* sp. pest of silkworm; lanes 10, 11, *P. hirsuta* and *A. holocericea*, pests of *Terminalia* species; lanes 12–17, Insect pests of *A. mylitta* including *X. pedator*, *V. orientalis*, *C. furcellata*, *B. zebina*, *S. collaris* and *H. bipapilla* respectively.

The size of the amplified *Wolbachia* DNA fragment ranges from 590 to 632 bp depending on the individual *Wolbachia* strains and the band size of our PCR results matches that of the other insects[26–28]. Among the sixteen tested individuals, 25% arthropod species were found positive for *Wolbachia* infection. This was in agreement with the result of Werren *et al.*[29], who obtained 16% of neotropical arthropod species infected by *Wolbachia*. In contrast, Jeyaprakash and Hoy[30] found that 76% insects species among sixty-two arthropod species tested positive for *Wolbachia* infection. In the present communication, *Wolbachia* infection was detected in the members of the new families Eurybrachidae and Curculionidae, orders Hemiptera and Coleoptera respectively, but its absence was recorded in the members of families Arctidae, Noctuidae, Amatidae and Pyralidae, order Lepidoptera and in the members of family Cerambycidae, order Coleoptera, the pests of *Morus* species and also absence in the pests of silkworm cocoon, *Dermestes* species (Coleoptera: Dermestidae). However, in the pests of food plant of tasar silkworm *A. mylitta*, two of the tested insect species belong to family Psyllidae, order Hemiptera and family Tachinidae, order Diptera proved positive for *Wolbachia* infection. Further, *Wolbachia* is absent in members of family Cerambycidae, order Coleoptera, families Ichnuemonidae and Vaspidae, order Hymenoptera, families Pentatomidae and Reduviidae, order Hemiptera and family Mantidae, order Dictyoptera (Table 1 and Figure 1).

Earlier, Werren *et al.*[29] found 26.1% infection in the members of the orders Hymenoptera followed by members of the orders Lepidoptera and Hemiptera. In the present study, higher *Wolbachia* infection was recorded in the order Hemiptera followed by Diptera and Coleoptera. The data presented here, which show the presence of *Wolbachia* in over 25% of arthropod species, further support the widespread occurrence of *Wolbachia* infection.

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Interactions between non-flying mammals and flowers of *Cullenia exarillata* Robyns (Bombacaceae), a canopy tree from the wet forests of Western Ghats, India

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Many non-flying mammals help in the pollination of plant species in tropical forests. However, this remains poorly documented from India. Here we demonstrate with *Cullenia exarillata*, a canopy tree in the Western Ghats, how a wide range of visitors frequent flowers of the tree and how among them a few select mammals are important in pollination. Since *Cullenia* also uses bats as a pollinating vector, we further discuss how the dual strategy of using non-flying mammals and bats could have evolved in the wet forests of the Western Ghats.

Keywords: *Cullenia exarillata*, non-flying mammals, pollination, Western Ghats.

POLLINATION by arboreal non-flying mammals is a rare phenomenon in tropical forests compared to other forms of pollination[1–3]. A recent study conducted in the wet forest of the Western Ghats showed that only 2 out of 89 species of trees were pollinated by mammals[1]. The few instances of non-flying mammal pollination systems that have been observed in tropical forests are interesting for several reasons. First, often flowers are the critical resource for animals during times of food scarcity in the forest and are considered as a keystone resource for them[4–6]. Second, they are often found in areas that are least conducive to other forms of pollination by insects, birds or bats because of cold and wet conditions[7]. Third, they are believed to occur in areas where bats as pollinators are non-existent[8].

Non-flying mammals pollinate trees in Australia, south and central Africa and tropical America[7]. This is not uncommon in countries of the southern hemisphere, mainly because members of the family Proteaceae that are pollinated by many marsupials and rodents apart from birds, are fairly abundant and species-rich[9]. In other parts of the world, families such as Bombacaceae, Combretaceae, Fabaceae and Melastomaceae are also visited by non-flying mammals[7,10,11]. Non-flying mammal pollinators also comprise of many diverse terrestrial mammals ranging from rat to giraffe[9,12–18]. Primates are more often viewed as flower predators because they inflict heavy damage to floral parts while handling flowers. However, Kress *et al.*[19] have

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