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WOLBACHIA INTERACTIONS THAT DETERMINE *DROSOPHILA MELANOGASTER* SURVIVAL

ADAM J. FRY[1] AND DAVID M. RAND
Department of Ecology and Evolutionary Biology, Brown University, Box G-W, Providence, Rhode Island 02912
[1]*E-mail: Adam_Fry@Brown.edu*

Abstract.—We have recently described a mutualistic symbiosis in which *Wolbachia* bacteria were shown to improve the fitness of some *Drosophila melanogaster* stocks. *Wolbachia* did not extend longevity in all *Drosophila* genotypes, even though 16s rDNA sequences indicated that our *Drosophila* stocks were infected with the same *Wolbachia* strain. Here, we use reciprocal hybrid crosses between two *Drosophila* strains, one that lived longer with *Wolbachia* (Z53) and one that did not (Z2), to investigate the inheritance of the survival phenotype and its dependence on the host genotype, sex, and mating conditions. *Wolbachia*'s positive effects were more apparent in hybrid flies than in parental flies, ruling out exclusive maternal inheritance or the dependence of the survival phenotype on *Wolbachia* strain differences. The *Wolbachia* survival effects were more apparent in single-sex cages, where courtship and mating were not permitted. In these cages, nearly all flies with *Wolbachia* lived longer than uninfected flies, even though strain Z2 showed no *Wolbachia* effect in mixed-sex mating cages. We used comparisons between single- and mixed-sex cages to estimate the cost of reproduction for both sexes. Our data suggest that *Wolbachia* infection may increase the inferred cost of reproduction, particularly in males. *Wolbachia* can even produce a positive survival effect almost as large as the negative survival effect associated with reproduction. We discuss the implications of our experiments for the study of insect symbioses.

Key words.—Cost of reproduction, hybrid, mortality, survival, symbiosis.

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Wolbachia are maternally inherited α-proteobacteria thought to infect millions of insect species (Werren et al. 1995a; Jeyaprakash and Hoy 2000; Werren and Windsor 2000; Jiggins et al. 2001a). Their widespread distribution among insects makes them one of the most common infectious microorganisms. These bacteria inhabit the reproductive tissues of their hosts (Dobson et al. 1999), where they induce a number of reproductive modifications intended to enhance their transmission through females. Their effects include the induction of parthenogenesis (Stouthamer et al. 1993), feminization of genetic males (Rousset et al. 1992), male-killing (Jiggins et al. 1998; Hurst et al. 1999), and cytoplasmic incompatibility (CI; Caspari and Watson 1959; Yen and Barr 1971; Fine 1978; Hoffmann et al. 1986, 1990). *Wolbachia* does not induce strong reproductive modifications in *Drosophila melanogaster*, and this has led to the suggestion that *Wolbachia* may benefit *D. melanogaster* fitness (Hoffmann et al. 1994, 1998; Solignac et al. 1994). Attempts to identify these benefits revealed that, in the field, *Wolbachia* could improve survival, but the positive survival effects depended on the population background and the location of the field site (Olsen et al. 2001). In the laboratory, we found that *Wolbachia* could significantly improve both survival and female fecundity, and these effects depended on the host genotype (A. J. Fry, M. R. Palmer, and D. M. Rand, unpubl. ms.). DNA sequences from 16s rDNA (O'Neill et al. 1992) and the *fts*Z cell cycle gene (Holden et al. 1993; Werren et al. 1995b) suggested that our *Wolbachia* strains were identical. However, recent reports of recombination between different *Wolbachia* strains preclude exact strain identification unless a large number of relevant loci are compared (Werren et al. 1995b; Werren and Bartos 2001; Jiggins et al. 2001b).

The positive fitness effects we found could have important implications for the maintenance of *Wolbachia* infection in *D. melanogaster* and may indicate that the symbiosis between *Wolbachia* and *Drosophila* is in transition from parasitic to mutualistic (A. J. Fry, M. R. Palmer, and D. M. Rand, unpubl. ms.). To better understand these survival effects, we used reciprocal hybrid crosses between two *Drosophila* strains, one that lived longer with *Wolbachia* (Z53) and one strain (Z2) that did not show a significant *Wolbachia* effect. This design allowed us to compare the survival effects of the *Wolbachia* strains found in Z53 and Z2 as each hybrid had a different *Wolbachia* strain. We also investigated *Wolbachia*'s effects in both inbred (Z53 and Z2) and outbred (hybrid) genotypes and we manipulated the mating conditions to understand *Wolbachia*'s interaction with reproduction. This was done because courtship and mating are known to affect *Drosophila* survival (e.g., Fowler and Partridge 1989; Partridge and Fowler 1990; Chapman et al. 1995) and could interact with *Wolbachia*'s effects on survival.

MATERIALS AND METHODS

Flies and Experimental Crosses

The Z53 and Z2 *D. melanogaster* stocks were originally collected in Zimbabwe, Africa. These laboratory strains were started as isofemale lines, have been in laboratory culture for several hundred generations, and have been maintained at moderate population size (100–200 pairs) in the laboratory of D. M. Rand. They also carry the endosymbiotic bacterium *W. pipientis*. Marc Tatar (Brown University) provided Ri-RedE, an uninfected control strain. We created paired *Wolbachia*-infected (W) and tetracycline-treated (T) experimental lines with a standard protocol (Hoffmann et al. 1994; Poinsot and Mercot 1997). The uninfected fly lines are called (T) because they were treated with the antibiotic tetracycline for two generations to remove *Wolbachia* (0.25 mg/ml tetracycline in water added to Carolina dry food in a 1:1 mix). The *Wolbachia*-infected (W) lines received identical food and

TABLE 1. Proportional hazards analysis of females from fly strains Z53, Z2, and their hybrids. Mating environments were mixed- or single-sex. *Wolbachia* refers to *Wolbachia*-infected or tetracycline-treated flies. Likelihood-ratio (LR) chi-squared values and associated probabilities are given.

Source	df	LR χ^2	$P > \chi^2$
Cross	3	764.24	$<10^{-3}$
Mating	1	562.02	$<10^{-3}$
Wolbachia	1	162.08	$<10^{-3}$
Cross × mating	3	21.22	$<10^{-3}$
Wolbachia × cross	3	26.02	$<10^{-3}$
Wolbachia × mating	1	9.79	$<10^{-2}$
Wolbachia × cross × mating	3	10.70	0.013

environment, except tetracycline was not added. The infection status of all lines was confirmed with *Wolbachia*-specific 16S rRNA polymerase chain reaction primers (O'Neill et al. 1992). To insure that differences between T and W lines were not due to tetracycline treatment, we used two controls. First, we treated an uninfected fly strain, RiRedE, with tetracycline to see if treatment affected survival. Second, we held experimental flies for two generations on standard corn-meal laboratory food after treatment, but before the experimental crosses, to minimize maternal effects of treatment.

Twenty-five pairs of parental flies from the T and W lines were placed into food bottles and held until approximately 120–150 eggs were deposited. We controlled egg density because W females from Z53 can lay more eggs than T females (A. J. Fry, M. R. Palmer, and D. M. Rand, unpubl. ms.), which could influence larval density and in turn affect *Drosophila* development time (Gonzalez-Candelas et al. 1990), survival (Buck et al. 1993), and *Wolbachia* infection levels (Hoffmann et al. 1998). Eggs were allowed to develop and virgin adults collected and paired into one of four cross types: two hybrid crosses, Z53 male × Z2 female and Z2 male × Z53 female, and two parental crosses, Z53 × Z53 and Z2 × Z2. To avoid confounding effects of cytoplasmic incompatibility on survival, all crosses were made with flies of the same infection status; that is, we did not cross T and W flies. Twenty-five pairs of adults from each cross were placed into food bottles until 120–150 eggs were deposited. Virgin F_1 adults from these crosses were collected over a 24-h period and used to initiate demography cages.

Demography Cages

The demography cages were constructed from quart-serving plastic containers with a screened lid; a side coupling the same dimension as a standard food vial; and a double-walled, rubber side entrance, made from bicycle inner tube. The demography cages were kept in a walk-in incubator on a 12L: 12D photoperiod at 25°C and 40% relative humidity. Fresh food vials we added to the cages every other day, when dead flies were removed with an aspirator, sexed, and counted. Because courtship and mating can affect *Drosophila* survival and could interact with *Wolbachia*'s effects on survival (Fowler and Partridge 1989; Partridge and Fowler 1990; Chapman et al. 1995), we scored survival in two different mating environments. Mixed-sex environments were constructed with 100 virgin F_1 males and 100 virgin females together in one cage. Single-sex environments were constructed with 100 virgin flies of only one sex per cage. There were three replicate cages for each cross, sex, and mating environment combination. Our treatment control, RiRedE, was reared in the mixed-sex environment only.

TABLE 2. Risk ratios of *Wolbachia*-infected (W) to tetracycline-treated (T) lines from two different mating environments, mixed- and single-sex, left columns. Ratios greater than one indicate W lived longer than T and measure *Wolbachia*'s survival benefits. The two right columns are risk ratios of survival in single-sex to mixed-sex cages for different infection states (T or W) and are a rough measure of the benefits to survival obtained by not reproducing. W and T or mixed- and single-sex cages were compared with log-rank tests and significance ($P < 0.001$) is indicated by an asterisk.

	Wolbachia/treated		Single-sex/mixed-sex	
Cross	Single-sex	Mixed-sex	*Wolbachia*	Treated
Female				
Z53	1.25*	1.25*	1.58*	1.47*
Z2	1.21*	1.01	1.47*	1.47*
Z53 × Z2	1.17*	1.15*	1.58*	1.54*
Z2 × Z53	1.15*	1.14*	1.55*	1.46*
Male				
Z53	1.21*	1.08	1.41*	1.26*
Z2	1.19*	1.06	1.60*	1.23*
Z53 × Z2	1.20*	1.21*	1.06	1.06
Z2 × Z53	1.08	1.05	1.00	1.03

In total, we monitored 78 demography cages and scored the survival of more than 10,000 flies. Statistical analyses were performed using the JMP statistical package (SAS Institute 1995) and a semiparametric proportional hazards statistical model (Cox 1972). In our model, the dependent variable was time of death measured to the nearest 48 h, with host genotype (four crosses), infection status (T or W), mating environment (mixed- or single-sex), and their interactions as predictors.

RESULTS

The survival of female flies from Z53, Z2, and their hybrids depended on interactions between *Wolbachia*'s effects on survival, the host genotype, and whether courtship and mating occurred (Table 1). A proportional hazards analysis of male survival was nearly identical except the three-way interaction was not significant (likelihood-ratio $\chi^2 = 5.82$, df = 3, $P = 0.12$). Due to the large number of pairwise comparisons between T and W flies, we show only some of the more important comparisons. The complete dataset is summarized in Table 2 using risk ratios.

The survival curves from the four crosses were significantly heterogeneous for females and males (Fig. 1), regardless of *Wolbachia* infection status, in both mixed- and single-sex cages. Hybrid flies survived longer than flies from either parental strain (Fig. 1). *Wolbachia* had a pronounced survival effect in hybrid flies and flies reared in the single-sex cages (Table 2). We found that *Wolbachia* affected the survival of hybrid flies more than parental flies. Hybrids that carry *Wolbachia* survived significantly longer than T hybrids, even though all hybrids had at least one parent (Z2) that did not show a significant *Wolbachia* survival effect (Fig. 2). The exception involves male hybrids with a Z2 father. Here, the

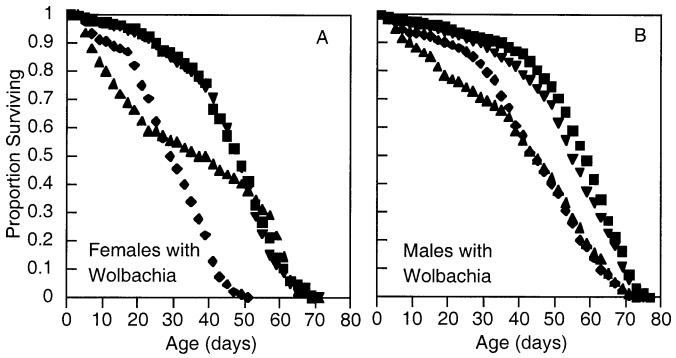

FIG. 1. Survival curves from females (A) and males (B) from parental crosses and hybrids. Data are from *Wolbachia*-infected (W) flies reared in mixed-sex mating cages. Symbols: Z53 × Z53, filled triangle; Z53 × Z2, square; Z2 × Z53, inverted triangle; Z2 × Z2, diamond. (A) Females are significantly heterogeneous by log-rank (LR) test ($\chi^2 = 254$, df = 3, $P < 10^{-3}$), (B) as are males (LR $\chi^2 = 270$, df = 3, $P < 10^{-3}$).

survival curves for T and W flies were not different in mixed-sex cages (Table 2). However, in single-sex cages, W flies lived longer than T flies, even if W and T flies were not different in the mixed-sex cages as was the case for Z53 males and both sexes of Z2 (Fig. 3). There were no significant survival effects associated with tetracycline (T) treatment in our treatment control, RiRedE ($P > 0.05$ for both sexes by log-rank test), indicating that tetracycline treatment was not directly responsible for the survival effects we observed.

DISCUSSION

Drosophila survival depended on strong interactions between *Wolbachia* infection, host genotype, and the mating conditions experienced by both male and female flies (Table 1). The parental strains Z53 and Z2 have been in laboratory culture for several hundred generations, they are inbred and have probably accumulated mutations affecting survival. Not surprisingly, hybrid flies were longer-lived than their parents in both mixed- (Fig. 1) and single-sex mating environments. With one exception, the hybrids also showed a significant positive *Wolbachia* effect, even though each hybrid had at least one parent (Z2) that did not. The presence of a *Wolbachia* effect in nearly all hybrids suggests that there may be a relationship between inbreeding and the expression of the *Wolbachia* survival effect. Other *Wolbachia* phenotypic effects such as the expression of cytoplasmic incompatibility and the fidelity of maternal transmission are more pronounced in laboratory stocks than in field populations (Hoffmann et al. 1990; Turelli and Hoffmann 1995). *Drosophila* fitness can be adversely affected by inbreeding (e.g., Miller et al. 1993; Fry et al. 1998; Aspi 2000). As *Wolbachia* infection spreads throughout a host population, genetic variation at other cytoplasmically inherited molecular markers can be reduced, such as mitochondrial DNA variation in *D. simulans* (e.g., Ballard 2000). Thus, the interaction between *Wolbachia* and inbreeding could be important for predicting the equilibrium frequency and spread of *Wolbachia* infection in natural populations.

There is little evidence to suggest that the positive survival effects associated with *Wolbachia* are uniparentally inherited.

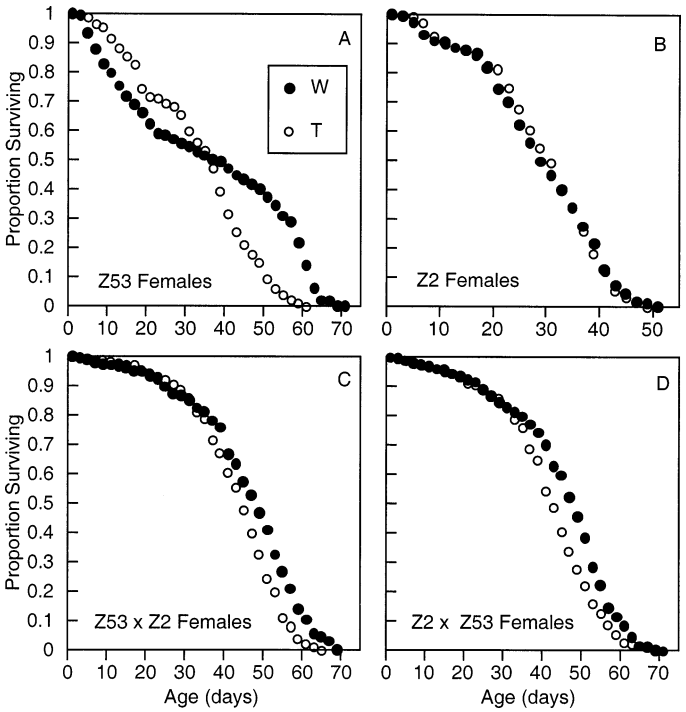

FIG. 2. Comparison of survival curves from *Wolbachia*-infected (W, closed circles) and tetracycline-treated (T, open circles) flies from four genotypes. Data are from females reared in mixed-sex cages. (A) LR $\chi^2 = 52.28$, df = 1, $P < 10^{-3}$; (B) LR $\chi^2 = 0.05$, $P = 0.89$; (C) LR $\chi^2 = 69.96$, $P < 10^{-3}$; (D) LR $\chi^2 = 15.59$, $P < 10^{-3}$.

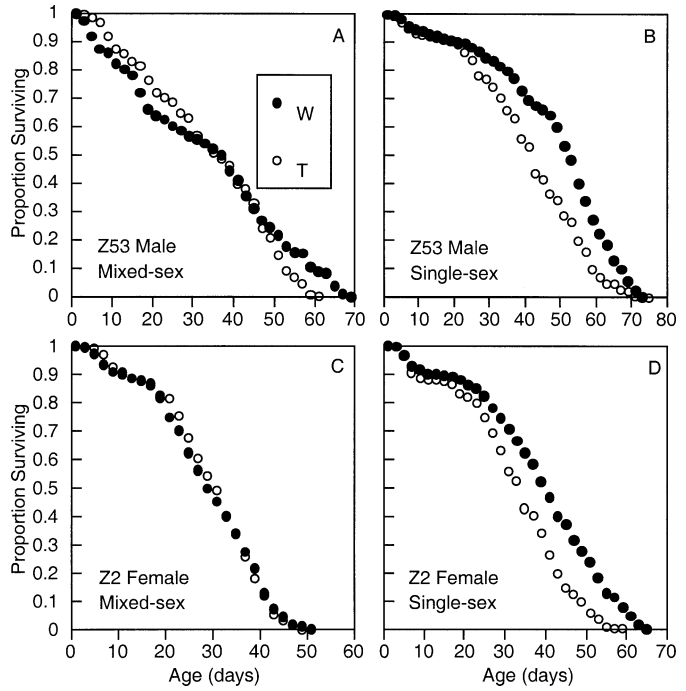

FIG. 3. Effects of *Wolbachia* in mixed- and single-sex mating cages. Comparisons between *Wolbachia*-infected (W, closed circles) and tetracycline-treated (T, open circles) flies. (A) LR $\chi^2 = 3.61$, df = 1, $P = 0.05$; (B) LR $\chi^2 = 35.40$, df = 1, $P < 10^{-3}$; (C) LR $\chi^2 = 0.05$, df = 1, $P = 0.89$; (D) LR $\chi^2 = 48.83$, df = 1, $P < 10^{-3}$.

TABLE 3. Mortality model analysis from Z53, Z2, and their hybrids from the mixed-sex mating cages. See Discussion for explanation of models and symbols. Values give the proportional contribution of that parameter to the total difference in mortality between tetracycline-treated (T) and *Wolbachia*-infected (W) flies.

	Model T	Model W	α	β	C
Female					
Z53	G	GM	0%	77.4%	22.6%
Z53 × Z2	G	G	0%	100%	
Z2 × Z53	GM	G	31.6%	53.5%	14.9%
Male					
Z53 × Z2	G	G	0%	100%	

TABLE 4. Mortality model analysis from Z53, Z2, and their hybrids from the single-sex mating cages. See Discussion for explanation of models and symbols. Values give the proportional contribution of that parameter to the total difference in mortality between tetracycline-treated (T) and *Wolbachia*-infected (W) flies.

	Model T	Model W	α	β	C
Female					
Z53	GM	GM	0%	100%	0%
Z2	GM	GM	91.6%	0%	8.4%
Z53 × Z2	GM	GM	0%	100%	0%
Z2 × Z53	GM	GM	0%	66.7%	33.3%
Male					
Z53	G	G	100%	0%	
Z2	GM	GM	0%	96.5%	3.5%
Z53 × Z2	GM	GM	100%	0%	0%

However, more crosses will be needed to determine the exact inheritance pattern. We reported previously that 16S rRNA sequences from our *Wolbachia* strains were identical, which implicated the host genome in the differential expression of these *Wolbachia* survival effects (A. J. Fry, M. R. Palmer, and D. M. Rand, unpubl. ms.). However, we note that recent reports of recombination between some *Wolbachia* strains (Jiggins et al. 2001b; Werren and Bartos 2001) make it necessary to obtain sequences from a large number of *Wolbachia* loci to determine if the strains are identical by descent. With complete *Wolbachia* genomes currently being sequenced (e.g., Slatko et al. 1999), it should soon be possible to determine the extent to which different *Wolbachia* strains and host genomes interact to produce the various *Wolbachia* phenotypes.

The *Wolbachia* survival effects in our experiment showed a strong dependence on the mating cage. W flies reared in single-sex cages lived longer than T flies, even if T and W flies were the same in the mixed-sex cages. The benefits of *Wolbachia* in the two environments can be compared using risk ratios (Table 2). For example, neither females nor males from Z2 show a significant *Wolbachia* effect in the mixed-sex environment (1.01 and 1.06 risk ratios, respectively). In the single-sex environment, however, there is a 21% and 19% benefit to *Wolbachia* infection. Aside from Z2 and Z53 males, however, the effect of *Wolbachia* on survival is about the same in the mixed- and single-sex cages. For example, in Z53 females, *Wolbachia*'s positive effect is about 25% under both mating conditions.

The right two columns of Table 2 compare fly survival in mixed- and single-sex cages. If we consider these risk ratios as a very rough index of the cost of reproduction, we can compare *Wolbachia*'s effects on fly survival with the effects of courtship, mating, and egg laying on fly survival. Inspection of these ratios indicates several things. First, nearly all of the flies experience a significant decrease in survival when courtship and mating occur. This has been well documented in *Drosophila* and presumably represents a physiological cost to reproduction (e.g., Fowler and Partridge 1989; Partridge and Fowler 1990; Chapman et al. 1995). Second, there is a trend suggesting that *Wolbachia* infections can contribute to the cost of reproduction. This cost appears much greater for the parental Z53 and Z2 males than for any female. Again, this effect could be related to the level of inbreeding in parental males because hybrid males show no cost of reproduction, infected or not (Table 2). Snook et al. (2000) showed that *Wolbachia* can decrease sperm production in *D. simulans*. It is unclear whether a similar mechanism increases the cost of reproduction in our W males. Our data reject the notion that *Wolbachia*'s positive survival effects are achieved by decreasing the cost of reproduction. This is consistent with our earlier result that found *Wolbachia* could improve survival without decreasing female fecundity. In fact, *Wolbachia* improved both survival and fecundity of female flies (A. J. Fry, M. R. Palmer, and D. M. Rand, unpubl. ms.). The final point to make about Table 2 is that by comparing *Wolbachia*'s effects on survival with the inferred cost of reproduction, we find that *Wolbachia*'s positive effects can be substantial. For example, the positive effect of *Wolbachia* (25%) is close to half the inferred cost of reproduction (58%) in Z53 females (Table 2). We suggest that *Wolbachia* effects of this magnitude are probably important determinants of life-history evolution in chronically infected host populations. Whether these positive and negative survival effects might be offsetting in natural populations is uncertain and requires additional field experiments.

To understand how *Wolbachia* affects survival, we used maximum-likelihood analyses (Pletcher 1999) to compare estimated mortality models from W and T flies (Tables 3, 4). In most cases, similar models described T and W mortality curves from the same genotype and sex. The inferred models were either two-parameter Gompertz models, which describe a simple exponential increase in mortality rate with age, or Gompertz-Makeham models (Vaupel and Yashin 1985) that include an additional parameter (*C*) to describe age-independent mortality. The proportional contribution of each parameter to the total difference in mortality was determined using the method of Pletcher et al. (2000). The results are shown in Table 3 for mixed-sex mating cages and Table 4 for single-sex cages. Our results indicate that *Wolbachia* infection can contribute to age-independent mortality. For example, 22.6% of the total difference in mortality between T and W flies for Z53 females is due to an age-independent (*C*) mortality contribution. More commonly, though, *Wolbachia* affected both the rate of aging (slope, β), and the initial mortality rate (intercept, α). Our data suggest that *Wolbachia* can affect the mortality schedules of flies in ways that are difficult to predict. Clearly, *Wolbachia* but must be controlled in experimental investigations of arthropod fitness.

Conclusions

We found strong evidence that survival in *D. melanogaster* depends on interactions between *Wolbachia* infection, host genotype, host sex, and whether reproduction occurs. *Wolbachia* had a significant positive influence on survival, and treatment to remove *Wolbachia* decreased survival by as much as 25% in mixed-sex cages where mating occurred. In the single-sex cages where mating was not permitted, *Wolbachia*'s effects on survival were even more pronounced and increased survival in all flies assayed. In addition to mating cage, the host genotype also determined whether *Wolbachia* affected survival. W hybrid flies survived longer than T flies, even though all hybrids had at least one parent that did not show a *Wolbachia* survival effect. Our data suggest there may be a relationship between the level of inbreeding in the host and the expression of *Wolbachia*-induced phenotypes. A comparison of *Wolbachia*'s effects on survival with the inferred costs of reproduction in our flies revealed that *Wolbachia*'s positive effect on survival can be almost as large as the negative effect of reproduction. There was also a trend suggesting that *Wolbachia* infection contributes to the cost of reproduction in both sexes, although this effect appears greater in males than in females, and a cost is not observed in hybrid males. Because *Wolbachia* can have both positive and negative effects on host survival (e.g., Min and Benzer 1997; Dobson et al. 2002; A. J. Fry, M. R. Palmer, and D. M. Rand, unpubl. ms.) and reproduction (e.g., Hoffmann et al. 1990; Snook et al. 2000; Dobson et al. 2002), the interactions we found could be important in the interpretation of fitness studies using *Drosophila* and other arthropods that carry *Wolbachia*. Future work should be directed toward identifying chromosomes or chromosomal regions in *D. melanogaster* that are involved in these *Wolbachia*-induced phenotypes. Such studies will prove useful for understanding how host and parasite genomes coevolve to produce more mutualistic symbioses from parasitic ones.

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Literature Cited

Aspi, J. 2000. Inbreeding and outbreeding depression in male courtship song characters in *Drosophila montana*. Heredity 84: 273–282.

Ballard, J. W. O. 2000. Comparative genomics of mitochondrial DNA in *Drosophila simulans*. J. Mol. Evol. 51:64–75.

Buck, S., M. Nicholson, S. Dudas, R. Wells, A. Force, G. T. Baker III and R. Arking. 1993. Larval regulation of adult longevity in a genetically-selected long-lived strain of *Drosophila*. Heredity 71:23–32.

Caspari, E., and G. S. Watson. 1959. On the evolutionary importance of cytoplasmic sterility in mosquitoes. Evolution 13: 568–570.

Chapman, T., L. F. Liddle, J. M. Kalb, M. F. Wolfner, and L. Partridge. 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products Nature 373: 241–244.

Cox, D. R. 1972. Regression models and life tables J. R. Stat. Soc. B 34:187–202.

Dobson, S. L., K. Bourtzis, H. R. Braig, B. F. Jones, W. Zhou, F. Rousset, and S. L. O'Neill. 1999. *Wolbachia* infections are distributed throughout insect somatic and germ line tissues. Insect Biochem. Mol. Biol. 29:153–160.

Fine, P. E. M. 1978. On the dynamics of symbiote-dependent cytoplasmic incompatibility in Culicine mosquitoes. J. Invertebr. Pathol. 30:10–18.

Fowler, K., and L. Partridge. 1989. A cost of mating in female fruit flies. Nature 338:760–761.

Fry, J. D., S. L. Heinsohn, and T. F. Mackay. 1998. Heterosis for viability, fecundity, and male fertility in *Drosophila melanogaster*: comparison of mutational and standing variation. Genetics 148:1171–1188.

Gonzalez-Candelas, F., J. L. Mensua, and A. Moya. 1990. Larval competition in *Drosophila melanogaster*: effects on development time. Genetica 82:33–44.

Hoffmann, A. A., M. Turelli, and G. M. Simmons. 1986. Unidirectional incompatibility between populations of *Drosophila simulans*. Evolution 40:692–701.

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

Hoffmann, A. A., D. J. Clancy, and E. Merton. 1994. Cytoplasmic incompatibility in Australian populations of *Drosophila melanogaster*. Genetics 136:993–999.

Hoffmann, A. A., M. Hercus, and H. Dagher. 1998. Population dynamics of the *Wolbachia* infection causing cytoplasmic incompatibility in *Drosophila melanogaster*. Genetics 148: 221–231.

Holden, P. R., J. F. Y. Brookfield, and P. Jones. 1993. Cloning and characterization of an *fts*Z cognate from a bacterial symbiont of *Drosophila melanogaster*. Mol. Gen. Genet. 240:213–220.

Hurst, G. D. D., F. M. Jiggins, J. H. G. Von Schulenburg, D. Bertrand, S. A. West, I. I. Goriacheva, I. A. Zakharov, J. H. Werren, R. Stouthamer, and M. E. N. Majerus. 1999. Male-killing *Wolbachia* in two species of insect. Proc. R. Soc. Lond. B 266: 735–740.

Jeyaprakash, A., and M. A. Hoy. 2000. Long PCR improves *Wolbachia* DNA amplification: *wsp* sequences found in 76% of sixty-three arthropod species. Insect Mol. Biol. 9:393–405.

Jiggins, F. M., G. D. D. Hurst, and M. E. N. Majerus. 1998. Sex ratio distortion in *Acraea encedon* (Lepidoptera: Nymphalidae) is caused by a male-killing bacterium. Heredity 81:87–91.

Jiggins, F. M., J. K. Bentley, M. E. N. Majerus, and G. D. D. Hurst. 2001a. How many species are infected with *Wolbachia*? Cryptic sex ratio distorters revealed to be common by intensive sampling. Proc. R. Soc. Lond. B 268:1123–1126.

Jiggins, F. M., J. H. G. Von Schulenburg, G. D. D. Hurst, and M. E. N. Majerus. 2001b. Recombination confounds interpretations of *Wolbachia* evolution. Proc. R. Soc. Lond. B 268:1423–1427.

Miller, P. S., J. Glasner, and P. W. Hedrick. 1993. Inbreeding depression and male mating behavior in *Drosophila melanogaster*. Genetica 88:29–36.

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

Olsen, K., T. Reynolds, and A. A. Hoffmann. 2001. A field cage test of the effects of the endosymbiont *Wolbachia* on *Drosophila melanogaster*. Heredity 86:1–7.

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

Partridge, L., and K. Fowler. 1990. Non-mating costs of exposure to males in female *Drosophila melanogaster*. J. Insect Physiol. 36:419–425.

Pletcher, S. D. 1999. Model fitting and hypothesis testing for age-specific mortality data. J. Evol. Biol. 12:430–439.

Pletcher, S. D., A. A. Khazaeli, and J. W. Curtsinger. 2000. Why

do lifespans differ? Partitioning mean longevity differences in terms of age-specific mortality parameters. J. Gerontol. 55: B381–389.

Poinsot, D., and H. Mercot. 1997. *Wolbachia* infections in *Drosophila simulans*: Does the female host bear a physiological cost? Evolution 51:180–186.

Rousset, F., D. Bouchon, B. Pintureau, P. Juchault, and M. Solignac. 1992. *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. Proc. R. Soc. Lond. B 250: 91–98.

SAS Institute. 1995. JMP statistics and graphics guide. Ver. 3.2 SAS Institute, Cary, NC.

Slatko, B. E., S. L. O'Neill, A. L. Scott, J. H. Werren, and M. L. Blaxter. 1999. The *Wolbachia* genome consortium. Microb. Comp. Genomics 4:161–165.

Snook, R. R., S. Y. Cleland, M. F. Wolfner, and T. L. Karr. 2000. Offsetting effects of *Wolbachia* infection and heat shock on sperm production in *Drosophila simulans*: analyses of fecundity, fertility, and accessory gland proteins. Genetics 155:167–178.

Solignac, M., D. Vautrin, and F. Rousset. 1994. Widespread occurrence of the proteobacteria *Wolbachia* and partial cytoplasmic incompatibility in *Drosophila melanogaster*. C. R. Acad. Sci. Paris 317:461–470.

Stouthamer, R., J. A. Breeuwer, R. F. Luck, and J. H. Werren. 1993. Molecular identification of microorganisms associated with parthenogenesis. Nature 361:66–68.

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

Vaupel, J. W., and A. I. Yashin. 1985. The deviant dynamics of death in heterogeneous populations. Soc. Methodol. 15:179–211.

Werren, J. H., and J. D. Bartos. 2001. Recombination in *Wolbachia*. Curr. Biol. 11:431–435.

Werren, J. H., and D. M. Windsor. 2000. *Wolbachia* infection frequencies in insects: evidence for a global equilibrium? Proc. R. Soc. Lond. B 267:1277–1285.

Werren, J. H., D. M. Windsor, and L. R. Gou. 1995a. Distribution of *Wolbachia* among Neotropical arthropods. Proc. R. Soc. Lond. B 262:197–204.

Werren, J. H., W. Zhang, and L. R. Gou. 1995b. Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. Proc. R. Soc. Lond. B 261:55–63.

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

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