

1 **Melanic mutation causes a fitness decline in bean beetles infected**  
2 **by *Wolbachia***

3

4 **Yuko Numajiri<sup>1\*</sup>, Natsuko I. Kondo<sup>2</sup> & Yukihiko Toquenaga<sup>3</sup>**

5

6 <sup>1</sup>Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba,  
7 Ibaraki, Japan, <sup>2</sup>Center for Environmental Biology and Ecosystem Studies, Tsukuba, Ibaraki,  
8 Japan National Institute for Environmental Studies, and <sup>3</sup>Faculty of Life and Environmental  
9 Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan

10

11 **\*Correspondence:** Yuko Numajiri, Graduate School of Life and Environmental Sciences,  
12 University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan. E-mail:  
13 numajiri@pe.ska.life.tsukuba.ac.jp

14

15 **Short title:** Fitness decline in melanic mutants with *Wolbachia*

16

17 **Key words:** *Callosobruchus analis*, cytoplasmic incompatibility, hierarchical Bayesian  
18 model, host fitness defect, melanism, microinjection, reproductive manipulation

19

20 **Accepted: 14 March 2017**

21

1 **Abstract**

2 *Wolbachia* cannot live outside a host, which is thought to be the reason for host-*Wolbachia*  
3 coevolution toward benign parasitism, especially because the fitness of *Wolbachia* is traded  
4 against its host's fitness. Insect melanism has been reported to have a positive effect on  
5 pathogen resistance, but melanic mutants of *Callosobruchus analis* (Fabricius) and  
6 *Callosobruchus chinensis* (L.) (Coleoptera: Chrysomelidae) are infected with *Wolbachia*.  
7 *Callosobruchus chinensis* is infected with CI-inducing *Wolbachia* and melanic mutants  
8 exhibit fitness decline. Interestingly, this decline is not observed in *C. analis* melanic mutants  
9 that are infected with CI-free *Wolbachia*. Our research question is whether the infection of  
10 CI-inducing *Wolbachia* causes fitness decline of melanic hosts in *C. analis*. We examined  
11 fecundity, fertility, and longevity of *C. analis* melanic mutants and compared them between  
12 uninfected and infected hosts with CI-inducing *Wolbachia*. Infected melanic mutants of *C.*  
13 *analis* exhibited fitness decline leading to reduced hatch rates even when parental  
14 combinations were compatible. *Wolbachia* can invade a host population by causing CI in  
15 order to decrease the fraction of uninfected hosts, but melanic mutant hosts decrease the  
16 number of infected hosts through fitness decline. Nevertheless, the melanism in hosts is not  
17 able to stop *Wolbachia* invasion in *C. analis*.

18  
19

## 1 **Introduction**

2 Symbiosis, first defined by de Bary (1879), broadly includes all interactions that have  
3 mutualistic, commensal, and parasitic effects on the host. Since symbionts with a vertical  
4 transmission mode share the same fate as their hosts, they should evolve to be harmless to the  
5 hosts (Ewald, 1987). However, vertically transmitted *Wolbachia*, an intracellular genus of  
6 bacteria detected by Hertig & Wolbach (1924), seems to strengthen parasitism. Yen & Barr  
7 (1973) found that cytoplasmic incompatibility (CI) is one of the reproductive manipulations  
8 that *Wolbachia* can induce. CI leads to early embryonic death in host offspring when female  
9 parents lack the same *Wolbachia* strain harbored by their mates (Werren et al., 2008). CI-  
10 inducing *Wolbachia* can invade a host population effectively because the relative infection  
11 frequency increases with CI (Turelli & Hoffmann, 1991; Turelli, 1994).

12 One antagonism against CI-inducing *Wolbachia* is reduced maternal transmission,  
13 which could indirectly contribute to reduced CI intensity (Turelli, 1994). Others are  
14 assortative aggregation of host eggs and pre-copulatory mate choice where hosts avoid  
15 choosing mates with which CI would occur (Vala et al., 2004; Jaenike et al., 2006). However,  
16 the evolution of these antagonisms is debatable because the number of hosts that exhibit such  
17 behaviors is very low, and they would be eliminated through drift in a population (Sahoo,  
18 2016).

19 Previous studies have reported that melanism had a positive effect on pathogen  
20 resistance (Wilson et al., 2001; Yassine et al., 2012; Dubovskiy et al., 2013). In *Spodoptera*  
21 *exempta* (Walker) (Lepidoptera: Noctuidae), melanic larvae exhibited lower mortality than  
22 non-melanic ones when they were exposed to pathogens (Wilson et al., 2001). Melanic  
23 *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) also have a higher tolerance to  
24 pathogen penetration than non-melanic ones, though their melanism is accompanied by  
25 fitness costs (Dubovskiy et al., 2013). *Infected Anopheles gambiae* (Giles) (Diptera:  
26 Culicidae) can delay internal pathogen proliferation through melanism (Yassine et al., 2012).

27 In *Callosobruchus* spp. bean beetles, the ease with which the melanic-body-color  
28 mutation can be seen enables its isolation from wild type beetles. Recessive mutations have  
29 been reported in *Callosobruchus chinensis* (L.) (Kashiwagi & Utida, 1972), but dominant,  
30 incomplete dominant and recessive mutations in *Callosobruchus maculatus* (Fabricius)  
31 (Breitenbecher, 1921; Eady, 1991; Mano & Toquenaga, 2011). The melanic mutation in  
32 *Callosobruchus analis* (Fabricius) found in the current study was recessive. As *Wolbachia*  
33 infection has been reported in *C. analis*, *C. chinensis*, and *Callosobruchus latealbus* (Pic)

1 (Kondo et al., 2011), recessiveness of melanic gene(s) in *Callosobruchus* may not have an  
2 effect on *Wolbachia* infection. However, Kashiwagi & Utida (1972) reported low fecundity  
3 and fertility among *C. chinensis* with the melanic-body mutation, concluding that the mutants  
4 could not survive in nature. As we do not observe such decline in melanic mutants of *C.*  
5 *analisis* infected with the wCana1 (accession no. AB545608) strain of *Wolbachia*, which is CI-  
6 free, and as *C. chinensis* is infected with a CI-inducing strain of *Wolbachia* (Kondo et al.,  
7 2002), we suspected that the decline is caused by an association between *Wolbachia*'s  
8 parasitism and host mutation. To investigate this association, we determined host fitness traits  
9 such as fecundity, longevity, and hatchability in *C. analisis* mutants with melanic bodies, and  
10 compared those traits between uninfected and infected mutant hosts with the CI-inducing  
11 wCana2 (accession no. LC090027) strain of *Wolbachia*. We hypothesized that fitness decline  
12 in infected melanic hosts may offset the reduction of uninfected hosts caused by CI. We built  
13 a mathematical model of the spread of the *Wolbachia* infection to examine whether  
14 *Wolbachia* can invade a host population with melanic mutants (see the supplementary  
15 information).

16

## 17 **Materials and methods**

### 18 **Source populations**

19 *Callosobruchus analisis* is a pest of stored legumes (Haines, 1989). Eggs are laid on the seed  
20 surface and hatched larvae grow inside a seed. As larvae exhibit contest-type resource  
21 competition (Toquenaga & Fujii, 1990; Mano et al., 2002), i.e. only one adult can emerge  
22 from a seed with multiple eggs, the population size of *C. analisis* is small. A population of *C.*  
23 *analisis* was derived from infested brown rice beans, *Vigna umbellata* (Thunb.) Ohwi & H.  
24 Ohashi (Fabaceae), at a market in 2005 in Colombo, Sri Lanka (S line). It is maintained on  
25 mung beans, *Vigna radiata* (L.) R. Wilczek, under laboratory conditions (30 °C, 60-70% r.h.,  
26 L24:D0) with about 180 adults per generation. The S line is naturally infected with a CI-free  
27 wCana1 (accession no. AB545608) strain of *Wolbachia* (Kageyama et al., 2010; Kondo et al.,  
28 2011). At least 30 generations after the establishment of the S line, two mated females with  
29 the melanic mutation were found (see Figure S1). All their offspring exhibited normal body  
30 color. Two females of the normal-colored offspring were crossed with two mutant males that  
31 were newly found. Their offspring exhibited both melanic and normal body colors; one  
32 normal-colored female produced 29 mutants out of 62 offspring and the other produced 28  
33 mutants out of 50 offspring. This indicates that the mutation was controlled by a single

1 recessive autosomal gene, establishing the mutant line as the melanic S (*b/b*) line. Wild type  
2 (*B/B*) and heterozygous (*B/b*) individuals exhibited normal body color.

3

#### 4 **Antibiotic treatment**

5 *Wolbachia* was removed from the S line using a tetracycline (TC) treatment. Larvae from the  
6 S line were fed with artificial beans containing 0.03% (wt/wt) TC. The artificial beans were  
7 made by mixing and kneading mung bean powder and TC, and pressing them to the bean size  
8 with a pollen press (6 mm diameter, 5 mm long). Artificial seed coats were added by dipping  
9 the artificial beans in collodion. Inseminated females from the S line were maintained and  
10 allowed to oviposit on the artificial beans. Emerged females were used to establish iso-female  
11 lines and were checked for infection using molecular identification of the wCana1 strain of  
12 *Wolbachia* with specific primers for the *wsp* gene as noted below ('Molecular identification  
13 of *Wolbachia*'). Uninfected females used to establish the non-infected (W-) line with *B/b* and  
14 *b/b* had been maintained in the laboratory for at least 21 generations.

15

#### 16 **Microinjection of *Wolbachia***

17 *Wolbachia* pellets (Braig et al., 1994; Grenier et al., 1998) were prepared by obtaining singly  
18 infected hosts with a wCana2 (accession no. LC090027) strain of *Wolbachia* as donors. The  
19 donor line singly infected with the wCana2 strain was established previously by injecting  
20 *Wolbachia* pellets, including both wCana1 and wCana2 strains from the Indonesian  
21 population of *C. analis*, into the uninfected S line. One injected host, which exhibited a  
22 single-strain wCana2 infection, was used to establish an iso-female S line. For the preparation  
23 of *Wolbachia* pellets, the whole body of the donor pupa was put into a 1.5-ml microtube and  
24 homogenized in 200  $\mu$ l of 30% sucrose in PBS (1.9 mM NaH<sub>2</sub>PO<sub>4</sub>, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, 175  
25 mM NaCl, pH 7.4). The homogenate was centrifuged at 1 509 *g* for 2 min to remove cellular  
26 debris. The supernatant was again centrifuged to remove any remaining debris. The  
27 supernatant then was centrifuged at 4 731 *g* for 5 min to pellet the *Wolbachia*. The resulting  
28 pellet was suspended in 5  $\mu$ l of PBS buffer by gently pipetting the solution on ice. *Wolbachia*  
29 pellet suspension (1  $\mu$ l) was microinjected directly in the ventral region between the thorax  
30 and abdomen of each uninfected pupa using glass needles with manually cut tips. After  
31 injections, emerged virgin females were mated with untreated males. We established an  
32 infected iso-female line (W+) by isolating a generation-one (G1) female that was infected.  
33 The single infection with the wCana2 strain of *Wolbachia* was checked using molecular  
34 identification with specific primers for the *wsp* gene as noted in the following section.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34

## **Molecular identification of *Wolbachia***

Infection status was confirmed before crossing experiments. For DNA extraction, live insects were first preserved in acetone (99.5%). One hind leg for each sample was put into a 0.2- $\mu$ l plastic tube with 100  $\mu$ l lysis buffer (1 mM EDTA, 10 mM Tris-HCl, 50 mM NaCl) and proteinase K, incubated at 55 °C for 1 h and 99 °C for 10 min, and preserved at 4 °C. The supernatant was used as the template in subsequent PCR reactions. PCR detection of *Wolbachia* from total DNA of *C. analis* was conducted using GoTaq hot-start green master mix (Promega) with specific primers for the *wsp* gene. A 361-bp fragment of the *wsp* gene in a wCana1 strain was amplified using Cana1F [5'-GCCTGCAGTACAATGGTGAA-3'] and Cana1R [5'-TGATCCTTAACTGCGTCAGC-3'] under a temperature profile of 95 °C for 10 min followed by 30 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min. The last extension step lasted 5 min at 72 °C. A 333-bp fragment of the *wsp* gene in a wCana2 strain was also amplified using Cana2F [5'-GTTTCGTTTGCAATATAATGGTGA-3'] and Cana2R [5'-GCTTACATACGCTGCACCAA-3'] under the same temperature profile. The PCR products were electrophoresed in TAE-agarose gels, stained with SYBR-safe DNA gel stain (Invitrogen, Carlsbad, CA, USA), and observed using a blue light transilluminator (Thermo Fisher Scientific, Inc., Waltham, MA, USA). In order to control for the failure of amplification with the primers, we tested whether the samples scored as negative for primers would result in positive amplification of the host mitochondrial cytochrome oxidase subunit I gene (*COI*) by amplifying a 608-bp fragment of the *COI* gene using CanaCOIF [5'-TCCTTTTATTACTTTCTCTACCCGTTT-3'] and CanaCOIR [5'-TTCCTGTAAATAAGGGGAATCA-3'] under a temperature profile of 95 °C for 10 min followed by 30 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 1 min.

## **Establishment of infected and uninfected melanic lines**

We used uninfected (W-) and wCana2-infected (W+) lines for all three genotypes (*B/b*, *B/B*, and *b/b*) produced as shown in the procedure illustrated in Figure 1. To obtain heterozygous (*B/b*) offspring, wild type (*B/B*) females of W- and W+ lines were crossed with melanic males from the S (*b/b*) line whose wCana1 was CI-free with three replicates each. All first-generation (F1) offspring exhibited a normal body color. Heterozygous F1 females from each replicate were crossed again with the melanic males from the S (*b/b*) line with three replicates. Second generation (F2) offspring included both *B/b* and *b/b* genotypes. The F2 offspring and *B/B* individuals from the W- and W+ lines were used to examine host fitness traits.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33

## Host fitness traits

Fecundity and hatch rates were examined by conducting four reciprocal crossings among *B/b* and *b/b* individuals and one crossing between *B/B* females and males. Fecundity was checked by depositing virgin adults onto beans within 24 h of emergence. Virgin males were individually mated with virgin females. Each of 10 to 24 pairs was set in a 10-cm-diameter dish filled with about 60 mung beans. Males and females were allowed to mate and oviposit until they died. Fecundity was assessed by counting the eggs that a female laid. Hatch rates were checked by counting hatched and unhatched eggs. Longevity of infected and uninfected *S* lines of all three genotypes was determined by counting days from the adult emergence to its death (13-77 replicates) in absence of food.

## Cytoplasmic incompatibility assay

To examine whether the *wCana2* strain of *Wolbachia* induced CI or hatch rate reduction, we investigated hatch rates of incompatible (i.e., uninfected female × infected male) parental combinations and of reciprocal combinations for three genotypes (10-15 replicates). Hatch rates were determined using the same procedure noted above. We excluded the crosses with no oviposition.

## Statistical analysis

We applied the Bayesian hierarchical model below with a joint scaling method (JSM; Mather & Jinks, 1982; Takano et al., 2001). JSM uses a design matrix (Table 1) to assign additive and dominance effects of a gene (Wolf et al., 2000), and other effects such as maternal and infection effects. For fecundity the following model was applied:

$$\text{Fecundity} \sim \text{Poisson}(\mu_i)$$

$$M_i = D_{\text{fec},i} \beta_{\text{fec}}^t$$

where  $D_{\text{fec},i}$  represents the design matrix for crossing type *i* as represented in Table 1,  $\beta_{\text{fec}}$  is the vector  $\beta_{\text{fec}} = [\beta_{\text{icp}}, \beta_A, \beta_D, \beta_M, \beta_I, \beta_{A \times D}, \dots, \beta_{A \times D \times I}, \dots, \beta_{A \times D \times I \times M}]$  of parameters for the effects of intercept (icp), additive (A), dominance (D), maternal (M), infection (I), and their interaction effects.  $\mu_i$  is the mean fecundity of crossing type *i*. Priors were assigned to  $\beta$ s and  $\sigma$ : Normal(0,  $\sigma^2$ ) and Uniform(0,100) (Gelman & Hill, 2006).

We applied the following model for hatch rates;

$$\text{hatched eggs} \sim \text{Binomial}(p_i, t)$$

1  $\text{logit}(p_i) = D_{h,i} \beta_h^t$ ,

2 where  $p_i$  is the hatching probability when crossing type  $i$ ,  $t$  is the total number of eggs  
 3 deposited,  $D_{h,i}$  represents a part of the design matrix for crossing type  $i$ , as in Table 1, and  $\beta_h$   
 4 is the vector  $\beta_h = [\beta_{icp}, \beta_A, \beta_D, \beta_M, \beta_I, \beta_{A \times D}, \dots]$  of parameters for the effects as explained  
 5 above. We assigned  $\text{Normal}(0, \sigma^2)$  to each  $\beta$  and  $\text{Uniform}(0, 100)$  to  $\sigma$ .

6 For longevity the following model was applied:

7  $\text{longevity} \sim \text{Normal}(\mu_1, \sigma^2)$

8  $\mu_1 = \text{intercept} + \beta_G + \beta_I + \beta_S + \beta_{G \times I} + \beta_{G \times S} + \beta_{I \times S}$ ,

9 where  $\beta_s$  represents host genotype (G), infection (I), and sex (S) effects and their interaction  
 10 effects.  $\mu_1$  is mean longevity.  $\text{Normal}(0, \sigma^2)$  and  $\text{Uniform}(0,100)$  were assigned to  $\beta$  and  $\sigma$ ,  
 11 respectively.

12 Samplings from posterior distributions of the parameters using Markov Chain Monte  
 13 Carlo (MCMC) methods were performed using the R2jags package (Su & Yajima, 2015) on  
 14 R v.3.3.1 (R Core Team, 2016). The posterior samples were obtained by running 10 000  
 15 iterations (the first 5 000 iterations were discarded as a burn-in) for each of four independent  
 16 MCMC chains. The convergence of MCMC calculations was confirmed by evaluating the  
 17 results of Gelman and Rubin's convergence diagnostic (Gelman & Rubin, 1992) for each  
 18 parameter by comparing within-chain and between-chain variances. We also calculated  $R^2$ ,  
 19 the proportion of variance explained by the hierarchical model at the data level (Gelman &  
 20 Hill, 2006).

21

## 22 **Results**

23 Declines in host fecundity, fertility, and longevity were observed when mutant hosts were  
 24 infected with the wCana2 strain of *Wolbachia*. Both wild type and heterozygous parents  
 25 showed few differences in fecundity between infected and uninfected groups (Figure 2).  
 26 However, reduced fecundity was observed in infected hosts compared to uninfected hosts  
 27 when  $b/b$  parents were included. In particular, infected  $b/b$  females produced fewer eggs than  
 28 the others. The mean fecundity was eight and 69 eggs, respectively, in infected and  
 29 uninfected  $b/b \times b/b$  (female  $\times$  male) crosses, and six and 69 eggs, respectively, in infected  
 30 and uninfected  $b/b \times B/b$  crosses. A slight reduction in fecundity was also observed in  
 31 uninfected  $b/b$  mutants compared to the other uninfected groups. Estimated parameters for  
 32 the examined effects of fecundity are shown in Figure 3. Negative values of estimates



1 indicate reduced host fecundity. The parameter for the intercept effect was more positive than  
2 the others (95% CrI: 3.2–5.5). Effects of infection (I) and its interaction with additive (A×I)  
3 were distributed positively (95% CrI: -0.2–2.1 for I, -0.1–2.1 for A×I), but its interaction  
4 effect with maternal (M×I) was distributed negatively (-4.5– -0.1). Thus, the loss of fecundity  
5 in the infected *b/b* females was caused by the M×I effect.

6 Reduced hatch rates were observed when parents were infected with *Wolbachia*  
7 compared to the hatch rates of uninfected parents (Figure 4). Mutant *b/b* females exhibited  
8 lower hatch rates than the others when they were infected with the wCana2 strain of  
9 *Wolbachia*. The mean hatch rate was 0.2 and 0.7, respectively, in infected and uninfected *b/b*  
10 × *b/b* (female × male) crosses, and 0.4 and 0.8, respectively, in infected and uninfected *b/b* ×  
11 *B/b* crosses. The distributions of parameters for host hatch rates are shown in Figure 5. The  
12 parameters for the I and M×I effects were distributed negatively (95% CrI: -1.1–0.1 for I, -  
13 3.8– -1.4 for M×I) whereas positive distributions were observed for the effects of intercept,  
14 A×I (0.2–1.4) and D×I (1.3–2.7). The hatch rate reduction observed in infected compared to  
15 uninfected hosts was caused by the I effect. The M×I effect contributed negatively to hatch  
16 rates when female parents were infected *b/b* mutants. The M effect partly contributed to the  
17 hatch rate reduction (95% CrI: -3.4–0.8), indicating that the maternal *b* allele also causes  
18 reduction independently of host infection status.

19 Both infected and uninfected males of *B/B* and *B/b* had shorter longevity than females  
20 (Figure 6). The mutant *b/b* hosts had more reduced longevity than the others, especially when  
21 hosts were *Wolbachia* infected. The mean longevity was 4 days in both infected *b/b* females  
22 and males. Estimated parameter distributions for host longevity are shown in Figure 7. Most  
23 of the parameters were distributed positively, but negative distributions were observed for the  
24 *b/b* effect (95% CrI: -7.6–1.1) and its interaction effects with female (*b/b*×female: -6.7–1.3)  
25 and infection (*b/b*×I: -9.3– -1.1). Thus, host longevity was shortened by the effects of *b/b*,  
26 *b/b*×female, and *b/b*×I.

27 Cytoplasmic incompatibility assays revealed that the wCana2 strain of *Wolbachia*  
28 induced almost complete CI ( $R^2 = 0.99$ ; Figure 8) with a mean hatch rate of 0. Note, however,  
29 that non-CI-occurring parental combinations (i.e., infected females × uninfected males) also  
30 exhibited hatch rate reduction (mean hatch rate: 0.2) when host genotypes were *b/b*. This is  
31 consistent with the results described above that infected *b/b* females had reduced hatch rates.  
32 The parameter estimation for the effects of CI is shown in Figure 9. Negatively distributed  
33 parameters of I (95% CrI: -8.3– -1.9) and D×I (-3.1–0.1) effects indicate reduced hatch rates  
34 due to these effects. The interaction effect of A×I was distributed positively (95% CrI: -0.7–

1 5.7).

2

### 3 **Discussion**

4 The combination of CI-inducing *Wolbachia* and host mutation of body color affected host  
5 fitness traits negatively. When infected with the wCana2 strain of *Wolbachia*, *b/b* mutants  
6 had reduced fecundity, fertility, and longevity. The fitness decline indicates that the *b/b*  
7 mutant hosts infected with wCana2 cannot survive in nature. The fitness decline observed in  
8 the infected *b/b* mutant hosts was caused by the interaction of hosts and *Wolbachia* because  
9 the decline was accompanied by the combination of host mutation and the infection of CI-  
10 inducing *Wolbachia*. CI assay revealed that the reduction in hatch rates occurred not only in  
11 the incompatible parental combinations but also in compatible combinations when hosts had  
12 *b/b* genotypes. Although the number of infected hosts was reduced by the infected *b/b*  
13 females, the model for *Wolbachia* infection spread suggested that the host *b/b* mutation could  
14 not stop the *Wolbachia* invasion (see the supplementary information).

15 Some studies have reported that mutations of melanic body color, controlled by a  
16 single recessive autosomal gene, are associated with a fitness decline in other Coleoptera  
17 species. Ebony mutants in the flour beetle, *Tribolium confusum* Jaquelin Du Val, exhibit  
18 reduced fecundity compared to heterozygous and wild type adults (Park et al., 1945). Black  
19 mutants in the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), exhibit low egg  
20 viability (Boiteau, 1985). In *C. chinensis*, declines in fecundity and fertility have also been  
21 reported (Kashiwagi & Utida, 1972). The fitness decline is accompanied by a mutation of  
22 melanic body color independently of CI-inducing *Wolbachia* infection because even  
23 uninfected mutants in *C. analis* had a slight decline in fitness traits (cf. uninfected *b/b*  
24 females in Figures 2 and 4; uninfected *b/b* in Figure 6:). Possibly, *Wolbachia* contributes to a  
25 worsening of the fitness decline caused by the *b/b* genotype in *C. analis*. Starr & Cline (2002)  
26 reported that host fitness was modified by *Wolbachia* infection. They found that removing  
27 *Wolbachia* induced an oogenesis deficit in *Drosophila* hosts and concluded that *Wolbachia*  
28 remedied the deficit. Although we did not examine oogenesis in *C. analis*, fecundity  
29 reduction in infected *b/b* females suggests that *Wolbachia* contributes to fecundity reduction.  
30 In this light, our results provide an example of the *Wolbachia* association with host oogenesis  
31 that contrasts with that reported by Starr & Cline (2002).

32 As the wCana2 strain induced CI in hosts, we emphasize that the reduction in  
33 examined host traits was caused by CI-inducing *Wolbachia*. The *b/b* mutants in *C. analis*

1 were first discovered in hosts originally infected with the wCana1 strain of *Wolbachia*. As the  
2 wCana1 strain was CI-free, the host fitness decline had an association with CI induced by  
3 wCana2 in *C. analis*. The relative percentage of the *b* allele can become high in a small  
4 population due to random drift. However, although the population size of *C. analis* in nature  
5 is thought to be small, the frequency of the *b* allele seems to be low: all of our *C. analis* lines  
6 obtained in nature exhibited an initially normal body color. Even if *b/b* mutants are fixed in a  
7 host population, incomplete sterility of infected *b/b* females produces a fraction of infected  
8 zygotes and the sterility reduces the fraction of infected *b/b* zygotes. Because of the reduction  
9 of infected *b/b* zygotes, *Wolbachia* can easily overcome the invasion threshold in the  
10 following host generation, leading to the fixation of *Wolbachia*. If the sterility of infected *b/b*  
11 females was complete and the *b/b* mutation was fixed in a host population by random drift  
12 (i.e., all hosts showed the *b/b* genotype) *Wolbachia* would not be able to invade the host  
13 population because infected *b/b* females would not produce viable infected offspring.

14 How the wCana1-infected hosts prevent the wCana2 invasion is unclear, because the  
15 sterility of infected *b/b* females is incomplete, meaning that it cannot be an antagonism  
16 against CI-inducing *Wolbachia* in *C. analis*. One possible explanation is that the wCana1  
17 strain was once CI-inducing *Wolbachia*, as is the wCana2 strain, and that CI was moderated  
18 after the wCana1 fixation by the host during the coevolutionary history between host and  
19 *Wolbachia*. Indeed, host infection experience has been reported to have an effect on the  
20 reduction of CI intensity (Poinsoot & Merçot, 2001). The wCana1-infected hosts may be  
21 eliminated by the wCana2-infected hosts in the future.

22 In conclusion, we found that *C. analis* body-color mutants exhibited a fitness decline  
23 in fecundity, fertility, and longevity when hosts were infected with a CI-inducing *Wolbachia*  
24 strain. The decline caused by infected *b/b* hosts cannot stop a *Wolbachia* invasion unless the  
25 sterility of infected *b/b* females is complete and the melanic mutants are fixed in a host  
26 population. If the CI-free wCana1 strain was caused by CI moderation during the  
27 coevolutionary history, the wCana2 strain may also become CI-free. To confirm the role of  
28 coevolutionary history in the ability of *Wolbachia* to induce CI, further investigations are  
29 needed.

30

### 31 **Acknowledgments**

32 This study was supported in part by Grant-in-Aids for Scientific Research from the JSPS  
33 (Nos. 14405003, 17405005, 17570014, 20405006, 23405008, 23570017, and 26304016 to  
34 YT).

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34

## References

- Boiteau G (1985) Bionomics and genetics of a black mutant Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Annals of the Entomological Society of America* 78: 663–666.
- Braig HR, Guzman H, Tesh RB & O'Neill SL (1994) Replacement of the natural *Wolbachia* symbiont of *Drosophila simulans* with a mosquito counterpart. *Nature* 367: 453–455.
- Breitenbecher JK (1921) The genetic evidence of a multiple (triple) allelomorph system in *Bruchus* and its relation to sex-limited inheritance. *Genetics* 6: 65–90.
- de Bary A (1879) *Die Erscheinung der Symbiose*. Verlag Karl J Trübner, Strasbourg, France.
- Dubovskiy I, Whitten M, Kryukov V, Yaroslavtseva O, Grizanov E et al. (2013) More than a colour change: insect melanism, disease resistance and fecundity. *Proceedings of the Royal Society of London B* 280: 1–10.
- Eady P (1991) Sperm competition in *Callosobruchus maculatus* (Coleoptera: Bruchidae): a comparison of two methods used to estimate paternity. *Ecological Entomology* 16: 45–53.
- Ewald PW (1987) Transmission modes and evolution of the parasitism–mutualism continuum. *Annals of the New York Academy of Sciences* 503: 295–306.
- Fine PE (1978) On the dynamics of symbiote–dependent cytoplasmic incompatibility in culicine mosquitoes. *Journal of Invertebrate Pathology* 31: 10–18.
- Gelman A & Hill J (2006) *Data Analysis Using Regression and Multilevel/Hierarchical Models*. Cambridge University Press, Cambridge, UK.
- Gelman A & Rubin DB (1992) Inference from iterative simulation using multiple sequences. *Statistical Science* 7: 457–472.
- Grenier S, Bernard P, Heddi A, Lassablière F, Jager C et al. (1998) Successful horizontal transfer of *Wolbachia* symbionts between *Trichogramma* wasps. *Proceedings of the Royal Society of London B* 265: 1441–1445.
- Haines C (1989) Observations on *Callosobruchus analis* (F.) in Indonesia, including a key to storage *Callosobruchus* spp (Col., Bruchidae). *Journal of Stored Products Research* 25: 9–16.
- Hertig M & Wolbach SB (1924) Studies on rickettsia-like micro-organisms in insects. *Journal of Medical Research* 44: 329–374.
- Jaenike J, Dyer KA, Cornish C & Minhas MS (2006) Asymmetrical reinforcement and *Wolbachia* infection in *Drosophila*. *PLoS Biology* 4: e325.

- 1 Mather K & Jinks JL (1982) Biometrical Genetics– The Study of Continuous Variation.  
2 Chapman and Hall, London, UK.
- 3 Kageyama D, Narita S, Imamura T & Miyanoshita A (2010) Detection and identification of  
4 *Wolbachia* endosymbionts from laboratory stocks of stored–product insect pests and  
5 their parasitoids. *Journal of Stored Products Research* 46: 13–19.
- 6 Kashiwagi M & Utida S (1972) A new mutant in *Callosobruchus chinensis* L. (Coleoptera:  
7 Bruchidae). *Applied Entomology and Zoology* 7: 95–96.
- 8 Kondo N, Ijichi N, Shimada M & Fukatsu T (2002) Prevailing triple infection with  
9 *Wolbachia* in *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Molecular Ecology*  
10 11: 167–180.
- 11 Kondo NI, Tuda M, Toquenaga Y, Lan YC, Buranapanichpan S et al. (2011) *Wolbachia*  
12 infections in world populations of bean beetles (Coleoptera: Chrysomelidae: Bruchinae)  
13 infesting cultivated and wild legumes. *Zoological Science* 28: 501–508.
- 14 Mano H & Toquenaga Y (2011) Contest–type competition between age classes in scramble–  
15 type *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Entomological Science* 14:  
16 166–172.
- 17 Mano H, Toquenaga Y & Fujii K (2002) Scramble competition in *Callosobruchus analis*  
18 (Coleoptera: Bruchidae). *Population Ecology* 44: 259–264.
- 19 Park T, Ginsburg B & Horwitz S (1945) Ebony: a gene affecting the body color and  
20 fecundity of *Tribolium confusum* Duval. *Physiological Zoology* 18: 35–52.
- 21 Poinot D & Merçot H (2001) *Wolbachia* injection from usual to naive host in *Drosophila*  
22 *simulans* (Diptera: Drosophilidae). *European Journal of Entomology* 98: 25–30.
- 23 R Core Team (2016) R: A Language and Environment for Statistical Computing. R  
24 Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>  
25 (accessed 31-07-2016).
- 26 Sahoo RK (2016) Why antagonistic traits against cytoplasmic incompatibility are so elusive.  
27 *Frontiers in Microbiology* 7: 392.
- 28 Starr DJ & Cline TW (2002) A host–parasite interaction rescues *Drosophila* oogenesis  
29 defects. *Nature* 418: 76–79.
- 30 Su Y & Yajima M (2015) R2jags: Using r to run jags. R package v.0.5-7. [https://CRAN.R-](https://CRAN.R-project.org/package=R2jags)  
31 [project.org/package=R2jags](https://CRAN.R-project.org/package=R2jags). (accessed 12-11-2015).
- 32 Takano M, Toquenaga Y & Fujii K (2001) Polymorphism of competition type and its  
33 genetics in *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Population Ecology* 43:  
34 265–273.

- 1 Toquenaga Y & Fujii K (1990) Contest and scramble competition in two bruchid species,  
2 *Callosobruchus analis* and *C. phaseoli* (Coleoptera: Bruchidae) I. larval competition  
3 curves and interference mechanisms. *Researches on Population Ecology* 32: 349–363.
- 4 Turelli M (1994) Evolution of incompatibility-inducing microbes and their hosts. *Evolution*  
5 48: 1500–1513.
- 6 Turelli M & Hoffmann AA (1991) Rapid spread of an inherited incompatibility factor in  
7 California *Drosophila*. *Nature* 353: 440–442.
- 8 Vala F, Egas M, Breeuwer JAJ & Sabelis MW (2004) *Wolbachia* affects oviposition and  
9 mating behaviour of its spider mite host. *Journal of Evolutionary Biology* 17: 692–700.
- 10 Werren JH, Baldo L & Clark ME (2008) *Wolbachia*: master manipulators of invertebrate  
11 biology. *Nature Reviews Microbiology* 6: 741–751.
- 12 Wilson K, Cotter SC, Reeson AF & Pell JK (2001) Melanism and disease resistance in  
13 insects. *Ecology Letters* 4: 637–649.
- 14 Wolf JB, Brodie ED & Wade MJ (2000) *Epistasis and the Evolutionary Process*. Oxford  
15 University Press, Oxford, UK.
- 16 Yassine H, Kamareddine L & Osta MA (2012) The mosquito melanization response is  
17 implicated in defense against the entomopathogenic fungus *Beauveria bassiana*. *PloS*  
18 *Pathogens* 8: e1003029.
- 19 Yen JH & Barr AR (1973) The etiological agent of cytoplasmic incompatibility in *Culex*  
20 *pipiens*. *Journal of Invertebrate Pathology* 22: 242–250.

21  
22

### 23 **Figure captions**

24 **Figure 1** Experimental flow for the preparation of wCana2-infected (W+) and uninfected (W-  
25 ) host lines with three genotypes. The recessive melanic mutation of body color is expressed  
26 as *b/b*. Wild type (*B/B*) and heterozygous (*B/b*) individuals exhibited normal body color.  
27 Wild type individuals of the W-line were established using antibiotic treatment for the wild  
28 type S line. Some of these were injected with the wCana2 strain of *Wolbachia* to establish the  
29 wild type W+ line. These *B/B* females were crossed with the mutant *b/b* males from the S line  
30 to obtain *B/b* offspring. The heterozygous first generation (F1) females were crossed again  
31 with the *b/b* males and the second generation (F2) *B/b* and *b/b* offspring were obtained. For  
32 experiments we used the six shaded host lines: W- (*B/B*, *B/b*, *b/b*) and W+ (*B/B*, *B/b*, *b/b*).

33

34 **Figure 2** Estimated fecundity (total no. eggs produced) is shown for the assigned parental

1 combinations ( $R^2 = 0.83$ ). Three genotypes represent wild type ( $B/B$ ), heterozygous ( $B/b$ ),  
2 and melanic mutant ( $b/b$ ) parents. Grey boxes indicate the *Wolbachia* infection and the others  
3 are infection-free. The thick lines represent the median value and the limits of the box  
4 represent inter-quartile range. The whiskers outside the box extend to the highest and lowest  
5 values within 1.5 times the inter-quartile range. Points outside the whiskers are outliers.  
6 Numbers along the x-axis indicate replicates.

7

8 **Figure 3** Estimated parameter distributions for the effects listed along the y-axis. Circles  
9 represent means of the estimated posteriors. The 50% (thick line) and 95% (thin line)  
10 posterior credible intervals are shown. Negative values represent reduced fecundity.

11

12 **Figure 4** Estimated hatch rates of offspring from the assigned parental combinations ( $R^2 =$   
13  $0.97$ ). Three genotypes represent wild type ( $B/B$ ), heterozygous ( $B/b$ ), and melanic mutant  
14 ( $b/b$ ) parents. Grey boxes indicate the *Wolbachia* infection and the others are infection-free.  
15 The thick lines represent the median value and the limits of the box represent inter-quartile  
16 range. The whiskers outside the box extend to the highest and lowest values within 1.5 times  
17 the inter-quartile range. Points outside the whiskers are outliers. Numbers along the x-axis  
18 indicate replicates.

19

20 **Figure 5** Estimated parameter distributions for the effects listed along the y-axis. Circles  
21 represent means of the estimated posteriors. The 50% (thick line) and 95% (thin line)  
22 posterior credible intervals are shown. Negative values represent reduced hatch rates.

23

24 **Figure 6** Estimated longevity of adult beetles ( $R^2 = 0.86$ ). Three genotypes represent wild  
25 type ( $B/B$ ), heterozygous ( $B/b$ ), and melanic mutant ( $b/b$ ) parents. The thick lines represent  
26 the median value and the limits of the box represent inter-quartile range. The whiskers  
27 outside the box extend to the highest and lowest values within 1.5 times the inter-quartile  
28 range. Points outside the whiskers are outliers. Numbers along the x-axis indicate replicates.

29

30 **Figure 7** Estimated parameter distributions are shown for the effects of genotype, infection,  
31 and sex. Circles represent means of the estimated posteriors. The 50% (thick line) and 95%  
32 (thin line) posterior credible intervals are shown. Negative values represent reduced longevity.

33

34 **Figure 8** Estimated hatch rates in offspring from parents with assigned genotypes ( $R^2 = 0.99$ ).

1 Three genotypes represent wild type ( $B/B$ ), heterozygous ( $B/b$ ), and melanic mutant ( $b/b$ )  
2 parents. Parents of infected ( $W+$ ) females and uninfected ( $W-$ ) males are non-CI occurring  
3 combinations, whereas the opposite combinations have CI. The thick lines represent the  
4 median value and the limits of the box represent inter-quartile range. The whiskers outside  
5 the box extend to the highest and lowest values within 1.5 times the inter-quartile range.  
6 Points outside the whiskers are outliers. Numbers along the x-axis indicate replicates.

7

8 **Figure 9** Estimated parameter distributions for the effects listed along the y-axis. Circles  
9 represent means of the estimated posteriors. The 50% (thick line) and 95% (thin line)  
10 posterior credible intervals are shown. Negative values represent reduced hatch rates.

11

12

### 13 **Supporting Information**

14 Additional Supporting Information may be found in the online version of this article.

15

### 16 **Model for *Wolbachia* infection spread**

#### 17 **Results for infection dynamics**

18

19 **Figure S1** Females (above) and males (below) of normal and melanic colored  
20 *Callosobruchus analis*.

21

22 **Figure S2** Infection status of zygotes.  $p$  is the proportion of infected hosts,  $q$  and  $r$  are  
23 frequencies of allele  $b$  in infected and uninfected hosts, respectively. Each box represents the  
24 relative proportion of zygotes produced by random mating between host females and males.  
25 Numbers in the boxes represent the  $b/b$  proportion among zygotes. The status of female  
26 parents is represented along the vertical axis and that of the males along the horizontal axis.  $s$   
27 and  $\beta$  are cytoplasmic incompatibility (CI) intensity and sterility level of  $b/b$  females,  
28 respectively. Zygotes fail to develop with the presence of CI and with  $b/b$  females.  $B/B$ , wild  
29 type;  $B/b$ , heterozygotes;  $b/b$ , melanic mutant.

30

31 **Figure S3** Parameter space of  $\Delta p$  when (A)  $s = 0.5$  and (B)  $b = 0.5$ . Combinations of ( $p, q$ )  
32 below the various dotted lines satisfy the condition to stop the *Wolbachia* invasion ( $\Delta p \leq 0$ ).  $p$   
33 is the frequency of infected zygotes,  $q$  is the frequency of the  $b$  gene in the infected zygotes,  
34 and  $s$  is the cytoplasmic incompatibility intensity (offspring survival rate).



1

2 **Figure S4** Parameter space of  $\Delta q$  ( $b = 0.8$ ,  $s = 0.5$ ). Combinations of  $(p,q,r)$  below dotted  
3 lines satisfy the condition  $\Delta q \geq 0$ .

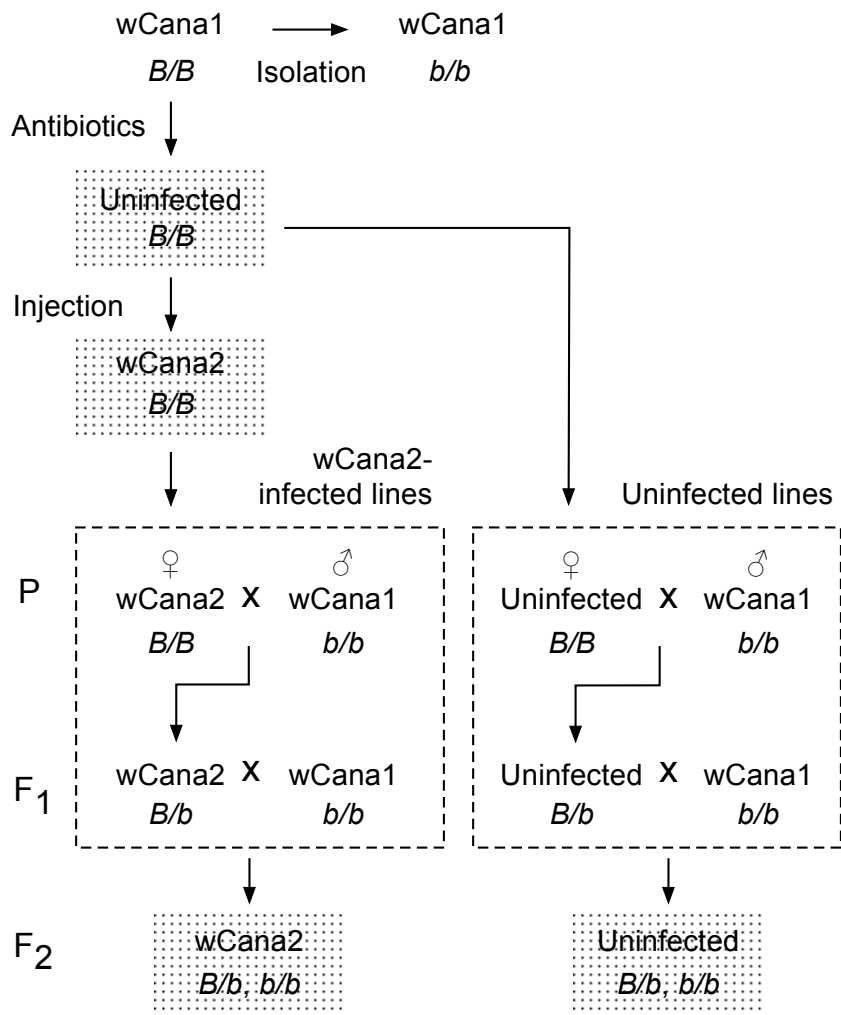
1 **Table 1** Design matrix for joint scaling method

Status	Cross type (female × male)	Intercept	A	D	M	I	A×D	A×I	A×M	D×I	D×M	I×M	A×D×I	A×D×M	A×I×M	D×I×M	A×D×I×M
Uninfected	<i>b/b</i> × <i>b/b</i>	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0
	<i>b/b</i> × <i>B/b</i>	1	1/2	1/2	1	0	1/4	0	1/2	0	1/2	0	0	1/4	0	0	0
	<i>B/b</i> × <i>b/b</i>	1	1/2	1/2	1/2	0	1/4	0	1/4	0	1/4	0	0	1/8	0	0	0
	<i>B/b</i> × <i>B/b</i>	1	0	1/2	1/2	0	0	0	0	0	1/4	0	0	0	0	0	0
	<i>B/B</i> × <i>B/B</i>	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Infected	<i>b/b</i> × <i>b/b</i>	1	1	0	1	1	0	1	1	0	0	1	0	0	1	0	0
	<i>b/b</i> × <i>B/b</i>	1	1/2	1/2	1	1	1/4	1/2	1/2	1/2	1/2	1	1/4	1/4	1/2	1/2	1/4
	<i>B/b</i> × <i>b/b</i>	1	1/2	1/2	1/2	1	1/4	1/2	1/4	1/2	1/4	1/2	1/4	1/8	1/4	1/4	1/8
	<i>B/b</i> × <i>B/b</i>	1	0	1/2	1/2	1	0	0	0	1/2	1/4	1/2	0	0	0	1/4	0
	<i>B/B</i> × <i>B/B</i>	1	-1	0	0	1	0	-1	0	0	0	0	0	0	0	0	0

2 Effects: A, additive; D, dominance; M, maternal; I, infection.

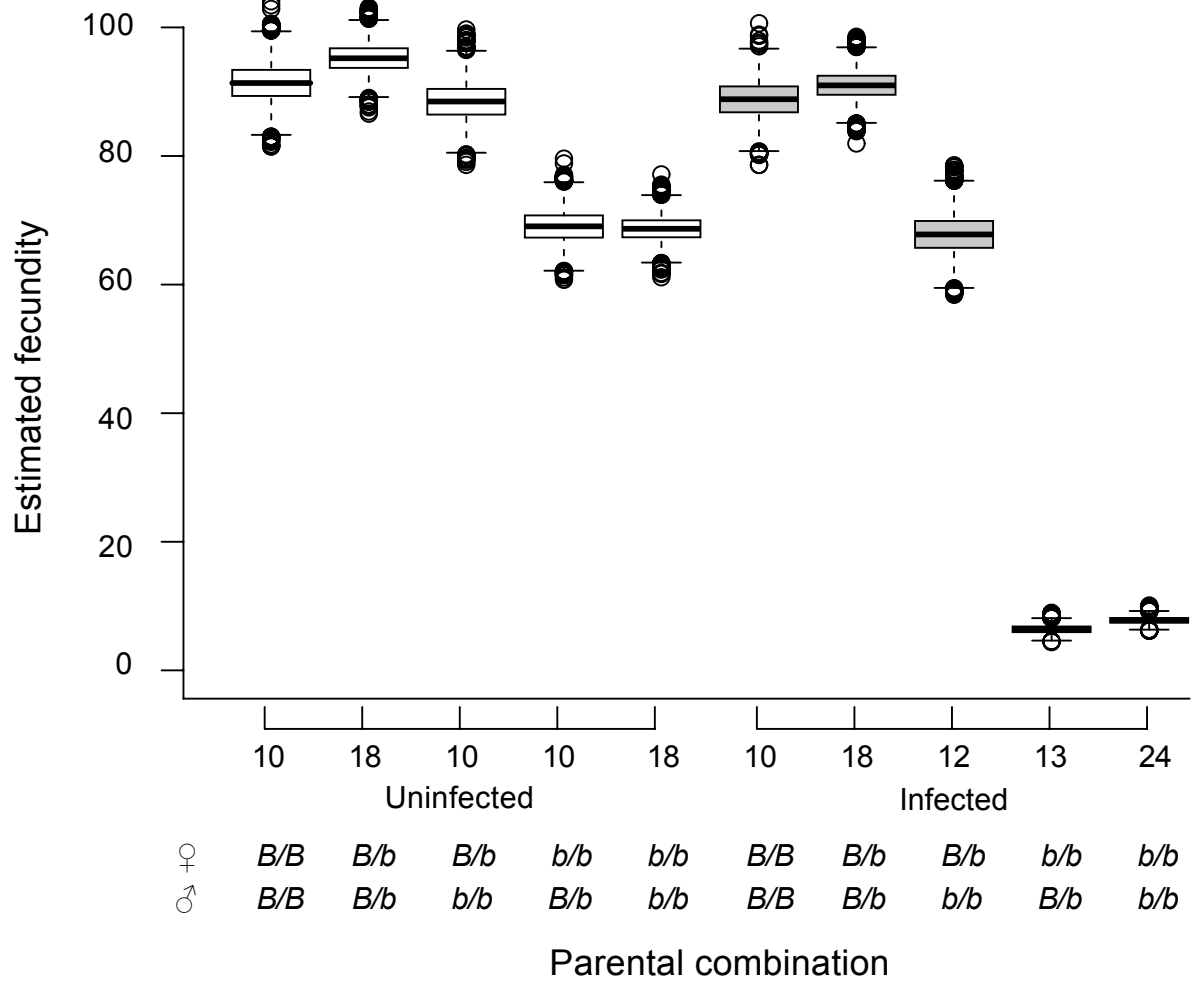
3

1 Figure 1



2  
3

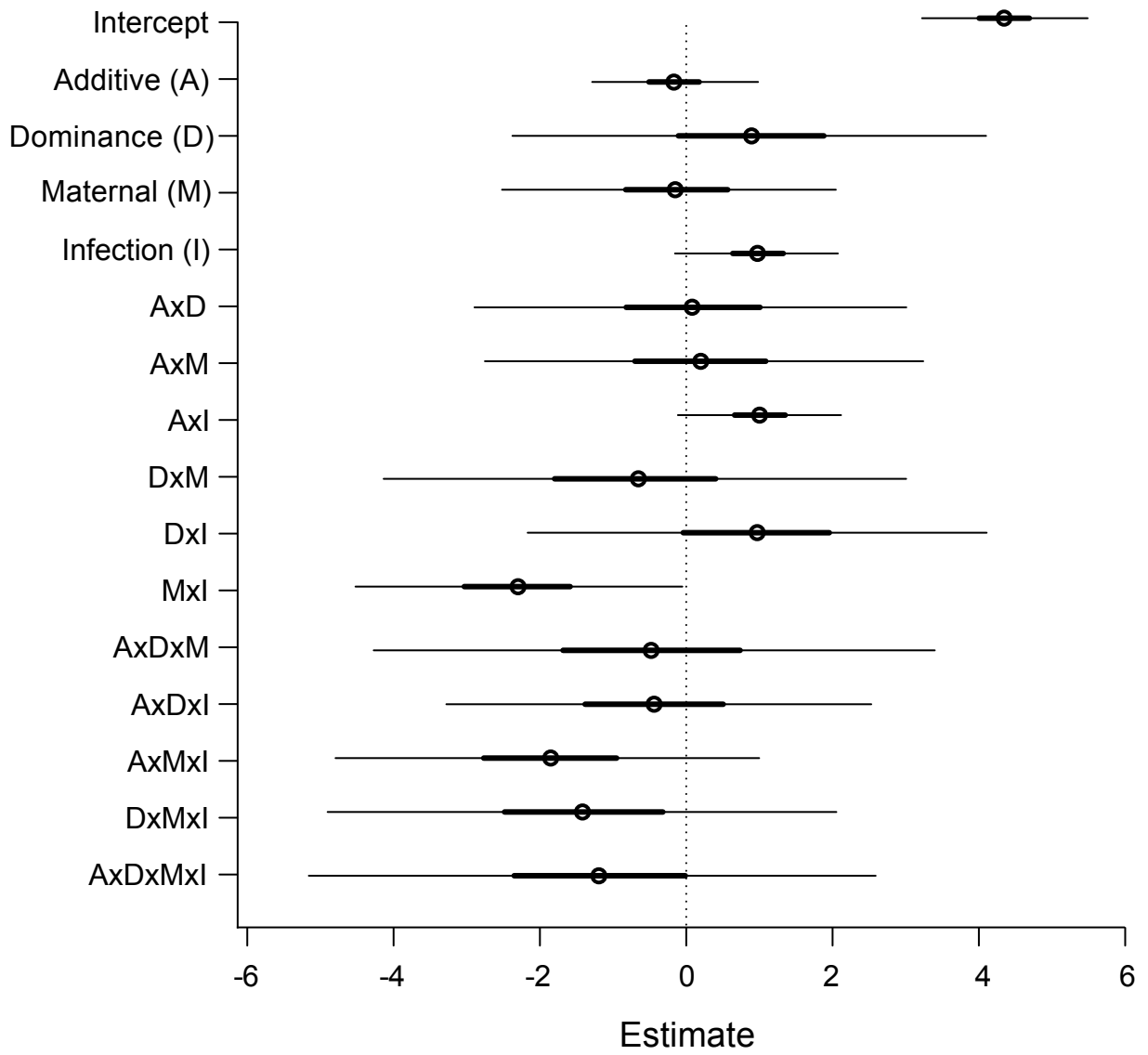
1 Figure 2



2

3

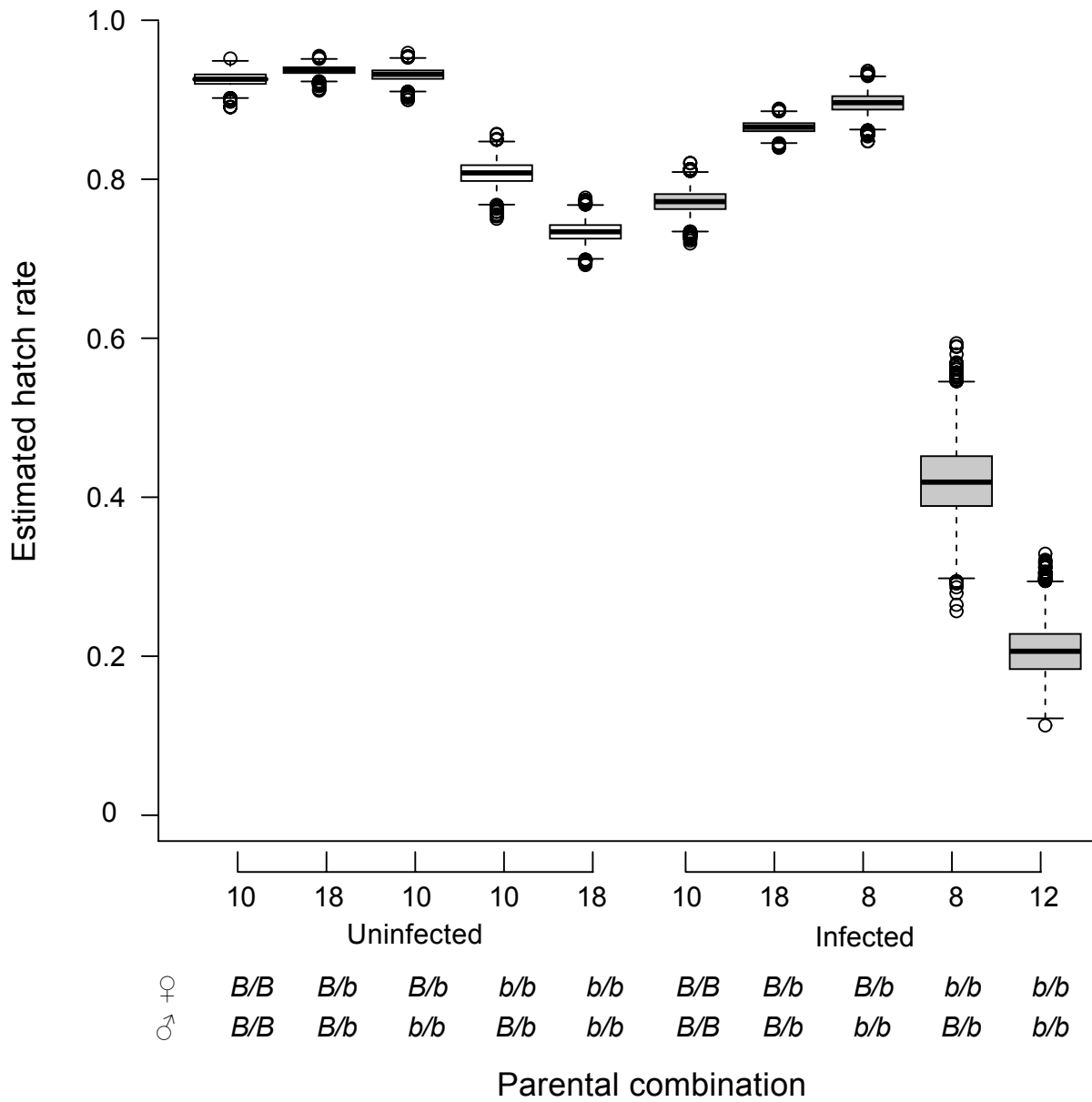
1 Figure 3



2

3

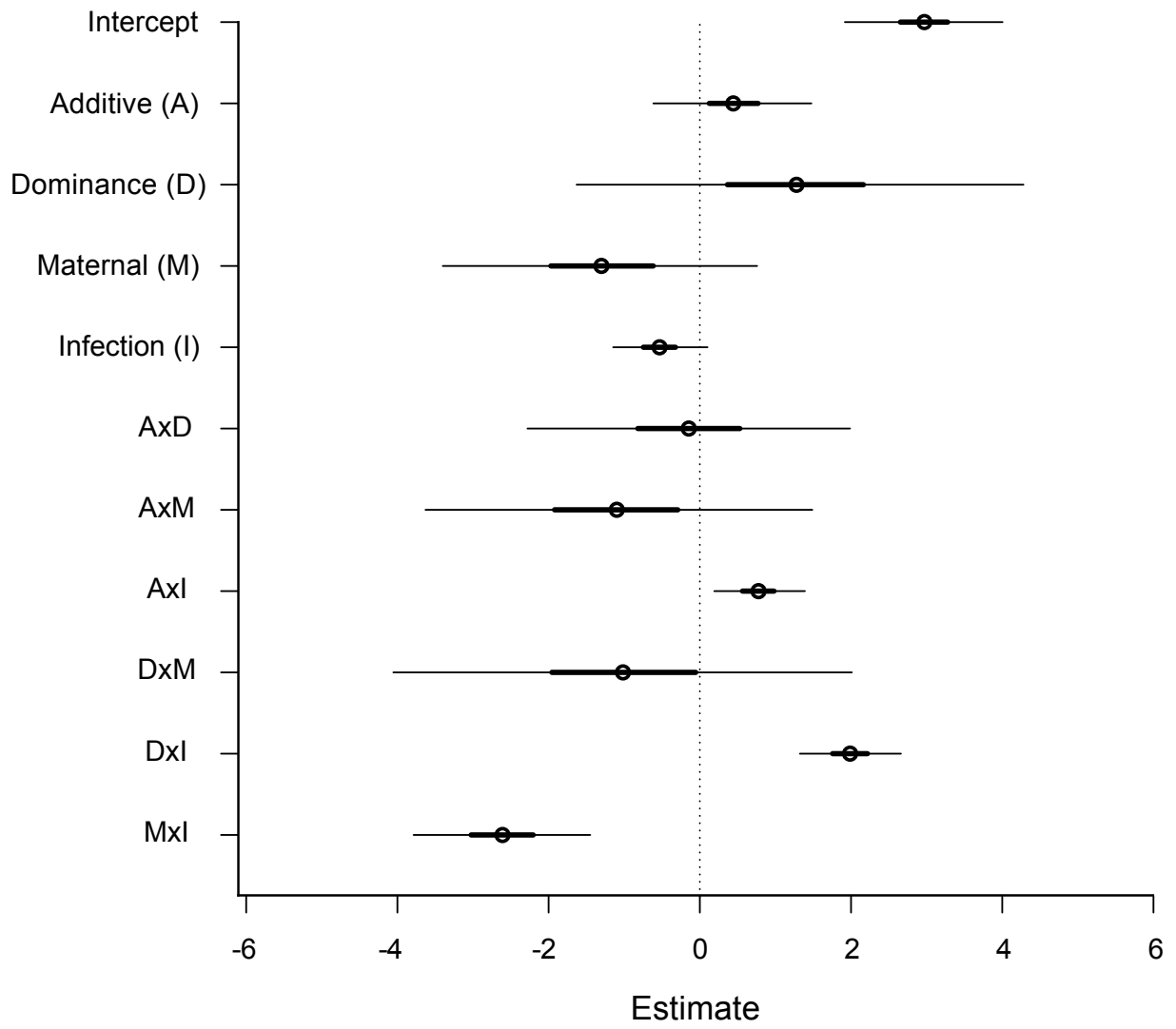
1 Figure 4



2

3

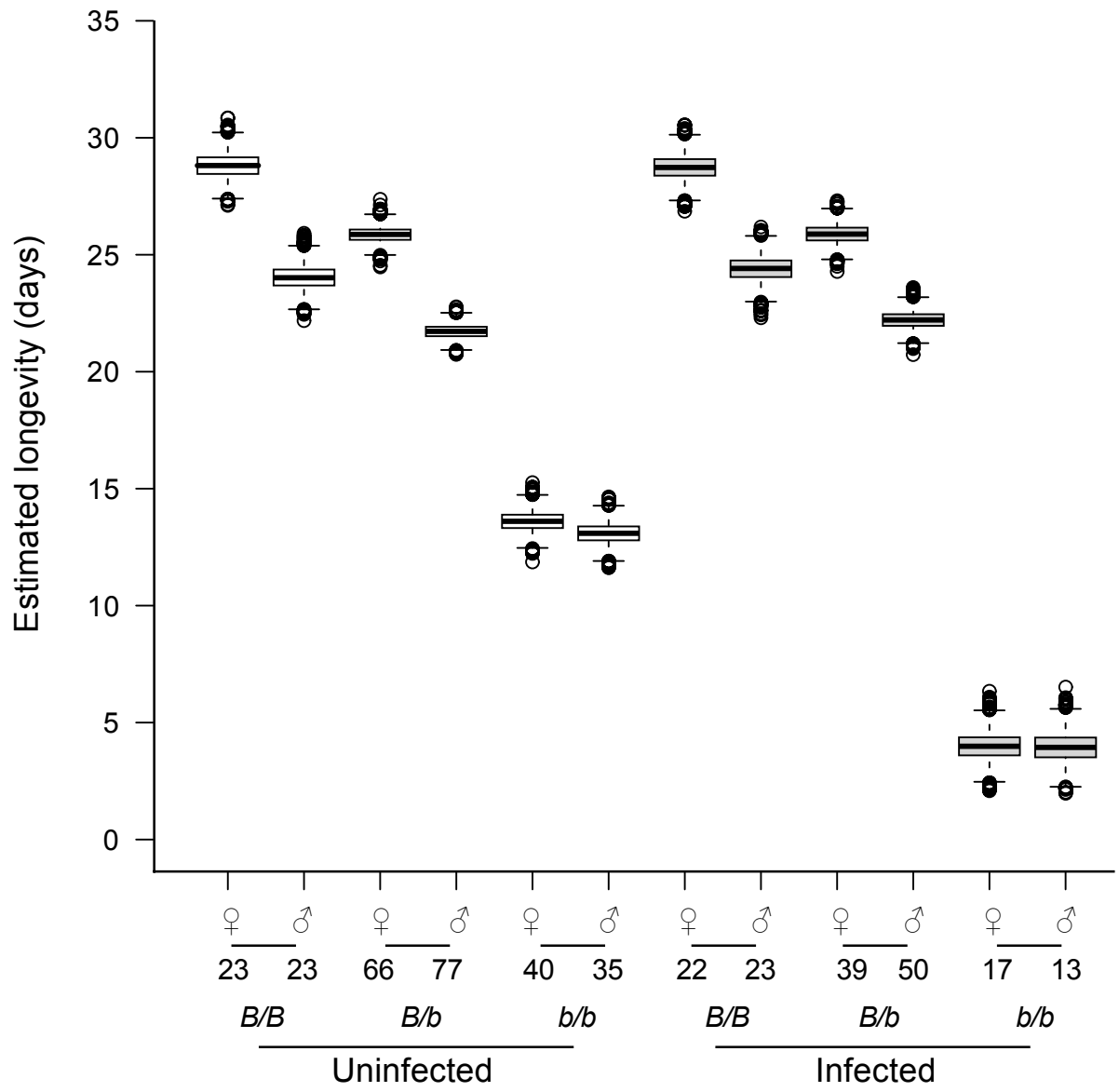
1 Figure 5



2

3

1 Figure 6

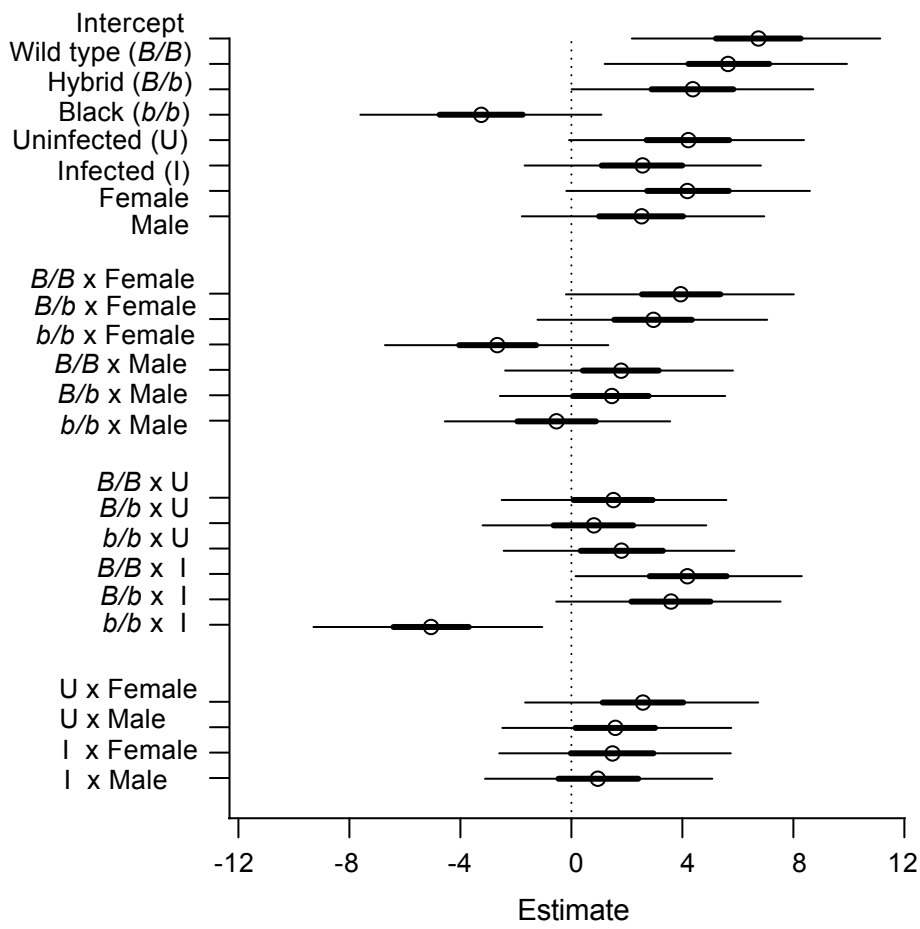


2

3



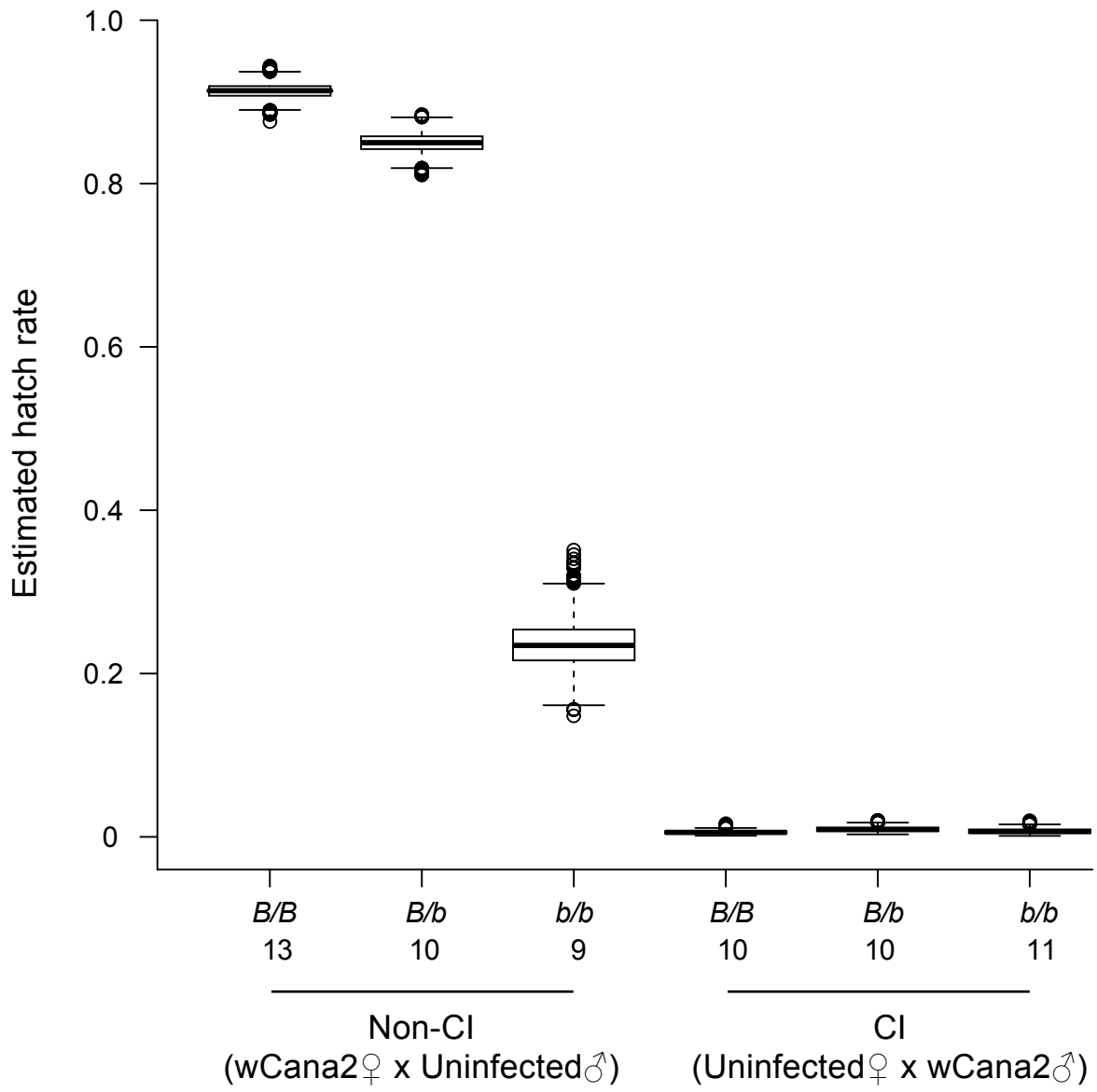
1 Figure 7



2

3

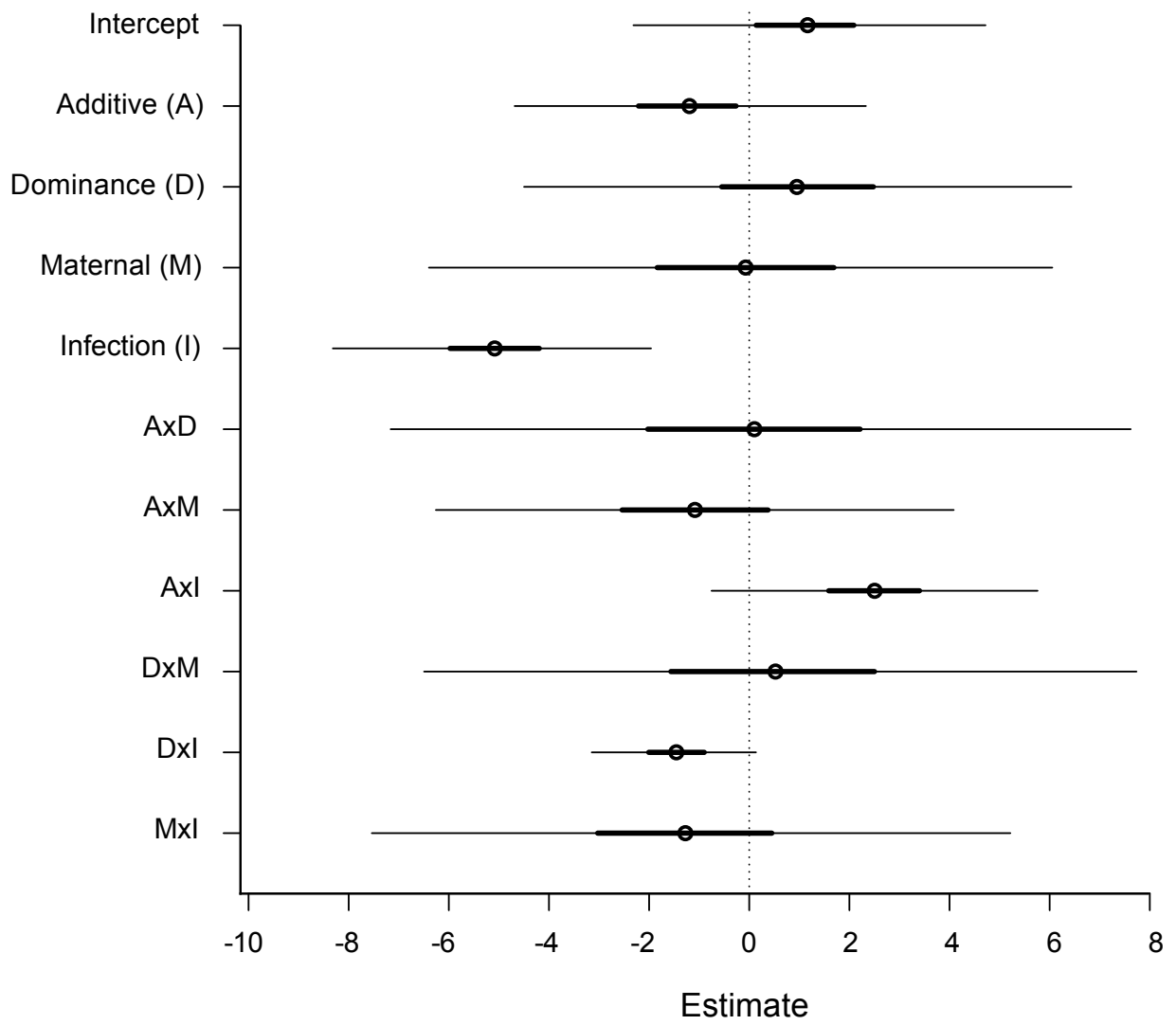
1 Figure 8



2

3

1 Figure 9



2

3

## 1 Supporting information

2

### 3 Model for *Wolbachia* infection spread

4 Cytoplasmic incompatibility enables the spread of *Wolbachia* by increasing the relative  
5 frequency of infected hosts. However, the sterility of infected *b/b* females reduces infected  
6 hosts. We modeled the infection dynamics considering both CI and incomplete *b/b* sterility  
7 based on the *Wolbachia* maintenance model proposed by Fine (1978). Figure S2 shows the  
8 schematic diagram of the relative proportions of compatible infected, compatible uninfected,  
9 and incompatible zygotes produced by a randomly mating population. In the figure, the  
10 numeric values inside the boxes represent proportions of *b/b* zygotes. We assumed that the  
11 proportion of *Wolbachia*-infected hosts was  $p_t$ , and that the gene frequency of the recessive  
12 melanic mutant at time  $t$  in infected and uninfected hosts was  $q_t$  and  $r_t$ , respectively. We also  
13 assumed that *Wolbachia* transmission failure was negligible but intensity of CI was  
14 incomplete ( $s \neq 0$ ). We introduced mortality ( $\beta$ ) of *Wolbachia*-infected zygotes that were  
15 reproduced by *b/b* females. The frequency of infected zygotes ( $p_t$ ), and the  $b$  frequency of  
16 infected ( $q_t$ ) and uninfected ( $r_t$ ) zygotes can be expressed as follows:

$$17 \quad p_{t+1} = \frac{p_t(1-\beta q_t^2)}{w_t},$$

$$18 \quad q_{t+1} = \frac{p_t(p_t(q_t-r_t)+q_t+r_t-\beta q_t^2(p_t q_t-p_t r_t+r_t+1))}{2w_t},$$

$$19 \quad r_{t+1} = \frac{(1-p_t)(p_t s(q_t+r_t)+2(1-p_t)r_t)}{2w_t}, \text{ and}$$

$$20 \quad w_t = 1 - p_t(1-p_t)(1-s) - \beta p_t q_t^2.$$

21

### 22 Results for infection dynamics

23 For infection dynamics, we considered difference in  $p$  between time  $t$  and  $t+1$ :

$$24 \quad \Delta p = p_{t+1} - p_t = \frac{p_t(1-p_t)(p_t(1-s)-\beta q_t^2)}{w_t}.$$

25 Figure S3 shows the combinations of  $p_t$  and  $q_t$  that satisfy the condition of  $p_t < \beta q_t^2/(1-s)$   
26 where *Wolbachia* invasion is stopped by *b/b* hosts when  $s = 0.5$  (Figure S3A) and  $b = 0.5$   
27 (Figure S3B). If  $q_t$  is constant, the above recursion equation has three points of equilibrium:  
28  $p_1^* = 0$ ,  $p_2^* = \beta q_t^2/(1-s)$ , and  $p_3^* = 1$ . Equilibrium points  $p_1^*$  and  $p_3^*$  are stable, but  $p_2^*$  is  
29 unstable. For a successful invasion, the initial frequency of *Wolbachia* needed to overcome  
30 the invasion threshold,  $p_2^*$ . However, as noted above,  $q_t$  is not constant. Thus, we considered  
31  $\Delta q$  as well:

$$1 \quad \Delta q = q_{t+1} - q_t = \frac{\beta p_t(2-p)q_t^3 + \beta p_t(p_t r_t - r_t - 1)q_t^2 + (p_t - 1)(2p_t s - p_t + 2)q_t + p_t(1-p_t)r_t}{2(1-p_t(1-p_t)(1-s) - \beta p_t q_t^2)}.$$

- 2 Figure S4 shows the combinations of  $p_t$ ,  $q_t$ , and  $r_t$  satisfying the condition of  $\Delta q \geq 0$ . The  
3 condition  $p_t < \beta q_t^2 / (1 - s)$  is easily violated after a couple of time steps because  $q_t^2$  soon  
4 diminishes to zero. Then  $\Delta p = 0$  has two equilibrium points (0 and 1), meaning that *b/b* hosts  
5 cannot stop the *Wolbachia* invasion.  
6

Wild type      Melanic mutant



1

2 **Figure S1** Females (above) and males (below) of normal and melanic colored

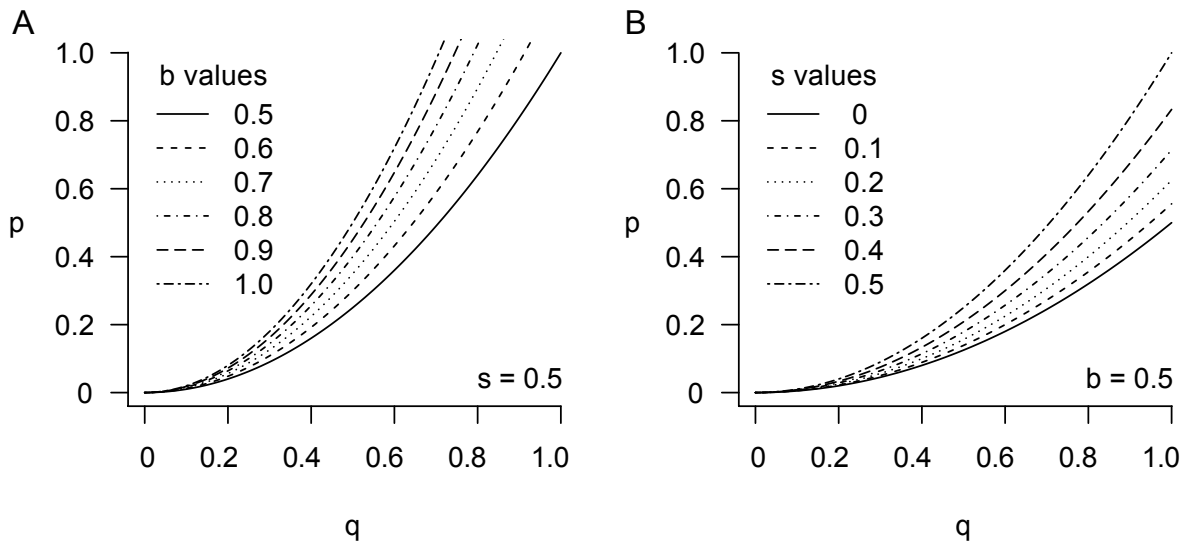
3 *Callosobruchus analis*.

4

 		Infected						Uninfected		
		p						1-p		
		1-s			s			B/B	B/b	b/b
		B/B	B/b	b/b	B/B	B/b	b/b	(1-y) <sup>2</sup>	2y(1-y)	y <sup>2</sup>
		(1-x) <sup>2</sup>	2x(1-x)	x <sup>2</sup>	(1-x) <sup>2</sup>	2x(1-x)	x <sup>2</sup>			
Infected	p	B/B	(1-x) <sup>2</sup>	Compatible infected						
		B/b	2x(1-x)	1/4	1/2	1/4	1/2	1/4	1/2	
		b/b	x <sup>2</sup>	1/2	1	1/2	1	1/2	1	
					Incompatible by infected b/b female					
Uninfected	1-p	B/B	(1-y) <sup>2</sup>	Compatible not infected						
		B/b	2y(1-y)	Incompatible by CI			1/4	1/2	1/4	1/2
		b/b	y <sup>2</sup>			1/2	1	1/2	1	

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11

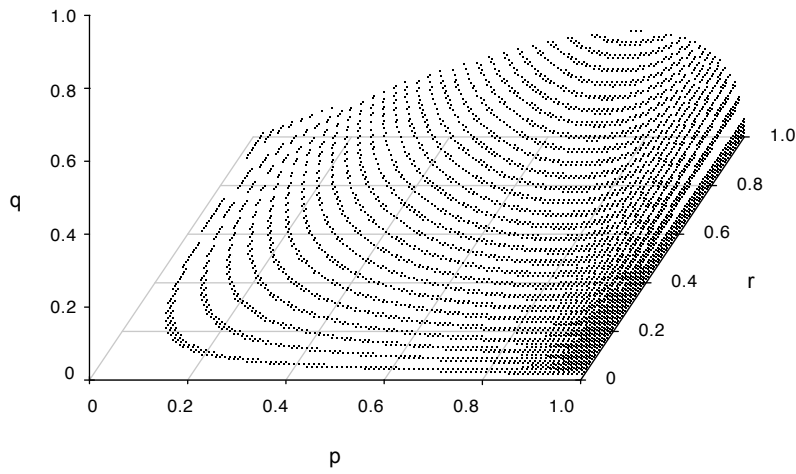
**Figure S2** Infection status of zygotes.  $p$  is the proportion of infected hosts,  $q$  and  $r$  are frequencies of allele  $b$  in infected and uninfected hosts, respectively. Each box represents the relative proportion of zygotes produced by random mating between host females and males. Numbers in the boxes represent the  $b/b$  proportion among zygotes. The status of female parents is represented along the vertical axis and that of the males along the horizontal axis.  $s$  and  $\beta$  are cytoplasmic incompatibility (CI) intensity and sterility level of  $b/b$  females, respectively. Zygotes fail to develop with the presence of CI and with  $b/b$  females.  $B/B$ , wild type;  $B/b$ , heterozygotes;  $b/b$ , melanic mutant.



1  
2  
3  
4  
5  
6  
7

**Figure S3** Parameter space of  $\Delta p$  when (A)  $s = 0.5$  and (B)  $b = 0.5$ . Combinations of  $(p,q)$  below the various dotted lines satisfy the condition to stop the *Wolbachia* invasion ( $\Delta p \leq 0$ ).  $p$  is the frequency of infected zygotes,  $q$  is the frequency of the  $b$  gene in the infected zygotes, and  $s$  is the cytoplasmic incompatibility intensity (offspring survival rate).





- 1
- 2 **Figure S4** Parameter space of  $\Delta q$  ( $b = 0.8$ ,  $s = 0.5$ ). Combinations of  $(p,q,r)$  below dotted
- 3 lines satisfy the condition  $\Delta q \geq 0$ .