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Wolbachia-Induced Cytoplasmic Incompatibility in Single- and Superinfected *Aedes albopictus* (Diptera: Culicidae)

STEPHEN L. DOBSON, ERIC J. MARSLAND, AND WANCHAI RATTANADECHAKUL

Department of Entomology, University of Kentucky, Lexington, KY 40546

J. Med. Entomol. 38(3): 382–387 (2001)

ABSTRACT Maternally inherited bacteria of the genus *Wolbachia* can cause cytoplasmic incompatibility resulting in the developmental arrest of early embryos. Previous studies have shown that both single- and superinfections of *Wolbachia* naturally occur in populations of *Aedes albopictus* (Skuse). Here, we report crossing experiments using three infection types occurring in *Ae. albopictus:* uninfected, single-infected, and superinfected individuals. Crosses were monitored over the lifetime of adults to detect possible effects of host age on cytoplasmic incompatibility levels and infection virulence. Both single- and superinfections induced high levels of cytoplasmic incompatibility throughout the lifetime of *Ae. albopictus*, demonstrating that both the single- and superinfections are well adapted for invasion of *Ae. albopictus* populations. Superinfected females were the longest lived and had the highest oviposition rates, whereas in males, uninfected individuals were the longest lived. These latter results demonstrate the need for additional experiments to better elucidate *Wolbachia* effects on host fitness in addition to cytoplasmic incompatibility.

KEY WORDS *Wolbachia pipientis*, *Aedes albopictus*, cytoplasmic incompatibility

CYTOPLASMIC INCOMPATIBILITY WAS described initially as a reproductive failure caused by a maternally inherited factor in *Culex pipiens* L. (Laven 1951). This discovery led to research focused on characterizing this reproductive failure and developing applied strategies that employed cytoplasmic incompatibility for the control of field populations (Laven 1967, Davidson 1974). A cytoplasmically inherited bacterium, *Wolbachia pipientis*, was shown subsequently to be the etiological agent of cytoplasmic incompatibility (Yen and Barr 1971). This rickettsia-like bacterium had been observed to infect reproductive tissues of *Cx. pipiens* over two decades before the description of cytoplasmic incompatibility (Hertig and Wolbach 1924).

Although the mechanisms underlying cytoplasmic incompatibility have not been defined, two distinct components currently are recognized. Within males, *Wolbachia* modifies nuclear components of the sperm (Presgraves 2000). After fertilization, the modified pronucleus fails to undergo karyogamy resulting in developmental arrest of the embryo (Dobson and Tanouye 1996). The second component of cytoplasmic incompatibility occurs in females, where *Wolbachia* infections rescue the modified pronucleus, so that normal karyogamy and development can continue. Typically, modification and rescue components are specific for different *Wolbachia* strains (i.e., one *Wolbachia* strain is often unable to rescue the modification of a different infection). In addition, *Wolbachia* strains are known that do not modify the male pronucleus and are only capable of rescue ($mod^{-}resc^{+}$) and that are incapable of both modification and rescue ($mod^{-}resc^{-}$; Bourtzis et al. 1998). Superinfections (i.e., co-infection with two or more *Wolbachia* strains) occur naturally (Sinkins et al. 1995b) and have been generated artificially (Rousset et al. 1999). These superinfections can have additive effects, such that a superinfected male is unidirectionally incompatible with both single-infected and uninfected females (Fig. 1). Therefore, crosses of hosts harboring different infection types provide numerous examples of both unidirectional and bidirectional cytoplasmic incompatibility (Hoffmann and Turelli 1997).

In addition to basic research interests, *Wolbachia* infections that induce cytoplasmic incompatibility in mosquitoes are of applied scientific interest as a target for *Wolbachia*-based population modification strategies (e.g., population replacement; Sinkins et al. 1997, Curtis and Sinkins 1998). The design of effective applied strategies requires an understanding of cytoplasmic incompatibility levels and other effects associated with *Wolbachia* infection. Previous models developed to describe *Wolbachia* infection dynamics in *Drosophila* (Hoffmann et al. 1990) indicate three parameters of primary importance in determining the invasion of *Wolbachia* infections into host populations: levels of cytoplasmic incompatibility, rates at which infections are maternally transmitted, and the effects of infection on host fitness. Although these parameters have been estimated for multiple infections in *Drosophila*, much of our current knowledge of infections in mosquitoes is derived from research conducted before the identification of *Wolbachia* as the etiological agent of cytoplasmic incompatibility.

0022-2585/01/0382–0387$02.00/0 © 2001 Entomological Society of America

Female \ Male	U	A	B	AB
U	*U*	-	-	-
A	*A*	*A*	-	-
B	*B*	-	*B*	-
AB	*AB*	*AB*	*AB*	*AB*

Fig. 1. Diagram of possible crossing compatibility between uninfected (U), single-infected (A or B) and superinfected (AB) lines. Dashes indicate cytoplasmically incompatible crosses in which egg hatch is reduced or eliminated. Letters within cells indicate the infection type of the resulting brood assuming maternal inheritance.

Aedes albopictus (Skuse) provides an ideal system in which to study *Wolbachia* infections in mosquitoes. Both single- and superinfections have been identified in field populations (Sinkins et al. 1995b), providing the opportunity to examine different strains of *Wolbachia* within the same host and to observe potential interactions between co-infecting strains. *Ae. albopictus* is also an important pest and disease vector (Hawley 1988, Moore and Mitchell 1997) and therefore a candidate for applied population modification strategies using *Wolbachia* infections.

Although cytoplasmic incompatibility has been shown to occur in superinfected strains of *Ae. albopictus* (Kambhampati et al. 1993, Otsuka and Takaoka 1997), cytoplasmic incompatibility levels caused by single-infections have not been examined. In addition, prior studies of cytoplasmic incompatibility levels in *Ae. albopictus* have been limited to a single time point in young mosquitoes. Recent studies in another infected mosquito *Armigeres subalbatus* (Coquillett) demonstrated that levels of cytoplasmic incompatibility vary depending on host age (Jamnongluk et al. 2000). In *Drosophila*, the *popcorn Wolbachia* infection has a significant pathogenic effect, decreasing host longevity (Min and Benzer 1997). This indicates the need for examining cytoplasmic incompatibility levels, potential pathogenic effects, and other possible *Wolbachia* effects on host fitness over the lifetime of the mosquito host. Here, we report cytoplasmic incompatibility levels induced by both single- and superinfections over the lifetime of *Ae. albopictus* adults. To detect potential fitness or pathogenic effects of *Wolbachia* infection, comparisons were made of the number of eggs laid and adult longevity.

Materials and Methods

Mosquito Strains, Experimental Crosses, and Maintenance. The superinfected Houston strain (*Hou;* Texas 1986) and single-infected Koh Samui (*Koh;* Thailand, pre-1970) and Mauritius (*Mau;* Mauritius, Indian Ocean, pre-1970) strains were generously provided by Scott O'Neill (Yale University). UjuTet (*UjuT*) is an uninfected strain artificially generated by tetracycline treatment (Otsuka and Takaoka 1997) and was generously provided by Yasushi Otsuka (Oita Medical University). For rearing and all experiments, mosquitoes were maintained using standard conditions at 28 ± 2°C, 75 ± 10% RH, and a photoperiod of 18:6 (L:D) h. Experimental crosses consisted of 10, 2-d-old virgin females and males (20 mosquitoes total).

In the first experiment, all possible crosses using the three infection types were examined. Four cage replications were set up for each of these nine crosses. One replication of *UjuT* females crossed with *Koh* females was excluded from the data due to the premature death of caged females. In the second experiment, crosses were set up using only the *Hou* and *UjuT* strains (five replications for each crossing type).

For all crosses, a constant supply of 10% sucrose was provided to adults. Females were provided a mouse weekly for blood feeding. An oviposition container was constantly available to females and changed weekly for egg collection. Eggs were dried over a 2-d period and then allowed to mature for 5 d at 28°C 80% RH. After drying and maturation, eggs were hatched by submerging them in a deoxygenated water/liverpowder solution. Larvae were reared at low density in an excess of a liverpowder/yeast food suspension. Numbers of surviving males and females were recorded weekly. Egg papers were collected and counted until females in the cage were dead. Although all brood collections were used for comparisons of fecundity, only egg papers with ≥100 eggs were used for determining cytoplasmic incompatibility levels, because small brood sizes reduce the accuracy of determining cytoplasmic incompatibility levels. Repeated measures analysis of variance and Bonferroni mean separation were used for comparisons of untransformed data. These analyses were used for comparisons over time and to perform multiple comparisons, respectively (PROC GLM, SAS Institute 1996).

Polymerase Chain Reaction Amplification. Infection type in mosquito strains initially was confirmed using diagnostic primers: *w*AlbA (primers 328 F and 691R) and *w*AlbB (primers 183 F and 691R) (Zhou et al. 1998). For samples failing to amplify using *Wolbachia*-specific primers (e.g., *UjuT* strain), 12S primers were used to amplify mitochondria DNA as a positive control for template DNA quality (O'Neill et al. 1992).

For polymerase chain reaction (PCR) amplifications, ovaries or testes from individual mosquitoes were isolated and homogenized in 100 μl STE (0.1 M NaCl, 10 mM Tris HCl, and one mM EDTA [pH 8.0]). Proteinase K was added to a final concentration of 0.4 mg/ml, and this mixture was incubated at 56°C for 1 h. Following heat inactivation at 95°C for 15 min, 1 μl of these samples were amplified in 50 mM KCl, 20 mM Tris HCl (pH 8.4), 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.5 mM primers, and 1 U *Taq* DNA polymerase in a total volume of 20 μl. Samples were denatured for 3 min at 94°C, cycled 35 times at 94°C, 55°C and 72°C (1 min each), followed by a 10-min extension at 72°C using a PTC-200 Thermal Cycler (MJ Research, Waltham, MA). Ten microliters of each amplification was separated on 1% agarose gels, stained with ethidium bromide and visualized under UV illumination.

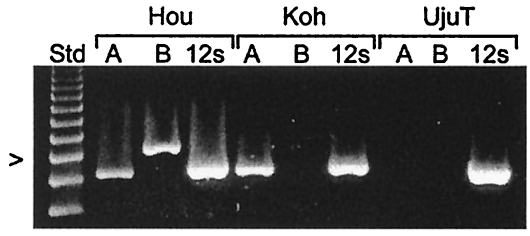

Fig. 2. Typical results of PCR amplifications of the *Hou*, *Koh*, and *UjuT* strains. Amplification using the *w*AlbA primers ("A") results in a 397 bp product in *Hou* and *Koh* (Zhou et al. 1998). Amplification using the *w*AlbB primers ("B") results in a 501 bp product in *Hou.* To verify template DNA quality, 12s mitochondrial primers ("12s") were used to amplify a 400 bp product. In the first lane, a molecular weight standard ("Std"; 123 bp ladder; Gibco, Rockville, MD) is shown. The arrow indicates 492 bp.

Results

The infection status of the three *Ae. albopictus* strains was verified by PCR before and during experiments (Fig. 2). As previously reported, the *Hou* strain is superinfected with the *w*AlbA and *w*AlbB infections, the *Koh* strain is single-infected with the *w*AlbA strain, and the *UjuT* strain is uninfected (Sinkins et al. 1995b, Otsuka and Takaoka 1997).

Single-infections of *w*AlbA induced high levels of cytoplasmic incompatibility, resulting in rare egg hatch. In crosses of *UjuT* females with single-infected *Koh* males, only 15 of 6,801 eggs hatched (Table 1). This hatch rate is similar to incompatible crosses of superinfected males and significantly lower than the reciprocal cross, crosses of superinfected females, and intrastrain crosses of the three infection types ($F = 278.59$; df = 8, 347; $P < 0.001$). Hatch rates were observed over the lifetime of females to monitor changes in incompatible levels (Fig. 3). For all three of the cytoplasmic incompatibility crossing types, low rates of egg hatch continued until the death of females, and no difference was observed in egg hatch rates from old versus young females. Although previous studies reported no egg hatch in crosses of superinfected males with single-infected or uninfected females (Kambhampati et al. 1993, Otsuka and Takaoka 1997), we observed rare egg hatch in all three of the incompatible crossing types.

Table 1. Average percent egg hatch (average ± SD; number of replicate cages [total eggs counted]) in cytoplasmically compatible and incompatible crosses

Females	Males		
	UjuT	*Koh*	*Hou*
	Experiment 1		
UjuT	65.3 ± 9.0; 4 (7,534)	0.28 ± 0.39; 3 (6,801)	0.00 ± 0.00; 4 (10,816)
Koh	83.6 ± 7.2; 4 (6,730)	85.5 ± 3.2; 4 (8,256)	0.03 ± 0.06; 4 (6,911)
Hou	82.5 ± 7.2; 4 (16,680)	76.3 ± 11.5; 4 (14,971)	78.6 ± 10.3; 4 (12,798)
	Experiment 2		
	UjuT	*Hou*	
UjuT	70.6 ± 8.5; 5 (14,198)	0.27 ± 0.43; 5 (13,263)	
Hou	88.8 ± 6.6; 5 (23,149)	79.1 ± 15.8; 5 (15,903)	

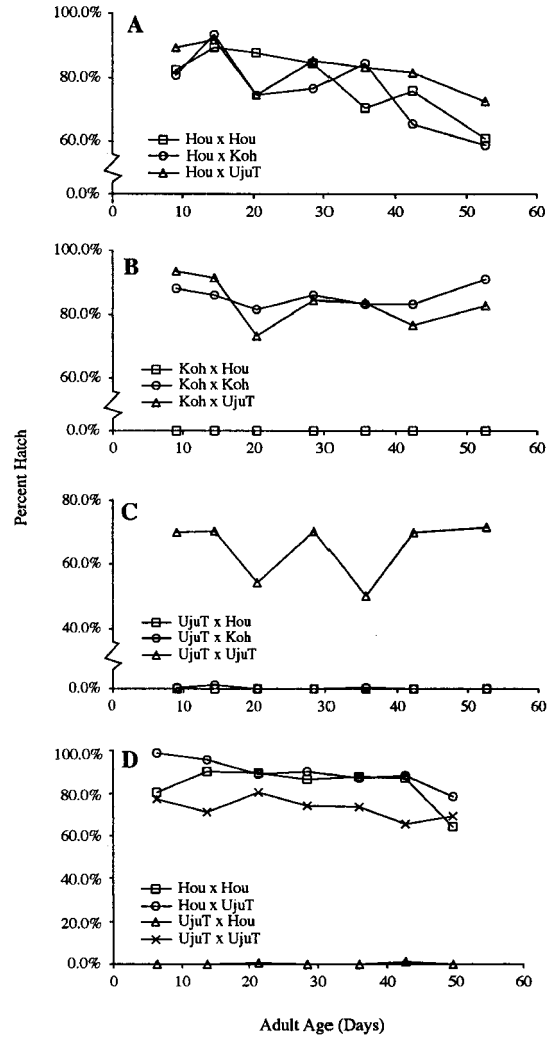

Fig. 3. Percent egg hatch (i.e., cytoplasmic incompatibility levels) from crosses of the three different *Wolbachia* infection types. Adult age is shown as days after emergence. The results of the first experiment are shown in A–C (grouped by female infection type). The results of the second experiment are shown in D. Crosses are female × male.

As expected, compatible crosses with high hatch rates resulted from matings in which the male did not harbor a unique *Wolbachia* infection type relative to the female (Table 1). However, comparison of these compatible crossing types demonstrated significant differences in the hatch rates. Crosses of superinfected *Hou* females mated with uninfected males resulted in significantly higher hatch relative to matings with infected males ($F = 278.59$; df = 8, 347; $P < 0.001$). In addition, compatible crosses of *UjuT* females resulted in significantly lower hatch rates relative to

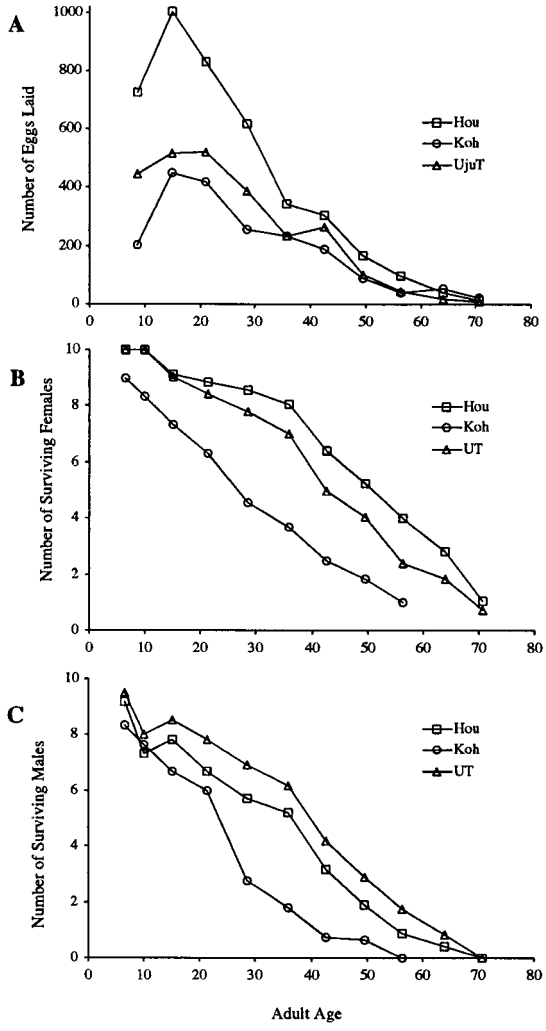

Fig. 4. Comparison of the (A) number of eggs laid and the longevity of adult (B) females and (C) males. Data are shown for both experiments, grouped by female infection type. Adult age is shown as days after emergence.

compatible crosses of *Hou* and *Koh* females (F = 278.59; df = 8, 347; $P < 0.001$).

In all three mosquito strains, oviposition peaked 2 wk after emergence and then declined with female age (Fig. 4). Although the pattern of egg laying was similar, the oviposition rate was significantly higher in *Hou* females (F = 53.77; df = 2, 561; $P < 0.001$). In comparisons of female survivorship (Fig. 4), significant differences were observed between each of the three strains (F = 24.53; df = 2, 449; $P < 0.001$). In contrast, male survivorship (Fig. 4) was similar in infected males, but higher in uninfected *UjuT* males (F = 10.09; df = 2, 449; $P < 0.001$). Combining fecundity (m_x) and survivorship (l_x), the realized fecundity (R_0) for *Hou* females (395.5 ± 141.3) was significantly higher (F = 18.07; df = 2, 63; $P < 0.001$) than that observed for females from the *Koh* (182.5 ± 112.1) and *UjuT* (239.2 ± 93.9) strains. No differences were observed in R_0 comparisons of *Koh* and *UjuT* females.

In previous crossing tests of *Ae. albopictus*, the *Mau* strain was used in crosses with *Hou* (Kambhampati et al. 1993). Unfortunately, the *Mau* strain was not available for our use until after completing the experiments described above. Previous PCR assays showed that the *Mau* and *Koh* strains harbor similar infections (Sinkins et al. 1995b). Our PCR assays using the diagnostic *w*AlbA and *w*AlbB primers confirmed that these strains harbored a similar *w*AlbA infection (data not shown). As further confirmation that the *Koh* and *Mau* strains harbor a similar infection, crosses were made between the *Mau* and *Koh* strains. We observed high hatch rates in crosses between *Mau* females and *Koh* males (92.5% hatch; 1,639 eggs) and in the reciprocal cross (96.1% hatch; 1,472 eggs), demonstrating that these strains harbor similar infection types and are compatible.

Discussion

In *Ae. albopictus*, both the single- (*w*AlbA) and superinfections (*w*AlbA and *w*AlbB) were capable of modification and rescue involved in cytoplasmic incompatibility (i.e., mod^+resc^+). Furthermore, high levels of incompatibility were observed over the lifetime of the host for both single- and superinfections. Crosses of *Koh* females and *Hou* males indicated that the *w*AlbB infection was also mod^+resc^+. However, these crosses cannot exclude potential synergistic interactions between co-infecting *w*AlbA and *w*AlbB. A strain harboring a single-infection of *w*AlbB must be identified or generated to directly examine cytoplasmic incompatibility levels induced by the *w*AlbB infection.

Rare egg hatch occurred in all three of the cytoplasmically incompatible crossing types. Egg hatch was more frequent in incompatible crosses of single-infected males relative to superinfected males. This was expected based on the hypothesis that rare hatch in incompatible crosses results from the occasional failure of the *Wolbachia* modification mechanism. Assuming that both the *w*AlbA and *w*AlbB infections were independently capable of inducing equivalent levels of cytoplasmic incompatibility, failure in superinfected males should occur at a rate approximately half of that observed in single-infected males. Combining both experiments, the rate of hatch for *UjuT* females mated with *Hou* and *Koh* males were 26/24,079 (0.1%) and 15/6,801 (0.2%), respectively.

Although rare egg hatch was distributed equivalently among cages of *UjuT* females crossed with *Koh* males and cages of *Koh* females mated with *Hou* males, hatch was not distributed evenly in crosses of *UjuT* females and *Hou* males. Combining experiments 1 and 2, hatch occurred in only two of the nine replications. In these two cages, 14 out of 1,702 (0.8%) and 12 out of 1,998 (0.6%) eggs hatched. None of the 20,279 eggs laid in the remaining seven replications hatched. Therefore, it appeared that a similar, singular event occurred in each of these two cages, resulting in

higher rates of egg hatch relative to the remaining seven replicate cages. It was unlikely that the increased hatch rates observed in these two cages resulted from males that lacked one or both infection types, due to either experimental error or failure of the infections to be maternally transmitted. For example, if one male in these cages was uninfected, then this male would be expected to produce hatch rates similar to compatible crosses (=65% hatch). Therefore, the total egg hatch in this cage would be expected to exceed 6%. The hatch rates observed in these cages also were difficult to explain as resulting from males harboring only the *w*AlbA infection, because the hatch rates in these two cages were both higher than that observed in incompatible crosses of *Koh* males (0.3%). Alternative reasons for the increased hatch rates in these two cages include the loss of the *w*AlbA infection, resulting in males that were single-infected with *w*AlbB; reduced *Wolbachia* density in males, resulting in testes that are mosaic for infection (Sinkins et al. 1995a, Perrot-Minnot and Werren 1999), an increased incidence of parthenogenic reproduction (Singh et al. 1976); or males harboring restorer genes (Rousset et al. 1991).

Significant differences in egg hatch rates were observed in comparisons of the different compatible crossing types. Interestingly, crosses of *Hou* females with uninfected males resulted in significantly higher hatch rates relative to matings with males harboring either of the two infection types. This reduced egg hatch rate resulting from matings with infected males could result from the occasional failure of the rescue mechanism in the embryos. For example, if some of the eggs failed to maternally inherit one or both of the infections, these embryos might not hatch due to their inability to rescue the modification occurring in their infected mates. This type of maternal transmission failure has been reported in infections occurring in multiple host species (Subbarao et al. 1977, Turelli and Hoffmann 1995).

Hatch rates in compatible crosses of *UjuT* females were significantly lower than that observed in the other compatible crosses. Although increased hatch could result from a fitness advantage associated with *Wolbachia* infection, this also might reflect differences between the host genetic backgrounds and not the *Wolbachia* infection type. Genetic variation also might explain the differences observed in the compatible crosses of *Hou* females described above. To directly address these questions, additional crossing experiments are needed, examining host strains that have similar genetic backgrounds (i.e., introgressed strains; Breeuwer and Werren 1993). Alternatively, tetracycline curing could be used to generate strains that are genetically similar but harbor different *Wolbachia* infection types. The latter would have the additional advantage of similar mitochondrial backgrounds.

Wolbachia infections in *Ae. albopictus* did not appear to reduce female fecundity or longevity. Superinfected *Hou* females were the longest lived and produced the highest number of eggs. In contrast, uninfected males were the longest lived, indicating a potential virulence of infections in males. However, as described above, these differences may reflect *Wolbachia* effects but are complicated by the differing genetic backgrounds of the hosts and demonstrate the need for additional crosses using host strains with randomized or uniform genotypes.

Although not examined directly, maternal transmission failure of infections to males was not detected in any of the crosses. For example, in crosses of *UjuT* females and *Koh* males, if the mother of one or more of the males failed to transmit the *w*AlbA infection, then the minimum egg hatch in this cage would be expected to exceed 6% (as described above). Because the hatch rates were below 0.28%, failure of maternal transmission was unlikely to have occurred in any of the mated males in the *Koh* crosses. This same reasoning also could be used for the *w*AlbB infection based on data from crosses of *Koh* females and *Hou* males.

These results demonstrate that both the single- and superinfection are well adapted for invasion and maintenance within populations of *Ae. albopictus.* A previous hypothesis for the existence of the superinfection in *Ae. albopictus* was that the superinfection invaded a population previously invaded by the *w*AlbA infection (Sinkins et al. 1995b). Our results showing that the single *w*AlbA infection can cause high levels of cytoplasmic incompatibility, lend support to this hypothesis by demonstrating that the single *w*AlbA infection might have spread into an uninfected population.

In addition to demonstrating the ability of both single- and superinfections to induce high levels of cytoplasmic incompatibility throughout the life of females, the results reported here also demonstrate the need for the generation of *Ae. albopictus* strains that harbor different *Wolbachia* infections within a uniform genotype. These strains, generated via introgression or tetracycline treatment, may elucidate the variation in host fitness described in this report and determine if these differences in host fitness result from *Wolbachia* infection. Future examination of incompatibility levels in *Ae. albopictus* also should include crosses of field-collected males. Previous studies of *Drosophila* populations have observed different cytoplasmic incompatibility levels and *Wolbachia* infection dynamics in laboratory and field populations (Hoffmann and Turelli 1988, Hoffmann et al. 1990, Turelli and Hoffmann 1995). Differing cytoplasmic incompatibility levels also were observed in comparisons of laboratory-reared and field-collected *Ar. subalbatus* (Jamnongluk et al. 2000). In this latter study, partial incompatibility was detected in laboratory populations but not in crosses of field collected males.

Acknowledgments

We thank Scott O'Neill and Yasushi Otsuka for supplying the mosquito strains used in these experiments. We thank John Webb for his assistance in maintaining the mosquito colonies and conducting experiments. Additional thanks go to Chuck Fox, David Wise, and Jordi Moya-Laraño for their

comments and improving the manuscript. This research was supported in part by United States Department of Agriculture NRICGP grant #9902683. This is publication 00-08-126 of the University of Kentucky Agricultural Experiment Station.

References Cited

Bourtzis, K., S. L. Dobson, H. R. Braig, and S. L. O'Neill. 1998. Rescuing *Wolbachia* have been overlooked. Nature (Lond.) 391: 852–853.

Breeuwer, J.A.J., and J. H. Werren. 1993. Effect of genotype on cytoplasmic incompatibility between two species of *Nasonia.* Heredity 70: 428–436.

Curtis, C. F., and S. P. Sinkins. 1998. *Wolbachia* as a possible means of driving genes into populations. Parasitology 116(suppl.): S111–115.

Davidson, G. 1974. Genetic control of insect pests. Academic, New York.

Dobson, S., and M. Tanouye. 1996. The paternal sex ratio chromosome induces chromosome loss independently of *Wolbachia* in the wasp *Nasonia vitripennis.* Dev. Genes Evol. 206: 207–217.

Hawley, W. A. 1988. The biology of *Aedes albopictus.* J. Am. Mosq. Control Assoc. 4(suppl. 1): 2–39.

Hertig, M., and S. B. Wolbach. 1924. Studies on rickettsia-like micro-organisms in insects. J. Med. Res. 44: 329–374.

Hoffmann, A. A., and M. Turelli. 1988. Unidirectional incompatibility in *Drosophila simulans:* inheritance, geographic variation and fitness effects. Genetics 119: 435–444.

Hoffmann, A. A., and M. Turelli. 1997. Cytoplasmic incompatibility in insects, pp. 42–80. *In* S. L. O'Neill, A. A. Hoffmann, and J. H. Werren [eds.], Influential passengers: inherited microorganisms and arthropod reproduction. Oxford University Press, Oxford.

Hoffmann, A. A., M. Turelli, and L. G. Harshman. 1990. Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans.* Genetics 126: 933–948.

Jamnongluk, W., P. Kittayapong, and S. L. O'Neill. 2000. *Wolbachia* infection and expression of cytoplasmic incompatibility in *Armigeres subalbatus* (Diptera: Culicidae). J. Med. Entomol. 37: 53–57.

Kambhampati, S., K. S. Rai, and S. J. Burgun. 1993. Unidirectional cytoplasmic incompatibility in the mosquito, *Aedes albopictus.* Evolution 47: 673–677.

Laven, H. 1951. Crossing experiments with *Culex* strains. Evolution 5: 370–375.

Laven, H. 1967. Speciation and evolution in *Culex pipiens,* pp. 251–275. *In* J. Wright and R. Pal [eds.], Genetics of insect vectors of disease. Elsevier, Amsterdam.

Min, K.-T., and S. Benzer. 1997. *Wolbachia,* normally a symbiont of *Drosophila,* can be virulent, causing degeneration and death. Proc. Natl. Acad. Sci. USA 94: 10792–10796.

Moore, C. G., and C. J. Mitchell. 1997. *Aedes albopictus* in the United States: Ten-year presence and public health implications. Emerg. Infect. Dis. 3: 329–334.

O'Neill, S. L., R. Giordano, A. M. Colbert, T. L. Karr, and H. M. Robertson. 1992. 16S *rRNA* phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. Proc. Natl. Acad. Sci. USA 89: 2699–2702.

Otsuka, Y., and H. Takaoka. 1997. Elimination of *Wolbachia pipientis* from *Aedes albopictus.* Med. Entomol. Zool. 48: 257–260.

Perrot-Minnot, M. J., and J. H. Werren. 1999. *Wolbachia* infection and incompatibility dynamics in experimental selection lines. J. Evol. Biol. 12: 272–282.

Presgraves, D. C. 2000. A genetic test of the mechanism of *Wolbachia*-induced cytoplasmic incompatibility in *Drosophila.* Genetics 154: 771.

Rousset, F., H. R. Braig, and S. L. O'Neill. 1999. A stable triple *Wolbachia* infection in *Drosophila* with nearly additive incompatibility effects. Heredity 82: 620–627.

Rousset, F., M. Raymond, and F. Kjellberg. 1991. Cytoplasmic incompatibilities in the mosquito *Culex pipiens:* how to explain a cytotype polymorphism? J. Evol. Biol. 4: 69–81.

SAS Institute. 1996. SAS-Stat User's Guide, release 6.12. SAS Institute, Cary, NC.

Singh, K.R.P., C. F. Curtis, and B. S. Krishnamurthy. 1976. Partial loss of cytoplasmic incompatibility with age in males of *Culex fatigans* Wied. Ann. Trop. Med. Parasitol. 70: 463–466.

Sinkins, S. P., H. R. Braig, and S. L. O'Neill. 1995a. *Wolbachia pipientis:* bacterial density and unidirectional cytoplasmic incompatibility between infected populations of *Aedes albopictus.* Exp. Parasitol. 81: 284–291.

Sinkins, S. P., H. R. Braig, and S. L. O'Neill. 1995b. *Wolbachia* superinfections and the expression of cytoplasmic incompatibility. Proc. R. Soc. Lond. (Biol.) 261: 325–330.

Sinkins, S. P., C. F. Curtis, and S. L. O'Neill. 1997. The potential application of inherited symbiont systems to pest control, pp. 155–175. *In* S. L. O'Neill, A. A. Hoffmann, and J. H. Werren [eds.], Influential passengers: inherited microorganisms and arthropod reproduction. Oxford University Press, Oxford.

Subbarao, S. K., B. S. Krishnamurthy, C. F. Curtis, T. Adak, and R. K. Chandrahas. 1977. Segregation of cytoplasmic incompatibility properties in *Culex pipiens fatigans.* Genetics 87: 381–390.

Turelli, M., and A. A. Hoffmann. 1995. Cytoplasmic incompatibility in *Drosophila simulans:* dynamics and parameter estimates from natural populations. Genetics 140: 1319–1338.

Yen, J. H., and A. R. Barr. 1971. New hypothesis of the cause of cytoplasmic incompatibility in *Culex pipiens* L. Nature 232: 657–658.

Zhou, W., F. Rousset, and S. O'Neill. 1998. Phylogeny and PCR based classification of *Wolbachia* strains using *wsp* gene sequences. Proc. R. Soc. Lond. (Biol.) 265: 509–515.

Received for publication 14 September 2000; accepted 21 December 2000.