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First Record of Wolbachia in Some Common Butterflies from Akola District of Maharashtra, India

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Maternally transmitted bacteria have been important players in the evolution of insects and other arthropods, affecting their nutrition, resistance, development and reproduction. Wolbachia are the best studied among these and typically the most prevalent. They are known to manipulate biology of their host by inducing male killing, feminization, parthenogenesis, cytoplasmic incompatibility and speciation through reproductive isolation. The current study aims to identify the presence of these bacteria in some of the common butterfly species from Akola district of in Maharashtra. In this study, a PCR screening method with specific primers was used to determine the occurrence of Wolbachia in five butterflies, Common grass yellow, Common emigrant, Mottled emigrant belonging to family Pieridae and Common tiger, Chocolate pansy, belonging to family Nymphalideae. A total of five samples for each butterfly species collected from five corners of the district and screened with Wolbachia-specific W-Spec primers. More than 80% of the samples of all the studied butterflies were found to be infected with Wolbachia spp. This is first record of Wolbachia in the studied butterflies from Akola district in Maharashtra.

Key words: Wolbachia, 16S rDNA, PCR, family Nymphalideae, family Pieridae.

Wolbachia are a group of endosymbionts bacteria found in reproductive tissues of many arthropods, first reported within the reproductive tissues of the mosquito *Culex pipiens* by Hertig and Wolbach in 1924. The name as Wolbachia *pipientis was* assigned *by Hertig* (1936). These bacteria are maternally transmitted through the cytoplasm of eggs and affects reproduction of their hosts, causing induction of reproductive incompatibility, parthenogenesis, feminization and male killing(O'Neill *et al.*, 1992; Rousset *et al.*, 1992; Werren *et al.*, 2008). Along with vertical maternal transmission Wolbachia are also transmitted horizontally (inter-taxon) among arthropod species. Wolbachia are extremely widespread among insects and found in over 16% of insect species, belonging to almost all the major orders (Werren *et al.*, 1995a and Werren and Jaenike, 1995). West *et al.*, (1998) revealed that 22% of British insects were infected. Jeyaprakash and Hoy (2000) predicted that Wolbachia infection levels may be as high as 76% of all insect species. Being infective there developed a widespread attention in Wolbachia to consider it as potent biological control agent in the form of a microbial "natural enemy", to boost productivity of natural enemies or as a vector for inculcating fatal genetic alterations in host pests (Werren and Jaenike,1995). Significant advances have been made in the study of these interesting endosymbionts due to recently available new study tools. PCR is one of the tools based on molecular techniques (Werren *et al.*, 1995a and Werren and Jaenike, 1995). According to Lo *et al.*, (2002) and Werren *et al.*, (2008) Wolbachia occurs in eight major clades or "super

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groups" (A to H) based on phylogenetic clustering of *ftsZ* gene sequences. A, B and E found in the arthropods; C and D in nematodes; G in spiders; H in termites and F in both nematodes and arthropods. The 16S rDNA and *ftsZ* studies have recognized many of useful molecular tools for such findings (John, 1997). These include general *Wolbachia* specific primers (Werren *et al.,* 1995b). Lepidoptera, are perhaps the most conspicuous and colorful insect in many ecosystem. The association of Wolbachia with order Lepidoptera is very well studied, but only a few species of butterflies are known to be infected by Wolbachia(Jiggins *et al.,* 2001: Dyson *et al.,* 2002 : Hiroki *et al.,* 2004 and Narita *et al.,* 2007). Maharashtra in India have a great butterfly diversity playing major role in biodiversity mentainence.Till now there is no report on the occurrence of Wolbachia in Butterflies from Maharashtra. Therefore the present study is carried out to determine the occurrence of Wolbachia in five butterflies: Common grass yellow, Common emigrant, Mottled emigrant belonging to family Pieridae and Common tiger, Chocolate pansy, belonging to family Nymphalideae collected from Akola district in Maharashtra.

MATERIALS AND METHODS

Females of five species of butterflies, i.e., Common grass yellow, Common emigrant and Mottled emigrant belonging to family Pieridae, Common tiger, and Chocolate pansy belonging to family Nymphalideae were collected during August 2013 December 2013 from different habitat in Akola district located at Punjabrao Deshmukh agriculture university campus- Tq.Akola., Katepurna sanctuary-Tq.Barsitakli, Melghat–Tq.Akot, Reverine sites-Tq.Balapur and National highway No.6-Tq.Murtizapur. The collection was done from the pesticides sprayed agriculture lands, in the morning hours from 8.30 a.m. to 1.00 p.m. The collected species were transferred to the laboratory carefully, identified with the help of the available keys and separated with respect to their sex. A total of 5 individuals from each species were subjected to PCR detection of Wolbachia. Before DNA extraction, the abdomen were dissected out in sterilized, deionized water on a sterile Petri dish and then serially rinsed in droplets of sterile water and air-dried for 15 min. For positive control samples known Wolbachia positive DNA sample from adult Weevils, were used. For negative control PCR reactions were performed on blanks using distilled water.

The DNA was isolated by the insect DNA Extraction Kit (Nucleopore Insect DNA Extraction Kit) as per the manufacturer's protocol. Polymerase chain reaction was performed using 16S rDNA to detect the presence of Wolbachia. The 16S rDNA primers used in the assay were W-Specf (5„-CATACCTATTCGAAGGGATAG) and WSpecr (5„-AGCTTCGAGTGAAACCAATTC) (Werren and Windsor, 2000).

According to the manufacturer's instructions (Peltier PCR Processor Model NEO - Bio Era Pune) PCR amplification was carried out with using 50 µl reaction volume consisting of 14 µl of master mix(Thermo scientific) (0.05U/ul Taq DNA polymerase, reaction buffer, 4mM MgCl2, 0.4mM of each dNTP (dATP, dCTP, dGTP, dTTP) containing 10_x Taq buffer with KCL(100 mM Tris-HCl pH 8.8, 500 mM KCl) 0.2 mM dNTPs, 2.5 mM MgCl2 and 0.5 U Taq DNA polymerase, 100 pm of 3 µl each of forward and reverse primers, 50 ng of 1.5 µl of template DNA, 23 µl of nuclease free water to make up 50 µl.

The reaction conditions consisted of an initial denaturation step at 95°C for 2 minutes, followed by 35 cycles of denaturation at 95°C for 30 Second, annealing at 60°C for 1 min and extension at 72°C for 45 seconds and a final extension at 72°C for 5 minutes. To determine the presence and size of the amplified DNA the amplified PCR products were separated through 1% agrose gel electrophoresis run in 1X TAE (40 mM Tris-HCl, 20 µM acetic acid and 1 mM EDTA) (Puregene genetix brand) buffer for a length of 5–6 cm at a constant 60V with a standard molecular weight marker DNA ladder (Thermo Scientific Gene Ruler 1 kb Plus DNA Ladder).

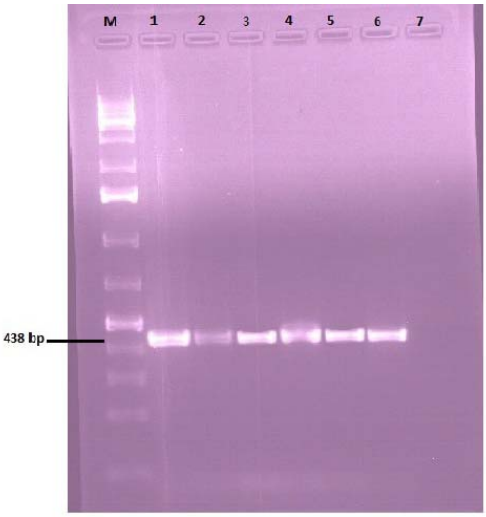
DNA bands were visualized by ethidium bromide staining and were documented with the gel documentation system. Blank sample did not have any DNA template. The size of the PCR product was determined by comparing with standard marker ladder. The samples yielding a product of the expected size (438 bp) were tentatively judged as having Wolbachia.

RESULTS AND DISCUSSION

A total of five different butterfly species from two families screened in the present study for Wolbachia by PCR amplification. The PCR amplification was conducted by Wolbachia 16S rDNA specific primers. As shown in the Photo Plate-I, Negative-control samples in PCR run did not amplified (Lane 7), while the 16S rDNA gene amplified at 438 bp, in all the experimental samples run (Lane1: Positive Control, Lane 2: Common grass yellow, Lane 3: Common emigrant, Lane 4: Mottled emigrant, Lane 5 : Common tiger, Lane 6; Chocolate pansy. Thus the results confirmed the occurrence Wolbachia in, Common grass yellow, Common emigrant, Mottled emigrant, Common tiger, Chocolate pansy collected from different corners of Akola district in Maharashtra(Table 1). This is the first record on the occurrence of Wolbachia in the studied butterflies from Maharashtra in India. Similar polymerase chain reaction methods were used by Werren and Windsor (2000) and reported, a large number (19.3%) of insect in North America are having Wolbachia. In accordance with the present methods West *et al.*, (1998) reported that 22% of British insects and Jayaprakash and Hoy, (2000) using same procedure predicted that 76% of all insect species hosting the Wolbachia as symbiotic.

Table 1. Screening of Butterfly species for Wolbachia using PCR

Butterfly	Number of individuals screened	Number of individual detected with Wolbachia
Eurema hecabe Common grass yellow	05	4
Catopsilia pomona Common emigrant	05	4
Catopsilia pyranthe Mottled emigrant	05	5
Danus genutia Common tiger	05	4
Junonia iphita iphita Chocolate pansy	05	5



Lane M: DNA marker.
Lane 1: Amplification of the 16S rDNA gene fragment Positive Control.
Lane 2: Amplification of the 16S rDNA gene fragment for Common grass yellow.
Lane 3: Amplification of the 16S rDNA gene fragment for Common emigrant.
Lane 4: Amplification of the 16S rDNA gene fragment for Mottled emigrant,
Lane 5 : Amplification of the 16S rDNA gene fragment for Common tiger,
Lane 6; Amplification of the 16S rDNA gene fragment for Chocolate pansy,
Lane 7 : Blank sample(negative control)

Plate 1. Detection of Wolbachia via amplification of specific gene fragment from butterflies. Wolbachia detection with primers of R and F for a fragment (438 bp) of the 16S rDNA gene

The results in the present study though provided the first record of Wolbachia in the studied butterflies from Akola district in Maharashtra, is based on a very few individuals collected from a narrow range of habitate. Therefore, the Wolbachia positive species reported were less likely to be infected and hence may underestimate the actual occurrence of Wolbachia in them from Maharashtra.

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