**Wolbachia-Induced Cytoplasmic Incompatibility Is Associated with Decreased Hira Expression in Male Drosophila**

Ya Zheng*, Pan-Pan Ren*, Jia-Lin Wang, Yu-Feng Wang*

Hubei Key Laboratory of Genetic Regulation and Integrative Biology, College of Life Science, Huazhong Normal University, Wuhan, People’s Republic of China

**Abstract**

**Background:** Wolbachia are obligate endosymbiotic bacteria that infect numerous species of arthropods and nematodes. Wolbachia can induce several reproductive phenotypes in their insect hosts including feminization, male-killing, parthenogenesis and cytoplasmic incompatibility (CI). CI is the most common phenotype and occurs when Wolbachia-infected males mate with uninfected females resulting in no or very low numbers of viable offspring. However, matings between males and females infected with the same strain of Wolbachia result in viable progeny. Despite substantial scientific effort, the molecular mechanisms underlying CI are currently unknown.

**Methodology/Principal Findings:** Gene expression studies were undertaken in *Drosophila melanogaster* and *D. simulans* which display differential levels of CI using quantitative RT-PCR. We show that *Hira* expression is correlated with the induction of CI and occurs in a sex-specific manner. *Hira* expression is significantly lower in males which induce strong CI when compared to males inducing no CI or Wolbachia-uninfected males. A reduction in *Hira* expression is also observed in 1-day-old males that induce stronger CI compared to 5-day-old males that induce weak or no CI. In addition, *Hira* mutated *D. melanogaster* males mated to uninfected females result in significantly decreased hatch rates comparing with uninfected crosses. Interestingly, *wMel*-infected females may rescue the hatch rates. An obvious CI phenotype with chromatin bridges are observed in the early embryo resulting from *Hira* mutant fertilization, which strongly mimics the defects associated with CI.

**Conclusions/Significance:** Our results suggest Wolbachia-induced CI in *Drosophila* occurs due to a reduction in *Hira* expression in Wolbachia-infected males leading to detrimental effects on sperm fertility resulting in embryo lethality. These results may help determine the underlying mechanism of CI and provide further insight into the important role *Hira* plays in the interaction of Wolbachia and its insect host.

**Introduction**

Wolbachia are endosymbiotic bacteria that infect many species of arthropods and filarial nematode [1,2]. The successful spread of *Wolbachia* can be partially attributed to their powerful ability to alter host reproduction by mechanisms such as cytoplasmic incompatibility (CI), parthenogenesis, male killing, and feminization. CI is the most common phenotype, which is expressed as embryonic lethality when *Wolbachia*-infected males mate with uninfected females or with females infected with a different *Wolbachia* strain [3].

Although the molecular mechanism of CI has not been elucidated, several studies suggest that sperm is modified by *Wolbachia* during spermatogenesis. This modification prevents the paternal chromosomes from entering the anaphase of the first division, thus leading to a defect in embryogenesis except that the same *Wolbachia* strain is present in the egg and rescue of CI occurs resulting in hatched embryos [3-5]. Studies of *Wolbachia* during spermatogenesis in *Drosophila* have led to a Wolbachia-Infected spermatocyte/spermatid hypothesis, which suggests that all CI-expressing strains have *Wolbachia*-infected spermatocytes/spermatids (WISS+cysts) [6]. However, subsequent work in other insect hosts suggests there is little correlation between *Wolbachia* density in spermatocytes/spermatid and the strength of CI. For example, in the parasitic wasp *Nasonia vitripennis*, *Wolbachia* are found only in around 25% of developing sperm, but induce almost complete CI with nearly 100% embryo mortality [7,8]. This is probably because *Wolbachia* may produce an unknown diffusible CI-inducing factor that can spread from infected to uninfected cells throughout the testis [7]. When the sperm derived from *Wolbachia*-infected males fertilizes uninfected eggs, the first mitotic division in the embryos is severely disrupted. As a result, when the fertilized egg proceeds to anaphase, paternal chromosomes either fail to segregate or appear as extensive chromosome bridging and

---

*E-mail: yfengw@mail.ccnu.edu.cn*

*These authors contributed equally to this work.*
fragmentation during segregation, indicating damaged or incompletely replicated chromosomes [9,10]. Tram and Sullivan observed that a delay in nuclear envelope breakdown and activation of cyclin-dependent kinase 1 (cdk1) in the male pronuclei occurred relative to that in the female pronuclei in *Nasonia* [11]. This delay is thought to slow down chromosome condensation in male pronuclei as cdk1 activation is required to drive chromosome condensation [12]. Recently, Landmann et al. found that CI delayed deposition of histone H3.3/H4 complex in the male pronucleus, which could be the cause of the chromosome defects present during the first mitotic division in CI embryos [13].

HIR/HIRA, a chaperone of histone H3.3, was first identified in yeast as a negative regulator of histone gene expression [14]. It contains a conserved family of proteins found in various organisms including *Drosophila*, *Xenopus*, mice and human and plays an essential role in development [15]. In *Drosophila*, a point mutation of *Hira* gene (*HiraΔ18*, originally called *swarm* gene) causes female sterility. When the eggs laid by homozygous *swarm* females are fertilized, the formation of male pronucleus is arrested in the late chromatin decondensing stage. Therefore the paternal chromatin cannot participate in the embryonic development [16]. Further studies have demonstrated that HIRA functions in replication-independent deposition of H3.3-H4 tetramers in the male pronucleus [17]. Loss of function allele (*Hira<sup>HRI</sup>*) reveals that the HIRA has the only essential role in the assembly of paternal chromatin during male pronucleus formation, since the mutation does not affect the viability of the flies [18].

As both CI embryos and *Hira* mutated flies result in defects in the formation of the male pronucleus, we investigated whether the strength of *Wolbachia*-induced CI is correlated with *Hira* expression level in *Drosophila* flies. Our results show that in both *Drosophila melanogaster* and *Drosophila simulans* males infected by *Wolbachia* strains that induce strong CI, *Hira* expression levels are significantly decreased compared to males exhibiting no CI or *Wolbachia*-uninfected males. In addition, *Hira* expression in 1-day-old *Wolbachia*-infected males inducing strong CI is also significantly reduced relative to 5-day-old *Wolbachia*-infected males exhibiting weak or no CI. Furthermore, we demonstrate that *Hira* mutated male flies mimic the CI phenotype, suggesting that *Wolbachia*-induced CI in *Drosophila* may occur by reducing *Hira* expression in male flies. These results provide an important insight into a novel pathway in which *Wolbachia* interacts with its insect hosts.

### Results

#### Reduced *Hira* expression in males expressing strong CI

To test the correlation of CI strength with *Hira* expression level, we initially tested the CI strength in 1-day-old *D. melanogaster* males reared under uncrowded conditions [19]. The results of crossing experiments show that CI is only induced in matings between 1-day-old *Dmel* azMel males and uninfected *Dmel* T females (hatch rate of 8.78±1.03). In contrast, no CI is induced by *Dmel* azAu 1-day-old males (hatch rate of 95.55±1.26) (Table 1).

To determine whether *Hira* expression is involved in CI level in *D. melanogaster* males, a quantitative RT-PCR assay was performed on 1-day-old male flies. As shown in Figure 1, *Hira* expression was significantly lower in azMel-infected males relative to uninfected males (*Dmel* azMel/Dmel T: 0.15±0.03) (*P<0.01*). In contrast, *Hira* expression was ~3 fold higher in azMel-infected females relative to uninfected females (*Dmel* azMel/Dmel T: 2.96±0.16) (*P<0.05*). Surprisingly, the non CI inducing azAu strain did not result in dramatically decreased *Hira* expression in males (*Dmel* azAu/Dmel T: 0.92±0.03). *Hira* expression was 1.90 fold higher in azAu-infected females relative to uninfected females (*Dmel* azAu/Dmel T: 1.90±0.07) (*P<0.05*) (Figure 1).

In order to determine if the correlation between CI level and *Hira* expression occurs in additional combinations of *Wolbachia* and their hosts, we examined *Hira* expression in *D. simulans* infected with azRi and azAu. Crossing experiments were undertaken with *D. simulans* lines and strong CI was observed to occur in matings between 1-day-old males infected with azRi and Dsim T females (hatch rate of 7.67±2.18). As in *D. melanogaster*, the azAu strain did not induce CI in *D. simulans* (hatch rate of 87.62±2.67) (Table 2).

### Reduced *Hira* expression in younger males expressing strong CI

Reynolds and Hoffmann reported that CI levels declined rapidly with increasing of male age in *Drosophila* strains infected by *Wolbachia* [20]. In order to further investigate the correlation of CI intensity with *Hira* expression in *Wolbachia*-infected male flies, we compared *Hira* expression levels between 1-day-old and 5-day-old males of *Dmel* azMel. As shown in Table 1, matings between *Wolbachia*-uninfected *Dmel* T females and 1-day-old *Dmel* azMel males result in significantly lower hatch rates (8.78±1.03) in comparison to that of the 5-day-old *Dmel* azMel males (69.51±1.33%) (*P<0.01*). However, in the reciprocal crosses between 1-day and 5-day old *Wolbachia*-uninfected males mated with the *Dmel* azMel females, there were no significant difference in hatch rates (91.66±2.92% and 92.99±1.36%, respectively) (*P>0.05*). This confirmed that in *D. melanogaster*, 1-day-old males induced strong CI, whereas 5-day-old males expressed no CI.

*Hira* gene expression, measured by quantitative RT-PCR, increases with age of male *Dmel* azMel flies (Figure 3). *Hira* expression in 1-day-old males was significantly lower than that in 5-day-old males (*P<0.01*) (Figure 3). However, *Hira* expression was not significantly different between 1-day and 5-day old *Wolbachia*-uninfected males (*P>0.05*) (Figure 5).

In crosses between *Dsim* azRi males and uninfected *Dsim* T females, hatch rates were also correlated with male age. When 5-day-old males were used in the crosses, the hatch rate of the embryos was 50.09±5.60%, which is significantly higher than that in crosses with 1-day-old males (7.67±2.18%) (Table 2). As expected, *Hira* expression in 5-day-old *Dsim* azRi male flies was also significantly increased compared to 1-day-old males (*P<0.05*). *Hira* expression between 1-day and 5-day old *Dsim* T males did not differ significantly (*P>0.05*) (Figure 4) confirming a similar effect in both *D. melanogaster* and *D. simulans*.

### Paternal effects of *Hira* mutation mimic CI in *D. melanogaster*

To examine the involvement of *Hira* expression in the CI phenotype, the crossing pattern of two *Hira*-mutated *D. melanogaster* lines (*Hira<sup>Δ18</sup>* and *Hira<sup>HRI</sup>*)) was assessed. Interestingly, we found
that both Hira-mutated males (1-day-old) mated to Wolbachia-uninfected Dmel T females resulted in significantly lower egg hatch rates (72.98 ± 5.10%, 74.34 ± 4.03%, respectively) relative to Dmel T males (92.44 ± 0.77%) (P < 0.05) (Table 3). However, the crosses between Hira-mutated males and wMel-infected females resulted in no significant differences of hatch rate comparing with uninfected crosses (96.01 ± 4.37%, 89.38 ± 0.06%, respectively) (Table 3). In the early embryos derived from the crosses of Hira-mutant males with Dmel T females, the asynchronous cleavage and chromatin bridges were observed (Figure 5, C) which is similar to the CI phenotype in D. melanogaster (Figure 5, B), while in the embryos derived from the uninfected flies the nuclear division was synchronous and the nuclei were evenly distributed (Figure 5, A). This suggests that the mutation of Hira in males may mimic the CI phenotype induced by Wolbachia.

Considering that Hira is on the X chromosome, we also examined the offspring sex ratio to see whether Hira mutation impacts sperm quantity. As shown in Table 3, mating with both Hira-mutant males resulted in significantly less female progeny in the next generation. In the progeny, the ratio of females to males derived from the crosses with Hira\textsuperscript{ssm} and Hira\textsuperscript{HR1} were 0.68 (±0.08) and 0.60 (±0.11), respectively. These sex ratios are significantly lower than those result from crosses with Dmel T males (P < 0.05), where the ratio was 0.98 (±0.10) (Table 3). These results suggest that many female progeny mortality was associated with Hira mutations on one X chromosome. Therefore we conclude that Hira mutation has negative effects on sperm quantity. In addition, the presence of the wMel strain of Wolbachia in females restored the sex ratio as shown by a sex ratio of ~1 when Hira-mutated males were crossed with Dmel wMel females (Table 3).

**Discussion**

Previous studies both in vitro and in vivo have shown that Wolbachia infection may affect the expression of various host genes, including those associated with immunity, fertilization, and development [21–23]. For example, wMelPop strain of Wolbachia has been shown to be capable of inducing immune upregulation in Anopheles gambiae and Aedes aegypti mosquitoes [23,24]. Even in a cell line naturally infected with Wolbachia, the expression of antioxidant proteins is also upregulated [22]. However, the association of CI strength induced by Wolbachia with the gene expression level in hosts is unknown. Here, we used two lines from both D. melanogaster and D. simulans infected with different Wolbachia strains to determine if Hira gene expression is correlated with CI. Our results demonstrate that for both Dmel wMel and Dsim wRi 1-day-old males, which express strong CI, the Hira expression levels are significantly decreased compared to Wolbachia-uninfected and wAu-infected males which induce either weak or no CI. Furthermore, increasing male age was correlated with increasing hatch rates (Tables 1, 2). Correspondingly, Hira expression was significantly lower in younger males (Figures 3, 4), suggesting that down regulation of Hira expression in male Drosophila might be causally linked to the CI strength.

The molecular mechanisms of abnormal embryo development in CI crosses are not fully known. Genetic and cellular evidences indicate that in CI embryos, the paternal chromosomes are improperly condensed when aligned at the metaphase plate of the first mitotic division after fertilization [9,10,25]. This could be attributed to the modification in the sperm of Wolbachia-infected...
males. Riparbelli et al. have described some malformations, including abnormal mitochondria and axoneme in the sperm developed within the infected testes [7]. Additionally, the amount of sperm produced by Wolbachia-infected D. simulans males is about 40% of that in uninfected males, especially in young males expressing strong CI, indicating that Wolbachia could affect male fertility through multiple ways [26]. Studies of spermatogenesis in the parasitic wasp Nasonia vitripennis and the beetle Chelymorpha alternans revealed that Wolbachia can modify sperm after not being present in developing sperm, suggesting that Wolbachia might alter expression and synthesis of gene products in the host, thus changing the products exported to the developing spermatids [8]. In this study, we show that Wolbachia strains that induce strong CI (wMel and wRi) significantly decrease Hira expression in male Drosophila flies, which may impair the sperm function at fertilization and result in the CI phenotype.

Chromatin remodeling is extremely significant in the late stage of spermatogenesis due to the necessity of histone replacement by male-specific transition protein (TP) and later by small arginine-rich proteins named protamines, ensuring the compacted sperm head configuration formation and promoting sperm getting into the female reproductive tract for fertilization [27]. It is known that HIRA is a chaperone of histone variant H3.3 and is involved in a DNA replication-independent pathway of nucleosome assembly [28]. In Drosophila, H3.3 is incorporated in specific regions in the early stages of spermatogenesis and then mostly disappears in condensed spermatid nuclei just before protamine deposition [18, 29]. H3.3 could play a role in spermatogenesis. Indeed, male mice carrying an impaired H3.3A gene have reduced fertility [30]. Considering that the process of spermatid differentiation is independent of DNA synthesis, the histone H3.3 chaperone HIRA might be involved in this process. In this study, we have shown that Hira mutations in young male Drosophila flies results in a significantly reduced hatch rates (Table 3) comparing with the uninfected flies, suggesting that HIRA could have an effect on male fertility by acting as a chaperone of H3.3. This is in contrast with the observations by Bonnefoy et al. [18], where they showed that Hira mutation had no effects on viability and male fertility. The contrasting results may be due to the fact that Hira mutated males do not completely lose their fertility but produce significantly less progeny.

Following fertilization, the paternal chromosomes are abnormally condensed during the first zygotic division in the embryos derived from CI crosses [4, 5]. Tram and Sullivan found that in CI crosses, the nuclear envelope breakdown and Cdk1 activation are

**Table 2. D. simulans crosses with different Wolbachia strains and host male ages.**

| Expected CI type | Cross ( male × female) | Egg counted | Egg hatch (%) |
|------------------|------------------------|-------------|---------------|
| **Compatible**   | Dsim T (1-day-old) × Dsim T | 355 | 92.76 ± 3.83 |
|                  | Dsim wRi (1-day-old) × Dsim wRi | 352 | 91.55 ± 3.08 |
|                  | Dsim wAu (1-day-old) × Dsim wAu | 301 | 90.69 ± 3.77 |
|                  | Dsim T (1-day-old) × Dsim wRi | 516 | 90.43 ± 1.25 |
|                  | Dsim T (1-day-old) × Dsim wAu | 393 | 90.01 ± 1.57 |
|                  | Dsim T (5-day-old) × Dsim wRi | 570 | 86.16 ± 3.56 |
| **Incompatible** | Dsim wRi (1-day-old) × Dsim T | 401 | 7.67 ± 2.18 * * |
|                  | Dsim wAu (1-day-old) × Dsim T | 347 | 87.62 ± 2.67 |
|                  | Dsim wRi (5-day-old) × Dsim T | 728 | 50.89 ± 5.56 * * |

Abbreviations: Dsim wRi, Drosophila simulans infected with wRi; Dsim wAu, Drosophila simulans infected with wAu; Dsim T, Drosophila simulans treated with tetracycline (without Wolbachia).

doi:10.1371/journal.pone.0019512.t002

**Figure 2. Hira gene expression in adult Drosophila simulans flies.** Quantitative RT-PCR was performed on 1-day-old males and 3–4-day-old females infected with the wRi and wAu strains of Wolbachia in addition to Wolbachia-uninfected flies (Dsim T). “T” represented the relative value; Bars indicated standard error; “**” indicated significant difference (P<0.05); “***” indicated extremely significant difference (P<0.01). Abbreviations: Dsim wRi, Drosophila simulans infected with wRi; Dsim wAu, Drosophila simulans infected with wAu; Dsim T, Drosophila simulans treated with tetracycline (without Wolbachia).
doi:10.1371/journal.pone.0019512.g002

**Figure 3. Hira gene expression in 1-day and 5-day-old D. melanogaster males.** Bars indicated standard error; “**” indicated significant difference (P<0.01).
doi:10.1371/journal.pone.0019512.g003
delayed in the male pronucleus relative to those in the female pronucleus [11]. Recent studies revealed a delay in loading H3.3 onto the paternal chromosomes, possibly causing disruption of replication in the male pronucleus of CI embryos [13]. As the chaperone of H3.3, HIRA has been demonstrated to be essential in sperm chromatin remodeling and specifically in assembling H3.3 containing nucleosomes during the formation of male pronucleus. Female flies homozygous for the null allele of Hira are sterile due to a defect in incorporated sperm nucleus decondensation [17,18]. In this regard, HIRA could be involved in the abnormal deposition of H3.3 to the paternal chromosome in CI embryos. It is possible that the low level of Hira in young Wolbachia-infected males results in a structural malformation of sperm nucleus. When sperm fertilize an uninfected egg, HIRA chaperoned maternal H3.3 may not be deposited promptly on the male nucleus during sperm chromatin remodeling. In this study, we have shown that in Wolbachia-infected female flies, Hira expression is dramatically increased in both Dmel wMel and Dsim wRi (Figures 1, 2). High level of HIRA in the females might be able to compensate for the shortage in sperm nucleus resulted from Wolbachia infection, thus speed up the deposition of H3.3 in male chromosones and rescue this defect at fertilization. Several models have been described to explain the rescue mechanism including the hypothesis that Wolbachia removes an essential component from the sperm nucleus and the same strain of Wolbachia may rescue the defects and result in high hatch rates. These mimic the CI phenotype induced by Wolbachia. Furthermore, it is the sperm- carrying X chromosome in Hira-mutants that leads to embryonic lethality, since there are significantly less females produced from these crosses (Table 3). Taken together, our results suggest that Wolbachia may induce CI by regulating the expression of some key factors, such as reducing Hira expression in males, which may influence sperm fertility and cause CI phenotype. For the compatible crosses of Wolbachia-infected females with uninfected males, it is possible that higher level of Hira in females has no effects on the embryogenesis. For bidirectional incompatibility, it is likely that different Wolbachia strains differentially impact Hira expression so that females infected with one Wolbachia strain can not rescue the deficiency in the sperm caused by another Wolbachia strain.

Materials and Methods

Fly lines

Fly lines were kept on a standard corn diet at a constant temperature of 25°C, with 8L : 16D (light : dark) cycle and were reared under non-crowded condition (200±10 eggs per 50 ml vial of media in 150 ml conical flask) [19]. The following Drosophila strains were used in the study: Wolbachia-infected Dmel wMel (D. melanogaster Brisbane nuclear background with introgressed Dmel from YW), Dmel wAu (D. melanogaster Brisbane nuclear background with injected wAu), Dsim wRi (D. simulans naturally infected with wRi), and Dsim wAu (D. simulans Coffs Harbou). Cured Dmel wMel and Dsim wRi were subsequently generated by tetracycline treatment following established protocols [32] and designated Dmel T and Dsim T, respectively. D. melanogaster of HiraHRI (a point mutant of Hira) [17] and HiraHR1 (a loss of function Hira allele) [18] were provided kindly by Dr. Lopin B. at the Université Claude Bernard Lyon I, France. These two Hira mutated lines were confirmed to be Wolbachia-free by PCR (data not shown).

CI Assays

CI tests were performed as previously described by Yamada et al. [19]. All the crossing schemes including expected compatible and incompatible crosses in this study are shown in Tables 1, 2, and 3. In all crosses, adult virgins were collected and crosses were undertaken with 30 females (3–4 days old) and 20 males (either 1-day or 5-day-old) at 25°C in bottles upturned on agar/grape juice plastic Petri dishes (ca.4cm²). After mating for around 10 h, all males were removed from the bottle to avoid diminishing CI

Table 3. Effect of Hira-mutant males (1-day-old) on egg hatch rates and progeny sex ratio.

| Expected CI type | Cross (male × female) | Egg hatch (%) | Sex ratio (F: M) | Total progeny |
|------------------|-----------------------|---------------|-----------------|--------------|
| Compatible       | Dmel T × Dmel T       | 94.24±0.77    | 1.03±0.20       | 320          |
|                  | HiraHR1 × Dmel wMel   | 86.81±4.37    | 1.04±0.17       | 334          |
|                  | HiraHR1 × Dmel wRi    | 89.38±8.06    | 1.17±0.26       | 219          |
| Reciprocal       | HiraHR1 × Dmel T      | 72.98±5.10*   | 0.68±0.08*      | 97           |
|                  | HiraHR1 × Dsim T      | 74.34±4.03*   | 0.62±0.05*      | 227          |

Egg hatch and the ratio of female to male were shown as average ± standard error; *indicates P<0.05.

doi:10.1371/journal.pone.0019512.t003
effects with increasing male age. Eggs were then collected for 6–8 h and incubated at 25°C and 45–70% humidity for 48 h. Hatch rates were determined by counting the number of hatched eggs to total eggs.

Quantitative RT-PCR Assay

Quantitative reverse transcription PCR (qRT-PCR) was performed to determine the relative Hira gene expression level in different fly lines. Total RNA was extracted from adults (males or females) using Trizol (Invitrogen). DNA contamination was removed with RTQ DNase (Promega). The first-strand cDNA was synthesized from 2 μg of total RNA using reverse transcriptase (RT) (Promega) and oligo dT15 primer (Takara) at 42°C for 1.5 h. Special primers were designed based on flybase for the Hira gene using the 20.15°C method: 

\[
Hira = \frac{C_{T (Hira)}}{C_{T (rp49)}} \times \frac{A_{rp49}}{A_{Hira}}
\]

where \( C_{T (Hira)} \) and \( C_{T (rp49)} \) are the threshold cycle numbers for Hira and rp49, respectively. The qPCR was performed using a Miniopticon system (BioRad) with a Platinum SYBR Green qPCR superMix (Takara). The reaction volume was 20 μl, containing 10 μl SYBR Premix Ex Taq (2×), 0.15 μl of forward and reverse primer (20 μM), respectively, and 7.7 μl ddH2O and 2 μl of cDNA template diluted by 10-fold. The qPCR procedure was consisted of 95°C for 2 min, followed by 95°C for 10 s, 61°C for 15 s and 72°C for 10 s per cycle for 40 cycles, then a melting curve analysis was carried out by a slow increase (0.2°C/s) from 55°C to 95°C, in purpose of examining if there were primer-dimers or nonspecific amplification. The relative expression ratio of Hira gene for samples A to B was calibrated against the 2\(^{ΔΔCT}\) calculation method: 

\[
ΔΔCT = (C_{T (Hira)} - C_{T (rp49)})_{A} - (C_{T (Hira)} - C_{T (rp49)})_{B}
\]

Statistical Analysis

Results are presented as means ± SE (n = 3). Differences among means were analyzed by one-way analysis of variance (one-way ANOVA). Differences were regarded as statistically significant when P < 0.05.

Acknowledgments

We thank Professor S.L. O’Neill (The University of Queensland, Australia) for kindly providing Dmel wMel, Dmel wAu, Dmel wHR1, and Dsim wAu flies. We thank Dr. B. Loppin (Université Claude Bernard Lyon I, France) for providing Hira\(^{ssm}\) and Hira \(^{HR1}\) flies. We are grateful to Dr. Thomas Walker (The University of Queensland, Australia) for critically reading the manuscript and giving helpful comments.

Author Contributions

Conceived and designed the experiments: Y-FW J-LW. Performed the experiments: YZ P-PR. Wrote the paper: Y-FW YZ J-LW.

References

1. Häglundsoeder K, Hammerstein P, Schlattmann P, Telschow A, Werren JH (2008) How many species are infected with Wolbachia? A statistical analysis of current data. FEMS Microbiol Lett 281(2): 215–220.
2. Serbus LR, Casper-Lindley C, Landmann F, Sullivan W (2008) The Genetic and Cell Biology of Wolbachia-host Interactions. Annu Rev Genet 42: 683–707.
3. Bourtzis, Dobson SL, Braig HR, O’Neill SL (1998) Rescuing Wolbachia have been overlooked. Nature 391: 852–852.
4. Tran U, Ferree PM, Sullivan W (2003) Identification of Wolbachia-host interacting factors through cytological analysis. Mbio Infect 3: 999–1011.
5. Ferree PM, Sullivan W (2006) A genetic test of the role of the maternal pronucleus in Wolbachia-induced cytoplasmic incompatibility in Drosophila melanogaster. Genetics 173(2): 839–847.
6. Clark ME, Veneti Z, Bourtzis K, Karr TL (2003) Wolbachia distribution and cytoplasmic incompatibility during sperm development: the cyst as the basic cellular unit of CI expression. Mech Dev 120(2): 185–198.
7. Riparbelli MG, Giordano R, Caiulli G (2007) Effects of Wolbachia on sperm maturation and architecture in Drosophila simulans Riverside. Mech Dev 124: 699–714.
8. Clark ME, Bailey-Jourdain C, Ferree PM, England SJ, Sullivan W, et al. (2008) Wolbachia modification of sperm does not always require residence within developing sperm. Heredity 101(5): 429–428.
9. Caiulli G, Dallai R, Riparbelli MG (1997) Wolbachia-induced delay of paternal chromatin condensation does not prevent maternal chromosomes from entering anaphase in incompatible crosses of Drosophila simulans. J Cell Sci 110: 271–280.
10. Tran U, Ferree PM, Sullivan W (2006) Paternal chromosome segregation during the first mitotic division determines Wolbachia-induced cytoplasmic incompatibility phenotype. J Cell Sci 119: 3653–3663.
11. Tran U, Sullivan W (2002) Role of delayed nuclear envelope breakdown and mitosis in Wolbachia-induced cytoplasmic incompatibility. Science 296: 1124–1126.
12. Royou A, McCusker D, Kellogg DR, Sullivan W (2008) Grapes (Chk1) prevents nuclear CDK1 activation by delaying cyclin B nuclear accumulation. J Cell Biol 183: 63–75.
13. Landmann F, Orsi GA, Loppin B, Sullivan W (2009) Wolbachia-Mediated Cytoplasmic Incompatibility Is Associated with Impaired Histone Deposition in the Male Pronucleus. PLoS Pathogens 5(3): e1000343.
14. Sherwood PW, Oseley MA (1991) Histone regulatory (hir) mutations suppress delta insertion alleles in Saccharomyces cerevisiae. Genetics 120(4): 729–738.
15. Wang YF, Du XZ (2005) Hira gene and development: from yeast to human. Heredity (Beijing) 27(6): 989–994.
16. Loppin B, Berger F, Couble P (2001) The Drosophila maternal gene s´e´aˆme is required for sperm chromatin remodeling at fertilization. Chromosoma 110: 430–440.
17. Loppin B, Bonnefoy E, Anselme C, Laurencon A, Karr TL, Couble P (2005) The histone H3.3 chaperone HIRA is essential for chromatin assembly in the male pronucleus. Nature 437: 1306–1309.
18. Bonnefoy E, Orsi GA, Couble P, Loppin B (2007) The essential role of Drosophila HIRA for de novo assembly of paternal chromatin at fertilization. PLoS Genet 3: 1991–2006.
19. Yamada R, Floate KD, Riegler M, O’Neil SL (2007) Male development time influences the strength of Wolbachia-induced cytoplasmic incompatibility expression in Drosophila melanogaster. Genetics 177(2): 801–808.
20. Reynolds KT, Hoffmann AA (2002) Male age, host effects and the weak of expression or non-expression of cytoplasmic incompatibility in Drosophila strains infected by the maternally inherited Wolbachia. Genet Res 80: 51–61.
21. Caron G, Govin J, Rousseaux S, Khochbin S (2005) How to pack the genome for a safe trip. Prog Mol Subcell Biol 38: 65–89.
22. Tagami H, Ray-Gallet D, Ahnoumi G, Nakatani Y (2004) Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. Cell 116(1): 51–61.
23. Ahmanova A, Miedema K, Wang Y, van Bruggen M, Berden JH, et al. (1997) The localization of histone H3.3 in germ line chromatin of Drosophila males as established with a histone H3.3-specific antiserum. Chromosoma 106(6): 335–347.
24. Coulthred C, Carlton MB, Nolan PM, Colledge WH, Evans MJ (1999) A retroviral gene trap insertion into the histone 3.3A gene causes partial neonatal lethality, stunted growth, neuromuscular deficits and male sub-fertility in transgenic mice. Hum Mol Genet 8(13): 2489–2495.
25. Poinot D, Charlat S, Mercot H (2003) On the mechanism of Wolbachia-induced cytoplasmic incompatibility: confronting the models with the facts. Bioessays 25(3): 259–265.
26. Hoffmann AA, Turelli M (1988) Unidirectional incompatibility in Drosophila simulans: inheritance, geographic variation and fitness effects. Genetics 119(2): 435–444.
27. Kambadi Z, Blagbrough AM, Pinto SB, Blagrove MS, Godfray HC, et al. (2010) Wolbachia stimulates immune gene expression and inhibits plasmodium development in Anopheles gambiae. PLoS Pathog 6(10): e1001143.
28. Moreira IA, Ibarbe-Ormaetxe I, Jeffery JA, Lo G, Pyke AT, et al. (2009) A Wolbachia symbiont in Aedes aegypti limits infection with dengue, Chikungunya, and Plasmodium. Cell 139: 1268–1278.
29. Reed KM, Werren JH (1995) Induction of paternal genome loss by the paternal sex-ratio chromosome and cytoplasmic incompatibility bacteria (Wolbachia); a comparative study of early embryonic events. Mol Reprod Dev 40: 408–418.