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Cytoplasmic Introgression and Diversity in *Wolbachia* infected Uzifly *Exorista sorbillans* (Diptera: Tachinidae)

N. M. Guruprasad, H.P.Puttaraju

ABSTRACT
Inherited bacterial symbionts from the genus *Wolbachia* have attracted much attention by virtue of their ability to manipulate the reproduction of their arthropod hosts. The *Wolbachia*-induced reproductive alterations bear greater effect on ecological, evolutionary and genetic aspects of a particular host. In the present investigation, we examined diversity in *E. sorbillans* infected with *Wolbachia*. The DNA finger printing of infected populations clearly showed diversity in *E. sorbillans* populations. We also, noticed that two *E. sorbillans* populations rejected the Null Hypothesis of homogeneity which clearly indicated diversity in populations. In Uzifly, population diversity results from effective migration rate of the Uzifly and crossing with different geographic populations that likely cause cytoplasmic introgression through hybridization events. *Wolbachia* through cytoplasmic introgression perhaps promotes the diversity in *E. sorbillans* populations. Overall, our study assessed the impact of *Wolbachia* on *E. sorbillans* diversity, population structure and dynamics.

Keywords: Uzifly, *Exorista sorbillans*, *Wolbachia*, cytoplasmic introgression, Diversity.

1. Introduction
Insects have been reported to be associated with a broad variety of microorganisms, affecting host biology in many different ways. Among them, *Wolbachia*, an obligate intracellular and maternally-inherited symbiont, has recently attracted a lot of attention. For decades, *Wolbachia* was known only from mosquitoes; the development of PCR-based screening methods clearly indicated that *Wolbachia* is widespread in nature [1-2]. It has been demonstrated that *Wolbachia* infects up to 76% of insect species, a large number of other arthropods including spiders, scorpions, mites, springtails, terrestrial isopods as well as filarial nematodes [3-5]. Several studies have shown that *Wolbachia* is mainly localised in the reproductive tissues of arthropods and is responsible for the induction of a number of reproductive alterations including feminization, thelytokous parthenogenesis, male-killing and Cytoplasmic Incompatibility (CI). These reproductive alterations have larger impact on ecological and evolution of particular *Wolbachia* infected-insect host [6-7].
Cytoplasmic incompatibility (CI), the most common effect on host reproduction, usually occurs between infected males and uninfected females (or females infected by a different incompatible *Wolbachia* strain), inducing progeny sterility or mortality [8]. CI is a sperm-egg incompatibility, which occurs in crosses between males and females with differing *Wolbachia* infection status. It can be either unidirectional when infected males mate with uninfected females, or bidirectional in crosses where both males and females are infected with different CI- inducing strains [9]. The modification or rescue model has been proposed, which assumes the existence of two distinct bacterial functions [10]: (a) the modification function expressed in the male germ line during spermatogenesis and (b) the rescue function expressed in the egg. At karyogamy, the modified paternal chromosomes are improperly condensed and lost during early embryo development, unless the egg expresses the rescue function corresponding to the *Wolbachia* strain present in the male [11]. Till date the Lock and Key model holds a good and satisfactory assumption for mod/res model. The Lock (modification factor in sperm) and Key (rescue factor in egg) involved in CI. The model involved in CI, the mod factor in males sperm by *Wolbachia* results in zygotic death unless it is rescued by resc factor in egg by *Wolbachia* of female [12]. This post-zygotic reproductive isolation can potentially cause or facilitate host speciation [13].

Cytoplasmic incompatibility has attracted attention as a possible ongoing mechanism of cytoplasmic introgression through hybridization events across the populations [14]. The cytoplasmic introgression through sibling taxa impact the global nuclear gene flow between populations serves as a powerful mechanism for penetration of useful alleles to permit divergence and eventual speciation [15-16]. The discovery that cytoplasmic introgression is caused by a widespread bacteria *Wolbachia* has revitalised interest in its potential role in speciation. Empirical studies are accumulating that are consistent with a possible role of *Wolbachia* as a major isolating mechanism in some recent speciation events and growing evidence that many insect species harbor different strains of *Wolbachia*, often in different geographic populations [17-18].

The Uzifly *Exorista sorbillans* (Diptera: Tachinidae) is a parasitoid reported to be a great menace to silkworm *Bombyx mori* L. (Lepidoptera: Bombycidae), which alone causes 15 to 20 % yield loss to sericulture industries in India [19]. It is known that this parasitoid harbours a *Wolbachia* endosymbiont and induces reproductive fitness effects in Uzifly populations [20-21]. Here we present the study carried out to explore the extensive level of polymorphism and population structure dynamics in the Uzifly population of different geographic regions. We made an attempt to infer the input of *Wolbachia*-induced cytoplasmic introgression in shaping population structure and diversity of *E. sorbillans.*

2. Materials and Methods

2.1 Collection and rearing of the *E. sorbillans*

The post-parasitic maggots of *E. sorbillans* were collected from silkworm cocoon markets in four states from South-East India: Karnataka, Andhra Pradesh, Tamil Nadu and from CSTRI Berhampur in West Bengal (Table-1, Figure-1), soon after their emergence from infested host silkworm cocoon. The maggots were brought to the laboratory in a perforated plastic container containing sand and allowed to metamorphose into pupae and then to adult in the wire mesh netted cages of 35 x 35 x 35 cubic cm [22]. In order to ensure virginity, male and female adult flies were separated based on genitalia and other morphological characters. Some of these flies were frozen at -80 ^{0}C or preserved in 70% ethanol until further use for DNA isolation for the RAPD assay. The remaining flies collected from different agroclimatic regions were reared by feeding with 8% glucose in distilled water soaked in cotton balls and maintained as follows until the crossing experiments.

Table 1: Populations from South-East India used in the study.

State	Sl. No. Marked in the Map	Locality	Acronym	Latitude	Longitude	Temp. (°C)
Karnataka	1	Bellary	KABE	15.22087	76.92446	23
	2	Chitradurga	KAC	14.27406	76.35224	28
	3	Sira	KASI	13.76413	76.88006	26
	4	Bagepalli	KABA	13.78166	77.93951	21
	5	Sidlaghatta	KAS	13.45153	77.89894	22
	6	Doddaballapur	KAD	13.33347	77.47451	21
	7	Kolar	KAKOL	13.16914	78.10728	27
	8	Hoskote	KAH	13.09230	77.85191	26
	9	Hassan	KAHA	13.00574	76.13774	27
	10	Kunigal	KAK	12.93899	77.00514	22
	11	Ramanagaram	KAR	12.75258	77.32424	25
	12	Chnnapatna	KACH	12.62827	77.18240	24
	13	Mandya	KAM	12.59679	76.86142	28
	14	Pandavapura	KAP	12.54838	76.64682	26
	15	Kollegal	KAKO	12.06077	77.38182	25
Tamil Nadu	18	Eroad	TNE	11.51964	78.20341	32
Andhra Pradesh	16	Chittoor	APC	13.46846	79.04593	31
	17	Kurnool	APK	15.53118	78.02709	29
	19	Hindupur	APH	13.90516	77.56629	29
West Bengal	20	Beharampur	WB	24.16013	88.25342	27

Temp. : mean temperature of each locality (±2 °C).

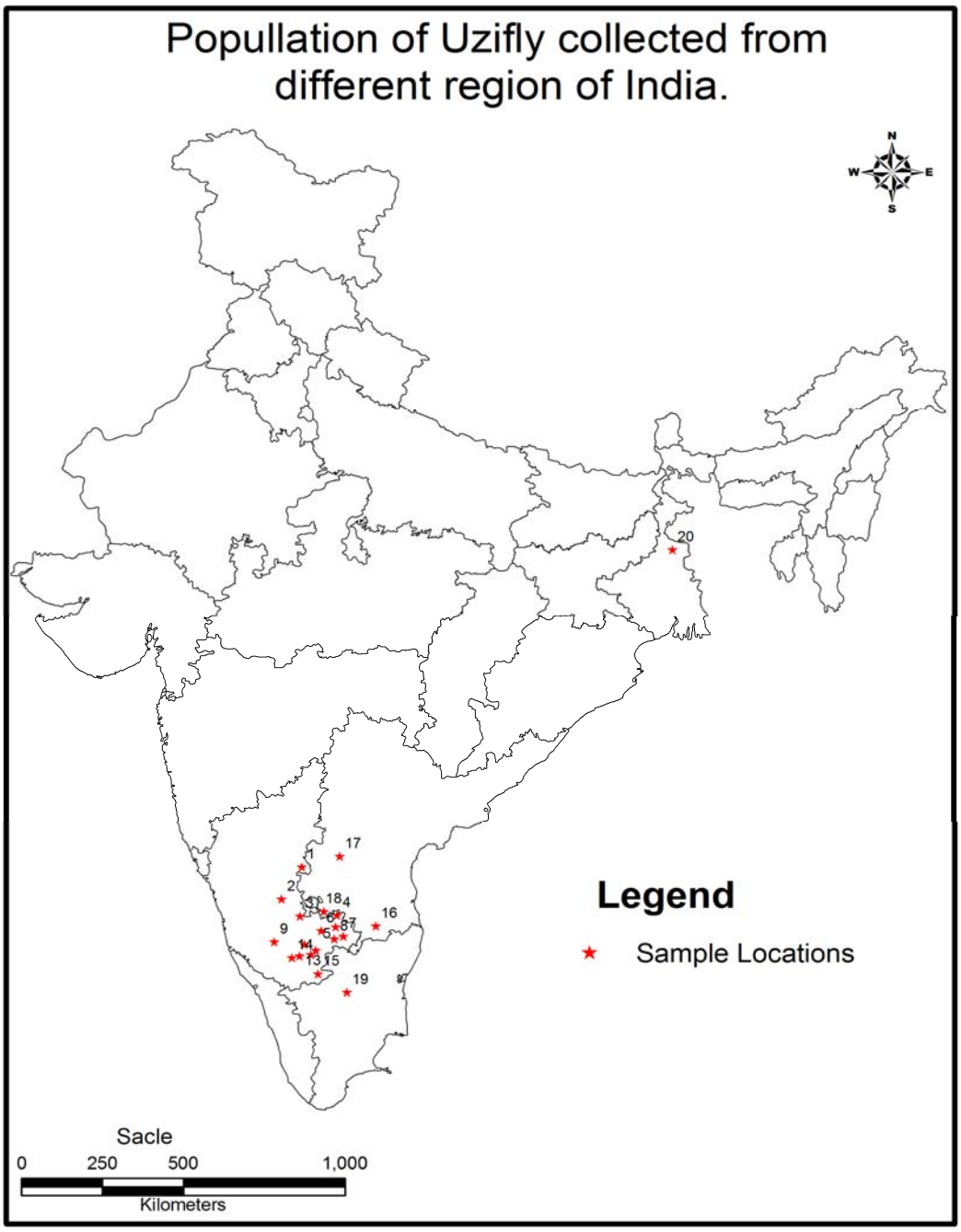

Fig 1: India map showing the locations of sample collected

20 populations were sampled from 4 states in East and South India, West Bengal, Andhra Pradesh, Karnataka and Tamil Nadu (SL No-name of place indicated in Table-1).

Early fifth instar larvae of silkworm, *Bombyx mori*, the hosts of the Uzifly, were placed in the cages to stimulate oviposition (Uzi flies prefer to lay their eggs on the body of silkworm) [22]. The silkworm larvae, which have Uzi eggs on their body, were taken out from cages and reared in the laboratory following the standard silkworm rearing technique [23]. The Uzi eggs which hatched, bored into the silkworm body, developed as endoparasitic maggots and then pierced out of the host body, were further collected and maintained in separate cages.

2.2 RAPD assay

The genomic DNA of the Uzi flies was extracted as described in Sambrook *et al.*, [24]. A set of 7 decamer random primers of OPT and OPR series (Operon Technologies, USA) was used for the DNA finger printing RAPD assay (Table 2). The PCR amplification was carried out in a total reaction mixture of 20 µL containing 15ng of the primer, 0.1 mM dNTPs, 1X Taq polymerase buffer, 1 unit *Taq* DNA polymerase (Biorad) and 40 ng of template DNA. The PCR reactions were conducted in an Eppendorf thermal cycler (Eppendorf) with the following conditions: an initial denaturation at 94 °C for 5 min followed by 35 cycles at 94 °C for 1 min, annealing at 36 °C for 1 min and extension at 72 °C for 2 min, and a final extension cycle of 10 min at 72 °C. RAPD products were analysed by electrophoresis on a 1.4% agarose gel in 1X TAE buffer and visualised by ethidium bromide staining using the Alpha Digi Doc gel documentation system.

2.3 Molecular analysis of RAPD data

The RAPD amplification products were scored as “1” or “0” for

presence or absence of the bands for each primer and the binary data were used for statistical analysis with the computer package "STATISTICA". The distance between populations was computed using Squared Euclidian Distance (SED) that estimated the genetic distances. A dendrogram was constructed by Unweighted Pair Group with Arithmetic Mean (UPGMA) method.

2.4 Crossing experiments

Two types of experimental crosses found realised: crosses between populations from the state of Karnataka, and crosses between populations collected in the four different states (Figure-1, Table-1). All crosses were set up as single pair mating between two-day old flies with a ratio of 1:1 males and females. These flies remained together for 24 h. For each cross, 20 to 24 replicates were done three times, and the fecundity (number of eggs laid), the hatching rate (proportion of adult emerging) and the sex-ratio (proportion of males in the offspring) were determined.

2.5 Statistical analysis

The fecundity, hatching rate and sex ratio were analyzed using the R statistical software (R-DCT 2006. http://www.R-project.org). Analyses were done after arcsine square root transformation for the hatching rate. Data normality and homoscedasticity were verified by Shapiro and Bartlett tests respectively. One-way ANOVA was done for multiple comparisons and pair wise student's t-tests were used for two- by- two comparisons (probability level of significance: 0.05).

Table 2: Nucleotide sequences of the 10-mers RAPD primers used in the present study and percentage of polymorphism in the sample

Primer Code	Primer sequence	Polymorphic bands	Polymorphism (%)
OPT-02	GGAGAGACTC	80	66
OPT-03	TCCACTCCTG	120	60
OPT-04	CACAGAGGGA	120	60
OPT-05	GGGTTTGGCA	80	57
OPT-06	CAAGGGCAGA	160	88
OPR-01	TGCGGGTCCT	60	60
OPR-02	CACAGCTGCC	80	80

Table 3: Squared Euclidean Distance of the 20 Uzi fly populations used in the study
Populations are named by the acronym of their originated locality (see Table 1).

State	Population	KAM	KAC	KAHA	KAKO	KACH	KAP	KAKOL	KASI	KAH	KAR	KABE	KAS	KABA	KAD	KAK	TNE	APC	APK	APH	WB
Karnataka	KAM	00	09	11	14	15	20	15	12	20	16	12	15	17	15	15	16	17	15	19	20
	KAC	9	00	12	13	12	17	10	13	15	13	13	16	14	10	16	15	16	12	16	15
	KAHA	11	12	00	19	20	21	16	11	19	13	13	16	18	16	20	15	16	14	16	15
	KAKO	14	13	19	00	11	12	15	8	16	18	12	9	9	15	5	12	13	11	9	16
	KACH	15	12	20	11	00	13	8	15	13	15	11	10	16	12	14	11	10	8	12	17
	KAP	20	17	21	12	13	00	15	14	12	24	14	13	15	15	11	18	17	17	17	16
	KAKOL	15	10	16	15	8	15	00	17	13	15	15	14	12	10	14	15	14	14	16	15
	KASI	12	13	11	8	15	14	17	00	20	16	8	11	15	15	9	14	11	11	11	14
	KAH	20	15	19	16	13	12	13	20	00	14	16	17	15	13	17	10	13	13	13	22
	KAR	16	13	13	18	15	24	15	16	14	00	14	15	15	13	19	10	15	11	15	18
	KABE	12	13	13	12	11	14	15	8	16	14	00	5	15	13	13	12	11	9	13	14
	KAS	15	16	16	9	10	13	14	11	17	15	5	00	10	12	10	11	12	8	10	13
	KABA	17	14	18	9	16	15	12	15	15	15	15	10	00	16	8	11	14	14	10	15
	KAD	15	10	16	15	12	15	10	15	13	13	13	12	16	00	16	11	14	10	16	13
	KAK	15	16	20	5	14	11	14	9	17	19	13	10	8	16	00	15	14	14	12	13
Tamil Nadu	TNE	16	15	15	12	11	18	15	14	10	10	12	11	11	11	15	00	7	5	7	18
Andhra Pradesh	APC	17	16	16	13	10	17	14	11	13	15	11	12	14	14	14	7	00	4	6	15
	APK	15	12	14	11	8	17	14	11	13	11	9	8	14	10	14	5	4	00	6	15
	APH	19	16	16	9	12	17	16	11	13	15	13	10	10	16	12	7	6	6	00	17
West Bengal	WB	20	15	15	16	17	16	15	14	22	18	14	13	15	13	13	18	15	15	17	00

3. Results and Discussion

3.1 Diversity revealed by RAPD in *E. sorbillans* populations

We examined for the diversity of *Wolbachia*-infected twenty specimens of *E. sorbillans* natural populations collected from different geographic regions of India. RAPD finger printing assays indicated the occurrence of diversity in all the examined twenty specimens. Taking into account the PCR-RAPD, in all seven primers we revealed that 35 horizontal loci, out of which 24 horizontal loci were polymorphic and 11 horizontal loci were monomorphic (monomorphic bands are present in all individuals, polymorphic ones are present in one or more but not in all individuals). All the seven primers detected variations with a total number of bands ranging from 60 (OPR-01) to 160 (OPT-06) with an average of about 100 bands per primer (Table-2). The primer OPT-06 was the most informative since it exhibited 88% polymorphism in RAPD banding patterns.

The Squared Euclidean Distance of the 20 populations showed a spatial variation range from 0.24 to 0.4 (Table-3). Here we found an important spatial variation in diversity of populations over wide range of geographic regions. The dendrogram generated based on UPGMA analysis divided the *E. sorbillans* genotypes into four distinct clusters and sub-clusters (Figure-2). The phylogenetic grouping of populations was clustered according to their originated collection site and geographic region. The sub-populations of Karnataka were closely related and form sub-clustered, and the Andhra Pradesh populations (APC, APK and APH) cluster together with the Tamil Nadu population. The population from the West Bengal (WB) formed a separate lineage. The pair wise multiple comparisons test revealed significant difference between WB population and two populations from Karnataka, KASI and KAM ($p<0.05$).This analysis clearly revealed that KASI and KAM populations rejected Null hypothesis of homogeneity and significantly indicated heterogeneity in *E. sorbillans* populations (Table-4).

Table 4: Result of pair wise multiple comparison between profile of WB and others, differentiating WB from other 19 Uzifly populations.

S. No.	Pairs	Mean	SD	SE	t	p- value
01	KAM - WB	-.28571	.71007	.12002	-2.380	.023
02	KAC - WB	-.08571	.65849	.11131	-.770	.447
03	KAHA - WB	-.20000	.63246	.10690	-1.871	.070
04	KAKO –WB	-.17143	.66358	.11217	-1.528	.136
05	KACH - WB	.08571	.70174	.11862	.723	.475
06	KAP- WB	-.11429	.67612	.11429	-1.000	.324
07	KAKOL - WB	.02857	.66358	.11217	.255	.800
08	KASI- WB	-.22857	.59832	.10113	-2.260	.030
09	KAH - WB	.05714	.80231	.13561	.421	.676
10	KAR - WB	.00000	.72761	.12299	.000	1.000
11	KABE - WB	-.05714	.63906	.10802	-.529	.600
12	KAS - WB	-.02857	.61767	.10440	-.274	.786
13	KABA - WB	-.08571	.65849	.11131	-.770	.447
14	KAD- WB	.08571	.61220	.10348	.828	.413
15	KAK- WB	-.20000	.58410	.09873	-2.026	.051
16	TNE- WB	.05714	.72529	.12260	.466	.644
17	APC – WB	.02857	.66358	.11217	.255	.800
18	APK – WB	.08571	.65849	.11131	.770	.447
19	APH- WB	-.02857	.70651	.11942	-.239	.812

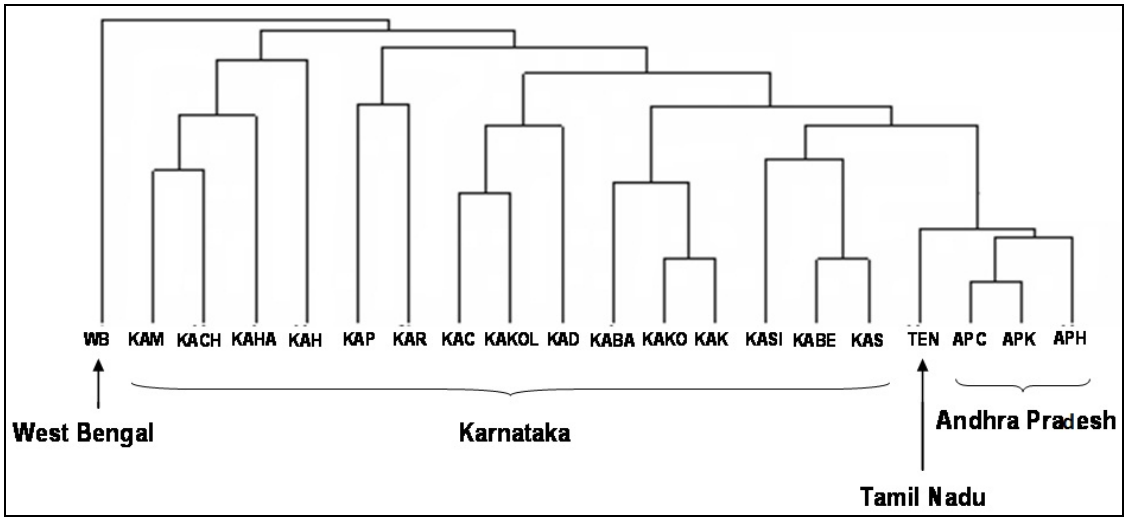

Fig 2: Dendrogram of 20 populations of the uzifly *Exorista sorbillans* based on 7 RAPD primers constructed according to UPGMA cluster analysis.

Populations are named by the acronym of their originated locality (see Table 1). The corresponding states are indicated in bold capital letters.

3.2 Crossing and cytoplasmic introgression in *E. sorbillans*

Cytoplasmic introgression experiments performed by crossing individuals from Uzifly populations collected from different geographic regions revealed that when infected males and females from the same population were crossed a very low level of cytoplasmic introgression was observed. However, when infected males and females from different geographic populations were crossed, a significant rate of reduction in progeny number was registered (Table-5).

Table 5: Results of the crossing experiments

5A. Crosses between populations from the state Karnataka; 5B. crosses between populations collected in the 4 different states. Mean values ± SE and statistical analyses of fecundity (number of eggs laid) and hatching rate (percentage of egg hatched) are shown (one-way ANOVA and pairwise student-t-tests). Analyses of the hatching rate were carried out after arcsine square transformation. The means marked with the same letter are not significantly different (probability level of significance: p=0.05). n indicate the number of pairs tested.

Table-5A.

Male	Female	n	Fecundity ± SE	Hatching ± SE (%)
Ramanagaram	Ramanagaram	24	372.3 ± 6.5 (a)	82.1 ± 0.7 (a)
Ramanagaram	Sidlaghatta	23	345.3 ± 3.8 (b)	65.5 ± 1.4 (b)
Sidlaghatta	Ramanagaram	24	346.0 ± 2.6 (b)	67.7 ± 0.4 (b)
Sidlaghatta	Sidlaghatta	24	363.3 ± 6.2 (a,b)	80.7 ± 0.2 (a)
ANOVA			**p=0.012**	**p<0.0001**
Ramanagaram	Ramanagaram	24	367.3 ± 4.1 (a)	82.1 ± 1.5 (a)
Ramanagaram	Sira	23	336.3 ± 3.3 (b)	66.4 ± 0.2 (b)
Sira	Ramanagaram	24	338.0 ± 1.5 (b)	65.6 ± 1.1 (b)
Sira	Sira	24	369.7 ± 3.7 (a)	78.5 ± 1.4 (a)
ANOVA			**p=0.0001**	**p<0.0001**
Ramanagaram	Ramanagaram	24	378.3 ± 10.4 (a)	83.4 ± 2.6 (a)
Ramanagaram	Mandya	22	340.3 ± 4.0 (b)	66.8 ± 1.6 (b)
Mandya	Ramanagaram	23	326.7 ± 2.8 (b)	63.9 ± 0.8 (b)
Mandya	Mandya	23	395.7 ± 2.3 (a)	81.0 ± 0.4 (a)
ANOVA			**p=0.0001**	**p<0.0001**
Ramanagaram	Ramanagaram	24	374.7 ± 3.2 (a)	78.4 ± 3.5 (a)
Ramanagaram	Hassan	24	337.3 ± 3.4 (b)	67.0 ± 1.7 (a,b)
Hassan	Ramanagaram	24	339.0 ± 2.8 (b)	65.9 ± 0.5 (b)
Hassan	Hassan	24	359.0 ± 9.0 (a,b)	76.9 ± 3.1 (a,b)
ANOVA			**p=0.0030**	**p=0.0180**

Table-5B.

Male	Female	n	Fecundity ± SE	Hatching ± SE (%)
Tamil Nadu	Tamil Nadu	23	417.6 ± 15.7 (a)	92.0 ± 2.2 (a)
Karnataka	Tamil Nadu	21	263.3 ± 4.8 (b)	51.5± 0.8 (b)
Tamil Nadu	Karnataka	22	292.3 ± 5.6 (b)	47.1 ± 0.9 (b)
Karnataka	Karnataka	24	427.9 ± 5.3 (a)	84.2 ± 4.7 (a)
ANOVA			**p<0.0001**	**p<0.0001**
Andhra Pradesh	Andhra Pradesh	24	357.2 ± 27.5 (a)	88.0 ± 2.9 (a)
Andhra Pradesh	Karnataka	23	211.5 ± 11.8 (b)	69.4 ± 5.3 (b)
Karnataka	Andhra Pradesh	23	215.6 ± 16.7 (b)	64.3 ± 4.4 (b)
Karnataka	Karnataka	24	363.0 ± 30.5 (a)	88.4 ± 2.4 (c)
ANOVA			**p=0.0016**	**P=0.0034**
Karnataka	Karnataka	24	369.5 ± 2.5 (a)	93.1 ± 2.3 (a)
Karnataka	West Bengal	23	216.4 ± 1.5 (b)	56.3 ± 1.2 (b)
West Bengal	Karnataka	21	219.2 ± 3.9 (b)	54.1 ± 1.7 (b)
West Bengal	West Bengal	23	337.8 ± 5.6 (c)	95.9 ± 0.6 (c)
ANOVA			**p<0.0001**	**p<0.0001**
Andhra Pradesh	Andhra Pradesh	24	363.9 ± 7.4 (a)	94.2 ± 2.8 (a)
Andhra Pradesh	West Bengal	23	212.0 ± 8.1 (b)	53.7 ± 4.7 (b)
West Bengal	Andhra Pradesh	20	216.4 ± 8.7 (b)	55.1 ± 1.2 (b)
West Bengal	West Bengal	24	354.6 ± 11.0 (a)	94.8 ± 1.7 (c)
ANOVA			**p<0.0001**	**p<0.0001**
Tamil Nadu	Tamil Nadu	23	382.0 ± 10.6 (a)	92.8 ± 2.2 (a)
Tamil Nadu	West Bengal	23	213.8 ± 10.0 (b)	56.4 ± 1.3 (b)
West Bengal	Tamil Nadu	22	214.1 ± 9.5 (b)	52.4 ± 0.1 (b)
West Bengal	West Bengal	23	358.2 ± 12.1 (a)	89.6 ± 2.0 (c)
ANOVA			**p<0.0001**	**p<0.0001**

We have investigated crosses that involved *Wolbachia*-infected *E. sorbillans* males and females of the Karnataka state populations (Ramanagaram, Sidlaghatta, Sira, Mandya and Hassan) and four different states (Karnataka, Andhra Pradesh, Tamil Nadu and West Bengal) populations. The results are significantly different among four state populations and among the Karnataka populations. The one-way ANOVA and pair wise student-t-test results revealed the difference in egg laying and hatching percentage. Results revealed that Karnataka state populations' females lay between 326 to 346 with lower hatching percentage rate between 63.9 to 67.7 ($P<0.0001$) which is significantly lower than the crosses of same population with females laying 378 eggs with higher hatching percentage rate of 83.4 (Table- 5A). On the other hand, results are significantly more different among the crosses of four different state populations where females lay between 211 to 292 eggs with lower hatching percentage rate between 47.1 to 69.4 ($P<0.0001$) when compared to same state populations females laying 417 eggs with higher hatching percentage of 92 (Table-5B). The sex-ratio was not significantly different in all the crosses with a value ranging from 50 to 52% (data not shown).

3.3 Diversity in *E. sorbillans* populations

The investigation clearly (Figur-2) revealed variability in *E. sorbillans* populations. The distinctness of the WB population clustering separate further strengthens the connection that this population has diverged populations from the southern states. Randomly Amplified Polymorphic DNA amplification results obtained were highly polymorphic, resulting in high variability in each individual population as observed in the OPT-06 Uzifly populations and displaying maximum polymorphic bands (Figur-3).

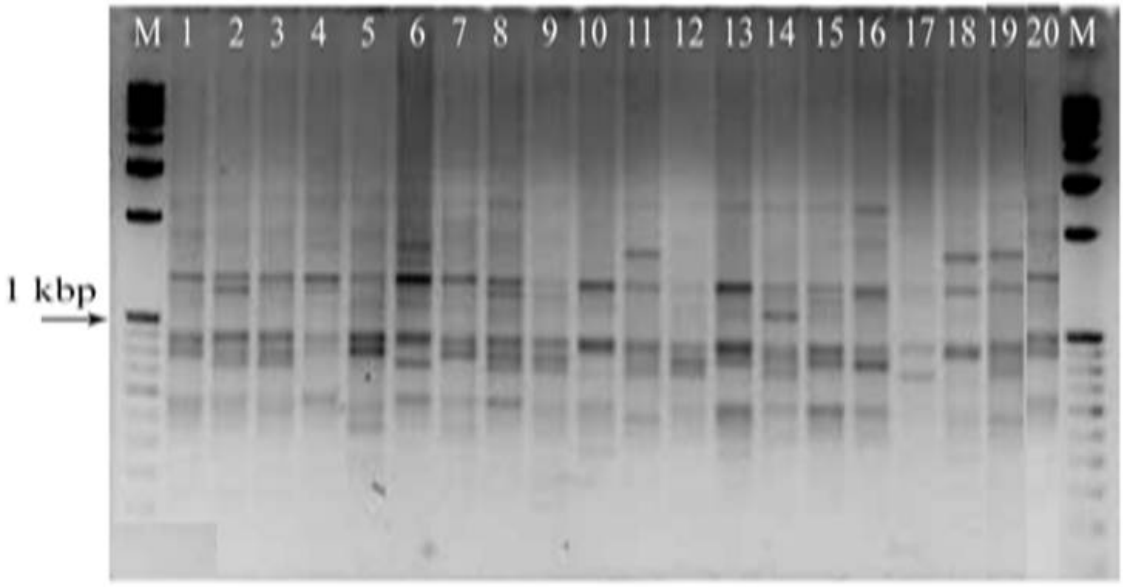

Fig 3: Randomly Amplified Polymorphic DNA (RAPD) finger printing of Uzi fly populations (Table-1) using OPT-06 primer

The seven RAPD primers used in the study revealed a clear clustering among individuals from the twenty Uzifly populations that were analysed. The West Bengal population was clustered separately and it clearly separated from the Uzifly populations of Karnataka, Andhra Pradesh and Tamil Nadu (Figur-2). This could be due to two distinct geographical origins between the West Bengal populations and South Indian populations [25]. Clear separation between clusters of the south Indian populations was also observed by forming three separate well-defined clusters among them. This clustering clearly indicates infusion of gene pool from West Bengal Uzifly populations to south Indian populations. It is a known fact that these Uzifly populations were transported to Karnataka from West Bengal along with the silkworm seed cocoons and then spread to all the sericultural areas of south India [19]. In addition, the availability of host silkworm crop grown around the year may cause Uzifly to migrate for shorter distances and crossing with the distantly related populations leads to the gene flow with the population in turn resulting in diversity within the populations [26-27].

Based on the geographic region and physical map it could be postulated that the Uzifly migrated from West Bengal to south India. However, the geo-climatic conditions play a key role in the diversity of the populations. It could have occurred under the influence of different agro- climatic conditions prevailing in different geographic regions. The results obtained through RAPD analysis suggest that the wide range of diversity is varying with the different geographic origin populations. Nevertheless, future studies on DNA barcoding may detect the exact extent of genetic diversity and microevolution coupled with migration events between the Uzifly populations.

The present investigation revealed a significant outcome that the two Uzifly populations rejected the Null hypothesis of homogeneity. The genotyping clearly indicated distribution of Uzifly populations which appears to be spatially structured and well-exemplified by Null hypothesis. Remarkably, present investigations have confirmed that Null hypothesis was rejected by the two Karnataka populations namely KASI ($P<0.03$) and KAM ($P<0.02$). One should expect that divergence in Uzifly populations may be due to effective migration rate and crossing with different populations of various geographic region. The persistence of *Wolbachia* infection may cause cytoplasmic introgression which has a large effect on divergence in *E. sorbillans* [27].

Moreover, Uzifly population structure vary in different seasons linked to temperature variations a fact that possibly reduces *Wolbachia* density [21, 28]. Density variation may be caused by cytoplasmic introgression in the Uzifly populations. It might be cytoplasmic introgression linked to hybridization events in *E. sorbillans*. It is thus a result of cytoplasmic introgression that hybridization distribution overlaps through sibling taxa. Hybridization can have impact on the host nuclear gene flow

between populations and so permit divergence between the populations, which could enhance the probability of speciation [29-30]. It remains to be seen whether cytoplasmic introgression has impact on host nuclear gene flow between populations to permit divergence and eventual speciation. The discovery that cytoplasmic introgression is caused by a widespread group of *Wolbachia* bacteria has revitalised interests for further research thus enhancing its potential role in microevolution and speciation [31-32].

3.4 Cytoplasmic introgression and its effect on divergence in *E. sorbillans*

These observations exhibit a variability in cytoplasmic introgression effects associated with *Wolbachia* infection in Uzifly populations. The concept of effective migration and crossing with different geographic region population allows the impact of cytoplasmic introgression on host gene flow. Migration of *E. sorbillans* populations takes place by transportation of Uzifly infested cocoons from neighbouring states of Andhra Pradesh and Tamil Nadu farmers who sell in Asia's largest cocoon markets Sidlaghatta and Mandya of Karnataka [25]. It is obvious that infusion of different geographic Uzifly populations and variation in *Wolbachia* density may have induced cytoplasmic introgression, which creates more genetic variability in *E. sorbillans* population. Further, growing of silkworm continuously in Uzifly infested region provides a host to complete several generations in a year. This would have facilitated the more rapid vertical transmission of *Wolbachia* infection and paved the way for faster rate of diversity [25].

The influence of environmental factors prevailing in different geographic regions can also be indirectly acting on the density of *Wolbachia* in *E. sorbillans*. It was shown that variation in *Wolbachia* density in host populations may cause the cytoplasmic introgression and reduce the gene flow between populations [33]. Cytoplasmic introgression coupled with genetic incompatibilities in the other crossing direction (e. g., premating discrimination) could be an important isolating mechanism in some mushroom feeding *Drosophila* [34]. The different geographic populations forms combinations of cytoplasmic introgression and nuclear incompatibility that will permit continued divergence and maintain stability of *Wolbachia* differences between (or closely related species) populations [35-40]. In Uzifly population the divergence may be due to effective migration rate of the fly and crossing with different geographic population that causes cytoplasmic introgression which can have large effect on divergence in the host. It is now clear that cytoplasmic introgression impact on nuclear gene flow has a significant effect on divergence between and within the different geographic populations of *E. sorbillans*.

4. Conclusion

Our analysis is now clear that *Wolbachia* is having a significant impact on diversity and population structure of *E. sorbillans*. *Wolbachia* acts through a potential reproductive manipulation while cytoplasmic introgression plays a vital role in divergence between the Uzifly populations. Overall, our study suggests that cytoplasmic introgression in different geographic region populations associated with hybridization event shapes the diversity and population structure with in the Uzifly *E. sorbillans*.

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