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Research Article

Wolbachia elimination induced cytoplasmic incompatibility, a latent tool for the management of uzi fly, *Exorista sorbillans* (Wied.) (Diptera: Tachinidae)

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ABSTRACT: The endosymbiotic bacteria *Wolbachia* that occurs commonly in arthropods, often manipulate host reproduction through cytoplasmic incompatibility (CI). The crosses between *Wolbachia* infected and uninfected uzi fly *Exorista sorbillans* populations (cured with antibiotic oxytetracycline) indicated strong CI, signifying their role in host reproduction. Significant reduction in brood hatch and increased sterility was observed.

KEY WORDS: *Wolbachia, Exorista sorbillans,* Cytoplasmic incompatibility, uziflies, fecundity, oxytetracycline.

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INTRODUCTION

Wolbachia are a group of rickettsial endosymbiotic bacteria in the alpha-proteobacteria. Infection with *Wolbachia* is found universally within various groups of insects, crustaceans, arachnids and nematodes (Werren *et al.,* 2008). *Wolbachia* alters reproductive biology of its hosts by inducing feminization, parthenogenesis, speciation, male killing and cytoplasmic incompatibilities (Werren, 1997). The bacteria are transovarially inherited, but may be transmitted horizontally between taxa (Heath *et al.,* 1994). *Wolbachia* has been detected using the polymerase chain reaction (PCR) in 15-70% of insect species sampled (Werren *et al.,* 1995; O'Neill *et al.,* 1997; Hilgenboecker *et al.,* 2008), although a method known as Long –PCR resulted in *Wolbachia* detection in 76% of sampled arthropod species (Jeyaprakash and Hoy, 2000; Sumithra *et al.,* 2012).

The most widespread and best known phenotypic effect of *Wolbachia* is cytoplasmic incompatibility (CI), wherein sperm from infected males cannot produce viable offspring with females that do not harbour the same bacterial strain (Hoffmann and Turelli, 1997). In its simplest form, cytoplasmic incompatibility occurs when sperm from an infected male fertilizes ova from an uninfected female, resulting reduced egg hatch. The mechanism of CI induction is currently unknown. However, the *mod resc* model allows interpretation of the various patterns observed so far (Werren, 1997; Werren *et al.,* 2008). It postulates the existence of two bacterial functions: *mod* (for modification) and *resc* (for rescue). The *mod* function acts on the nucleus in the males germline, before *Wolbachia* are shed from maturing sperm (Broutzis *et al.,* 1998; Presgraves, 2000). If sperm is affected by *mod*, zygote development will fail unless *resc* is expressed in the egg.

The uzi fly *Exorista sorbillans* (Diptera: Tachinidae), a parasitoid of silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae), alone causes 15 to 20% yield loss to sericulture industry in India (Narayanaswamy and Devaiah, 1998). It is known that this parasitoid harbours a *Wolbachia* endosymbiont (Madhu and Puttaraju, 2001; Guruprasad *et al.,* 2011a). The current study explores the strength of CI expression induced when *Wolbachia* is eliminated in the lab populations of *E. sorbillans*.

MATERIALS AND METHODS

Uziflies rearing and crossing experiments

The post parasitic maggots of uzi fly *E. sorbillans* were collected from the Ramanagaram silkworm cocoon market (Karnataka) and reared in the wire mesh netted cages of 35 x 35 x 35 cubic cm at 26 ± 1°C, 65 ± 5% relative humidity and 14-10 (L-D) photoperiod. After the

emergence, the adult male and females were separated immediately based on the morphological sex characters (Manjunatha, 1993). Uziflies were reared by feeding with glucose (8%) in distilled water soaked with cotton swabs to maintain nutritional uniformity among the replications. *Wolbachia* infections were cured by feeding the uzi flies with antibiotic oxytetracycline (0.02 mg/ml with 8% glucose). After seven generations, both the control (infected) and treated (uninfected) uziflies were crossed (@ 2 female:1 male). The following crosses were made mating with a period of 24 h.

1) Cured males x Uncured females
2) Uncured males x Cured females
3) Cured males x Cured females
4) Uncured males x Uncured females

For each cross, 22 to 24 replicates were done three times. The antibiotic treated flies are referred in the present study as *Wolbachia* cured (C) population and control flies as *Wolbachia* uncured (UC) population. Early fifth instar silkworm larvae were placed in respective uzi flies cages, the uzi flies were allowed to oviposit on silkworm. After 24 hours of oviposition, the fecundity on each silkworm larvae was recorded. The percentage of eggs hatched was determined by the number of black scars on the silkworm larva upon hatching of uzi fly eggs. The host silkworm larvae were reared in the laboratory until the uzi maggots emerged from the silkworm larvae. The collected maggots were allowed to pupate for adults emergence.

DNA extraction and PCR assay for *Wolbachia*

The DNA of *E. sorbillans* was extracted by proteinase – K and SDS lysis method as in Sambrook *et al.,* (1989). The genomic DNA was resuspended in 50ml of TE (10 mM Tris-HCl. 1 mM EDTA , P^H 8.0). The polymerase chain reaction (PCR) assay was carried out based on specific amplification of the *Wolbachia* wsp (*Wolbachia* surface protein) gene primer wsp81F – 5^1TGGTCCAATAAGTGATGAAGAAAC3^1 and wsp 691R – 5^1AAAAATTAA ACGCTACTCCA3^1 (Sigma Aldrich, India). The PCR was carried out with PTC 200 of MJ Research Thermocycler with 25ml reaction mixture containing 2.5ml of 10 x PCR buffer, 0.5ml of dNTP's (10mM each), 2.5ml of 2.5mM $MgCl_2$ and 0.5 U Taq DNA polymerase (New England Biolabs, England), 1ml of both forward and reverse primer (5 pmol), 20 ng template DNA; and final volume of milique water to make up 25ml. The PCR was carried out with a cyclic condition of initial denaturation step at 94°C for 5 min followed by 35 cycles with denaturation step at 92°C for 1 min, annealing 55°C for 1.30 min extension 72°C for 1.15 min and final extension at 72°C for 10 min. The PCR amplified products were checked by electrophoresis on 1.5% agarose gel running in 1 x TBE (89.2mM Tris HCl, 88.9mM Boric acid and 2mM disodium EDTA) buffer for a length of about 5 cm with a constant voltage of 70V. The gel was stained with ethidium bromide 0.5 mg/ml prior to casting. Gel documentation was done by using Alpha digi doc documentation system.

Statistical analysis

Student's t-test for paired samples and single factor ANOVA for multiple results was done by using SPSS15 version software.

RESULTS AND DISCUSSION

Effects of *Wolbachia* curing on Uzi fly population using antibiotic oxy-tetracycline

It took up to 6 generations to eliminate *Wolbachia* with oxytetracycline. The *Wolbachia* elimination was confirmed through PCR using wsp general primer (Fig. 1).

Effect of *Wolbachia* on fecundity

The average number of eggs from the cross between curec males x uncured females and from cured males x uncured females were 422±6.5 (n=24) and 450±6.6 (n=24), respectively. While the crosses between uncured males x cured females and between uninfected males x cured

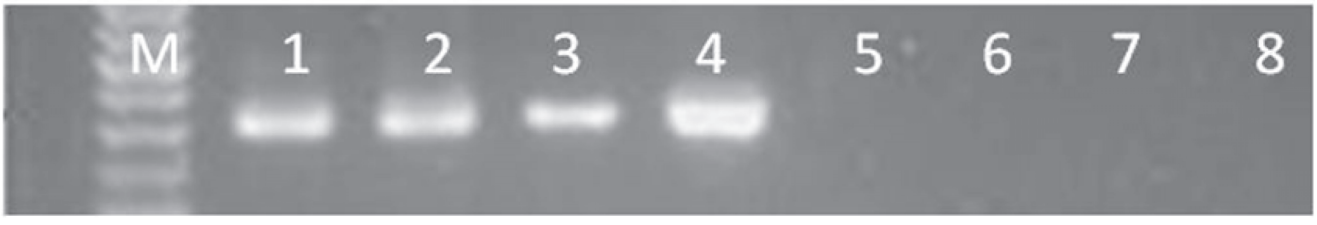

Fig. 1. PCR amplification of wsp primer from the *Wolbachia* infected and tetracycline treated populations of *Exorista sorbillans* lane M is marker, lane 1-4 is *Wolbachia* infected uzi flies and lane 5-8 is tetracycline treated uzi flies.

females it was found to be 172±2.7 (n = 23) and 170±2.5 (n = 22), respectively (F = 1006.40 df = 92, *P*<0.001) at 95% confidence interval (Table 1). The Post Hoc Test (Dunnett t) Multiple comparisons with control crosses between uncured males x uncured females and from the crosses uncured males x uncured females was 27.6(*) (P>0. 0.009). While the crosses between uncured male x cured females [–250.00(*) (P<0.001)] and between cured male x cured females was [–252.66(*) (P<0.001)] significant (Table 2).

Effect of *Wolbachia* and cytoplasmic incompatibility (CI) on uzi fly population size

Uzi flies displayed a meager amount of embryonic mortality from crosses of uncured males x uncured females and from cured male x uncured female which was 396 ± 5.1 (n = 24) and 408 ± 6.8 (n = 24) with a hatching percentage of 93.8 and 90.6, respectively indicating 6.2 and 9.1 percent sterility. However, in the crosses between uncured males x cured females and cured males x cured females, it was 37 ± 1.4 (n = 23) and 37 ± 4.5 (n = 22), respectively with hatching percentage of 21.5 and 21.7 and a significant increase in the rate of sterility 78.5 and 78.3 percent was recorded, respectively (F = 1847.18 df = 92, P<0.001) (Table 1). The Post Hoc Test (Dunnett t) multiple comparisons of control cross between uncured males x uncured females from the crosses cured males x uncured females was 12.66 (P>0.234) and uncured male x cured female was –358.33(*) (P<0.001) and from cured males x cured female –359.00(*) (P<0.001) were significant (Table 2).

The experimental plot and results obtained provide the necessary evidence that the endocellular bacterium, *Wolbachia* is responsible for the reproductive manipulation Cytoplasmic Incompatibility (CI) in uzi flies, *E sorbillans*. It is assumed that the in the infected males, the *Wolbachia* modifies the sperm while the same strain of *Wolbachia* in female egg rescues the sperm modification. However, in uninfected eggs the sperm modification cannot rescue therefore it leads to sterility (Broutzis and O'Neill, 1998; Ioannidis and Bourtis, 2007). Apart from the established mechanism of CI in uziflies, the results clearly emphasize that *Wolbachia* infection increased female fecundity (Guruprasad *et al.,* 2011b). Such positive host fitness effects have been previously reported for CI inducing *Wolbachia* infections in *Drosophila*, however these fitness have been shown to be transient (Poinsot and Mercot, 1997). A contrasting host fitness in effects have also been observed in *Wolbachia* infected *Nasonia*

Table 1. Fecundity and incompatibility in crosses between *Wolbachia* infected (UC) and uninfected (C) populations

Sl. No.	Crosses Male x Female	Replications	Fecundity ± SE	Hatchability ± SE	Hatching %	CI%
1	C x UC	24	450 ± 6.6	408 ± 6.8	90.6	9.1
2	UC x C	23	172 ± 2.7	37 ± 1.4	21.5	78.5
3	C x C	22	170 ± 2.5	37 ± 4.5	21.7	78.3
4	UC x UC	24	422 ± 6.5	396 ± 5.1	93.8	6.2
F – value			1006.40	1847.18		
p – value			<0.001	<0.001		

Table 2. Post Hoc Tests Multiple Comparisons in Dunnett t -tests treat one group as a control, and compare all other groups against it

Dependent Variable	(I) Factor	(J) Factor	Mean Difference (I–J)	SE	*P* – value
Fecundity	C x UC	UC x UC	27.66667(*)	6.84349	0.009
	UC x C	UC x UC	–250.00000(*)	6.84349	0.001
	C x C	UC x UC	–252.66667(*)	6.84349	0.001
Hatchability	C x UC	UC x UC	12.66667	6.93622	0.234
	UC x C	UC x UC	–358.33333(*)	6.93622	0.001
	C x C	UC x UC	–359.00000(*)	6.93622	0.001

vitripennis (Bordenstein and Werren, 2000; Bordenstein and Werren, 2007). Although prior crossing studies and crosses described suggest a fecundity advantage of infected females, certain limitations exist. A major bottleneck of a symbiont-associated reproductive incompatibility for control of pests is the necessity of employing an efficient release of uninfected females (Ioannidis and Bourtis, 2007).

In summary, *Wolbachia* infection increases female fecundity and elimination induces CI in *E. sorbillans*. A increase in cytoplasmic drive rates in population cage studies that corresponds to increased fecundity associated with *Wolbachia* infection (Kaiser *et al.,* 2010). The results of our experiments are consistent with the expectations for a mutualistic, CI inducing *Wolbachia* infection. However, the mechanism of fecundity enhanced due to *Wolbachia* remains unraveled and may could also be due compensatory evolution in the host. The present findings have broader implications on suppressing the menace of uzi fly on silkworm *Bombyx mori* L. *Wolbachia* is therefore a latent tool for the biological control of insect pests and disease vectors of agriculture, veterinary and medical importance.

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