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Niche heterogeneity associated with *Wolbachia* in the Uzi fly, *Exorista sorbillans* Wiedemann (Diptera: Tachinidae)

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ABSTRACT
The Uzi fly, *Exorista sorbillans* Wiedemann (syn. *E. bombycis* Louis), a tachinid endoparasitoid of silkworm, *Bombyx mori* L. is known to exhibit patterns of geographic variation among populations and suspected to consists of several very closely related cryptic species. The Uzi fly harbours *Wolbachia* which induces cytoplasmic incompatibility, sex ratio distortion, fecundity reduction and abnormalities in oogenesis of uninfected female, irrespective of whether the males are infected or not. In our study we have investigated the effects of ecological factors on *Wolbachia* induced reproductive incompatibility and fecundity. Crossing experiments suggest high impact of ecological variations on induction of cytoplasmic incompatibility and diversification of Uzi fly, *E. sorbillans* among different populations.

Keywords: Climatic variations; population structure and dynamics; *Wolbachia*; bidirectional cytoplasmic incompatibility; *Exorista sorbillans*; silkworm parasitoid; cryptic species.

1. Introduction
The Uzi fly, *Exorista sorbillans* (syn. *E. bombycis* Louis), a tachinid endoparasitoid of silkworm, *Bombyx mori* L. was introduced to South India around thirty years ago through anthropogenic means by way of unauthorized transportation of silkworm seed cocoons from West Bengal to Karnataka [1, 2]. It was originally reported to be rampant in the gangetic plains of West Bengal and other adjacent regions extending up to present day Myanmer [3]. During the last 30 years of its entry into south India, it has a great confused taxonomic history, and is currently denoted as *E. sorbillans* (Wiedemann). It represents important geographic variations and is considered to be polytypic species. Based on morphological and cytological criteria, many cryptic species/ecoraces have been recognized in south India [4, 5]. However, the validity of these cryptic species/ecoraces remains questionable.
The studied populations of Uzi fly *E. sorbillans* are known to harbour *Wolbachia* endobacteria [6-10] with multiple infections [10, 11]. *Wolbachia* are maternally inherited intracellular alpha-proteobacteria inducing an array of reproductive anomalies in arthropod and filarial nematodes [12-14]. In the Uzi fly, *E. sorbillans, Wolbachia* induces cytoplasmic incompatibility (CI), fecundity reduction and sex ratio distortion and also affects oogenesis [8, 10, 15] in uninfected females irrespective of the presence of *Wolbachia* in males. In this paper we have explored the effects of drought on *Wolbachia* induced reproductive anomalies, inturn estimate the population structure and dynamics with respect to the microevolution of Uzi fly, *E. sorbillans.*

2. Materials and Methods
2.1. Experimental Population and rearing technique
The Uzi fly, *E. sorbillans* (Diptera: Tachinidae) develops as a larval endoparasitoid of silkworm, *Bombyx mori* L. The population representing the geographic region 12-14° N and 77-79° E in South India was collected from Bangalore bivoltine silkworm cocoon market every month from June 2002 – May 2004. These seed cocoons were brought from a single area called seed area. The maggots that came out from these cocoons were brought to the Laboratory and maintained in cages [9]. Immediately after emergence, the male and female flies were separated to avoid mating and provided 8% sucrose as food. The flies were maintained at temperature of 25±1 °C with 70-75% relative humidity and 12: 12 Light: Dark conditions.

2.2. Genomic DNA isolation and PCR amplification

The ovaries and testis from Uzi flies were dissected in insect ringer solution and used for extraction of DNA by standard proteinase K and SDS lysis method. The gonads were washed in sterile distilled water and homogenized separately in 600 µl lysis buffer and incubated at 65 ºC for 30 minutes following the addition of 15 µl of proteinase-K. The supernatant was incubated on ice for one hour following the addition of 100 µl of 3M potassium acetate and finally DNA was precipitated with the addition of two volume of ice-cold ethyl alcohol. DNA was quantified in spectrophotometer and stored at −20 ºC.

The PCR assay was used to amplify *Wolbachia* A and B group specific ftsZ primers [11] in an Eppendorf thermocycler. The 25 µl PCR reaction volume contained 2 µl of 1x PCR buffer, 2 µl of 0.2 mM dNTP's, 2 µl of 2.5 mM $MgCl_2$, and 0.5 unit *Taq* DNA polymerase (MBI-Fermentas, USA), 2 µl of 0.1µM of each forward and reverse primer, 2 µl of (20 ng) template DNA and final volume of sterile water to make up 25 µl. The cyclic conditions were initial denaturation at 94 ºC for 5 minutes followed by 36 cycles with denaturation at 94 ºC for 1min, annealing at 55 ºC for 1min, primer extension at 72 ºC for 2 min and final extension at 72 ºC for 10 minute. The quality of extracted DNA was checked through an insect specific 18S rDNA primer pair amplifying a 555 bp of the host [16]. The PCR products were separated through 1.2% agarose gel stained with 0.5 µg/ml gel of ethidium bromide just prior to casting. A standard 10 kb molecular weight marker was used to estimate the molecular weight of amplified band size.

2.3. Crossing experiments

One day after emergence, single male was crossed with single female fly for about twenty four hours, after which thirty, fifth instar silkworm larvae were provided for oviposition. These were changed at a regular interval of twelve hours for five consecutive days. Seven replications were maintained for each cross and observed for fecundity and hatchability [7]. Controls sets of laboratory population [7, 8, 9] with seven replications were also maintained along with month wise experimental crosses. The control set was averaged across two years.

2.4. Data analysis

All the experimental data obtained were pooled and calculated for mean, standard deviation and standard errors using computer program functions (f_x) in MS Excel of Windows 10. The analysis of experimental results for significance at Alpha 0.05 and 0.01 levels was done with single factor ANOVA and multiple comparisons was performed using Tukey's HSD test for comparing difference between means of fecundity and hatchability of different months [17].

3. Results

The PCR results showed that the Uzi fly harbours A and B super group *Wolbachia* (Fig. 1). Further, during experimental period we have received severe drought condition that provided excellent opportunity to study and understand the effect of *Wolbachia* and ecological variation on field populations of *E. sorbillans*. Although we have not detected any uninfected flies during these drought conditions, all sampled individuals were PCR positive for *Wolbachia*. The fecundity and egg hatchability of field populations have exhibited high variation throughout the experimental period (Fig. 2 and 3).

3.1. Fecundity

Significantly high variation in fecundity was observed throughout experimental duration (F=17.95; p=1.6E-16). Least fecundity was recorded in the month of May (235.86±34.08) and a steady increase from June to November (313.71±15.21; 406.57±14.44; 454.86±21.12; 511.57±31.10; 574.71±38.04 and 798±65.82, respectively). Thereafter, fecundity showed a gradual reduction from 695.86±49.34 to 235.86±34.08 from December to May (Fig. 2).

3.2. Egg hatchability

As Uzi fly harbours A and B super group *Wolbachia* we assume that the expressed phenotype was due to bidirectional cytoplasmic incompatibility. We observed huge variation in the percentage of egg hatchability. The average least percentage of hatching was recorded in the month of May followed by June and July (3.69%; 38.39% and 55.38%, respectively). However, in all the other months the hatching rate was recorded between 57 to 80% (Fig. 2 and 3). The minimum CI level was seen in the month of September and highest in the month of May (Fig. 3).

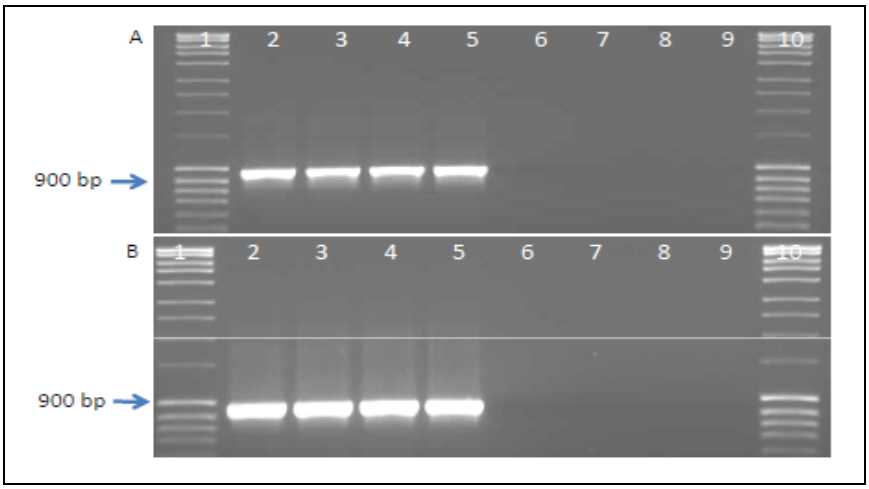

Fig 1: *Wolbachia* specific *ftsZA* (A) and *ftsZB* (B) group specific primer that amplifies at around 950 bp; lanes 1 and 10, molecular weight marker (10 kb ladder MBI-Fermentas, USA), lanes 2 and 3, *Wolbachia* in male Uzi flies, lanes 4 to 5, *Wolbachia* in female Uzi flies

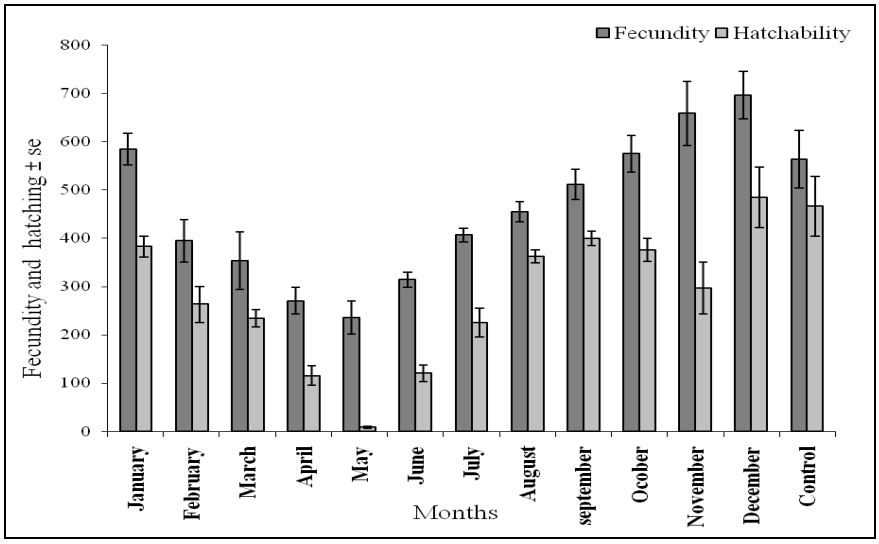

Fig 2: Monthly variation in fecundity and hatchability of Uzi fly from June 2002 to May 2004

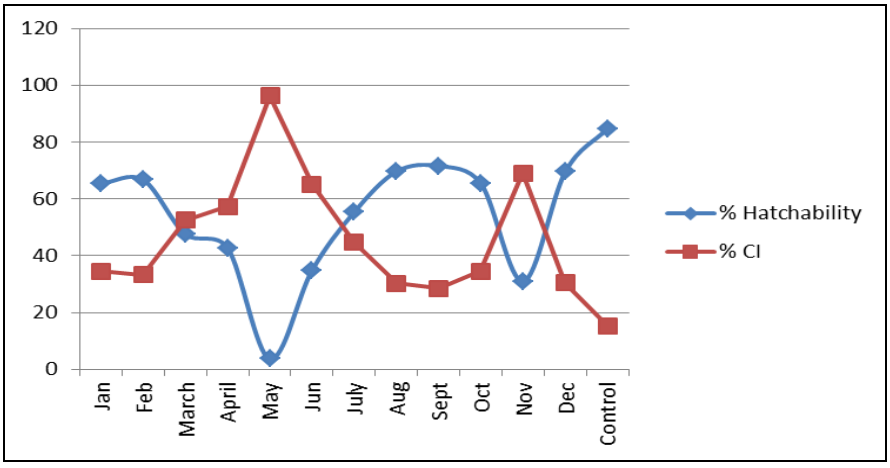

Fig 3: Percentage of Egg hatchability and bidirectional cytoplasmic incompatibility for different months in *E. Sorbillans*

4. Discussion

The results revealed high variation in fecundity and egg hatching rate perhaps due to the prevailing severe drought condition with the recorded atmospheric temperature ranging from 28 to 40 ºC. The observed results deviated from the results obtained by other workers [18, 19] and demonstrate the role played by ecological factors on the *Wolbachia- E. sorbillans* interaction, which induced cytoplasmic incompatibility and decreased fecundity. However, we could not rule out the interaction of the host verses environment on the fecundity and hatchability of eggs in Uzi flies during these conditions.

It seems that climatic variation has great bearing on the expression of *Wolbachia* induced phenotypes (Fig. 2 and 3). The rate of hatching showed tidal variation among different months of the year (Fig. 3). Minimum hatchability was recorded in the month of May and maximum in the month of September. The cyclic nature of fecundity and hatchability in Uzi fly may be due to variation in temperature leading to *Wolbachia* density variation. Variation of *Wolbachia* density was found to be associated with high degree of *mod/resc* (Modification/rescue) factor (Fig. 4) which led to expression of different levels of bidirectional cytoplasmic incompatibility, thus affecting population structure and dynamics of the Uzi fly. In rainy season, the temperature was recorded in the range from 25 to 28 ºC, which might have been congenial for Uzi fly reproduction and also *Wolbachia* multiplication. It could be also due to maternal effects, where in maternal and grand maternal stress condition in previous generations affects offspring performance positively [20]. In line with earlier studies in *Aedes* sp [12, 21] and *Drosophila simulans* [22] high temperature in the environment was observed to promote partial or complete curing of *Wolbachia* in single infected populations, while in double infected species like Uzi fly, *Wolbachia* density might have varied (Fig. 4).

In Fig.4, the model shows five types of bidirectional cytoplasmic incompatibility likely to be operating in Uzi fly. Our hypothesis is that in super infected insect populations, if the density of *Wolbachia* are high in males when compared to females leading to zygotic lethality due to either unidirectional or bidirectional cytoplasmic incompatibility, thus corresponding variation in *mod/resc* factor. The cyclic nature of environmental variation perhaps induces cyclic population dynamics possibly associated with *Wolbachia* in Uzi fly. Yet, we could not dissect out the decreased fecundity of Uzi fly in summer months. This may be due to temperature, which induces some physiological modifications; where more resources are allocated towards maintenance then reproduction, thus lower fecundity and survivorship or *Wolbachia* may eliminated partially or completely during summer months

leading to less fecundity and hatchability [7, 8, 9]. Moreover, our earlier studies indicated that antibiotic curing of *Wolbachia* in Uzi fly leads to production of around 20% less fecundity and more than 70% sterility [7, 15]. This corroborated the role of temperature on egg production in Uzi fly, though we established the involvement of *Wolbachia* in oogenesis, but not known whether A or B group *Wolbachia* have an effect on oogenesis.

Usually, genotype interacts with the environment to give rise to specific phenotype and subsequent fitness. In special cases like Uzi fly, the *Wolbachia* genome also interacts with environment and gives rise to *Wolbachia* specific phenotype. Therefore it is very difficult to say the influence of temperature alone on the induction of reproductive abnormalities in Uzi flies, such as lower fecundity and hatchability. Fig. 5 illustrates the possibility of complex interaction associated with *Wolbachia*, environment and host genotype. The environmental factors affect either individual genotype (*Wolbachia* or host genotype) or both leading to expression of complex phenotype. One would dissect out the effect of environment on *Wolbachia* or host genotype individually, but it could be very difficult to dissect out complex interaction by *Wolbachia*, host and environment. Therefore, the complex interplay among these may lead to diversification of Uzi fly *E. Sorbillans*.

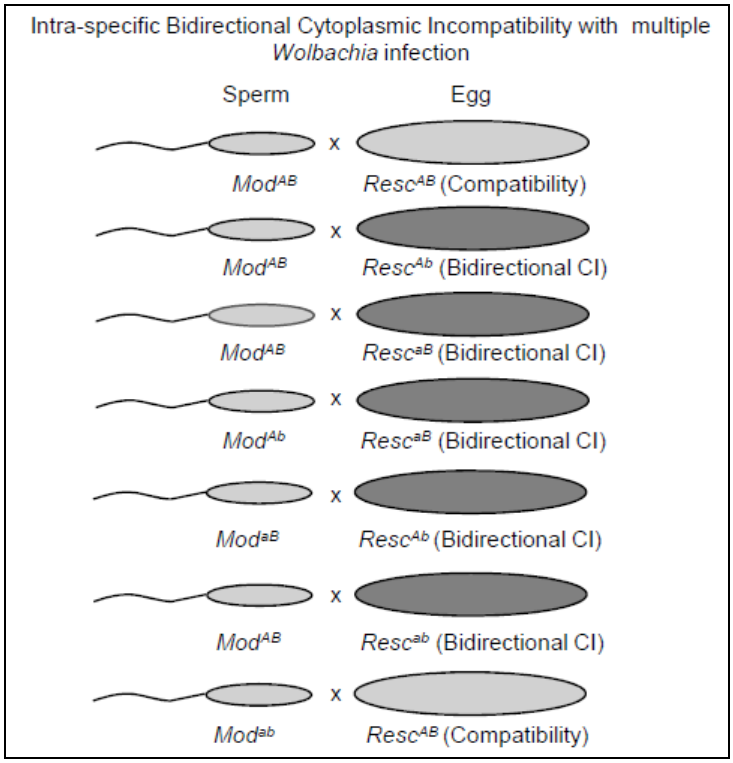

Fig 4: Diagrammatic representation of hypothetical variation on density of *Wolbachia* and variation in Bidirectional Cytoplasmic incompatibility in the Uzi fly, *E. sorbillans*. *Mod*: sperm modification *Wolbachia* in male; *Resc*: sperm modification rescue type in egg. Capital letters (A & B)- higher density of A and B group *Wolbachia*; Small letters (a & b)-lower density of A and B group *Wolbachia*

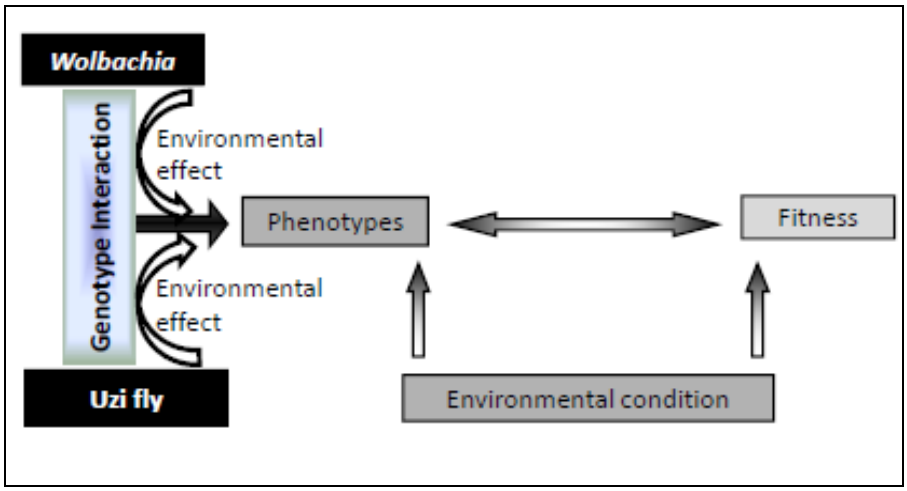

Fig 5: Effect of genotype-cytotype-environmental interaction in Uzi fly with respect to induction of *Wolbachia* specific phenotypes and fitness

5. Conclusion

The *Wolbachia* induce partial bidirectional cytoplasmic incompatibility in Uzi fly in nature. This induces cyclic population dynamics in different seasons of the year due to varied temperature. The varied temperature might have affect interaction of *Wolbachia* and Uzi fly genotype and subsequent phenotype and thus induce diversification.

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7. Reference

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