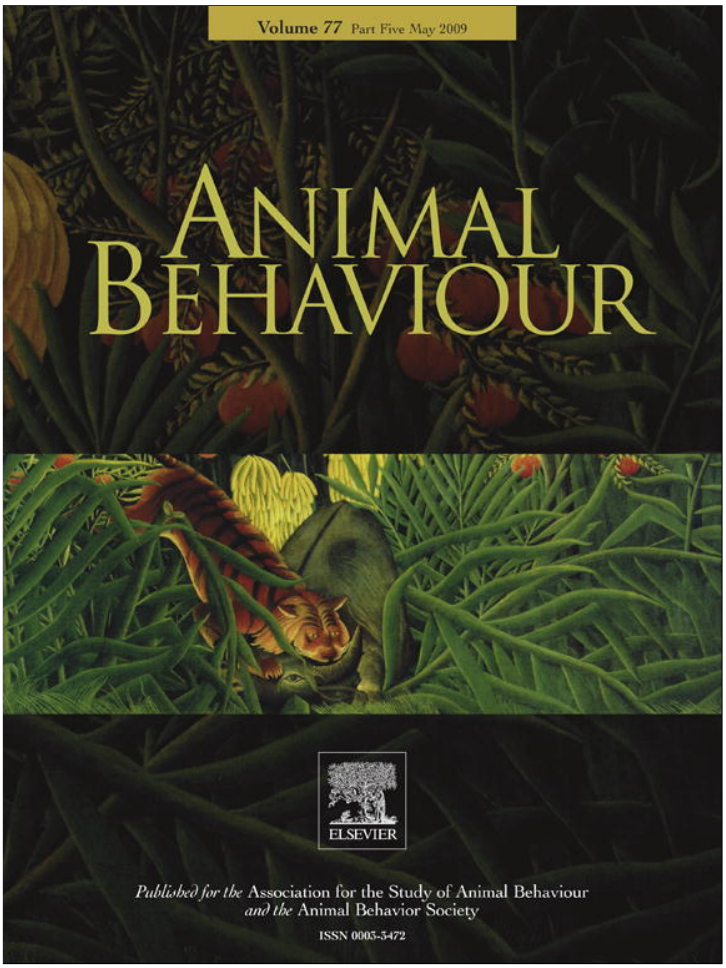

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Symbiotic bacteria affect mating choice in *Drosophila melanogaster*

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Mating preferences depending on *Wolbachia* infection were studied in two genotypically different strains of *Drosophila melanogaster*. Females from both strains carry two attached X chromosomes. Males from the red-eyed strain (R) have the wild-type X chromosome compared to males from the white-eyed strain (W), whose X chromosome contains two deleterious mutations (*white* and *singed*). Three types of competition tests showed that assortative mating depends on genotype, infection status and their combination in the mating partners. Males of strain R, genetically closer to the wild type, were more successful than males of strain W. *Wolbachia* infection increased the mating ability of W males but did not affect that of R males. Strain W showed positive assortative mating (preference for 'self') with regard to genotype and infection status. In strain R, negative assortative mating (preference for 'nonself') was observed. Moreover, the most affected flies (infected W) showed higher preference for 'self', while the least affected ones (uninfected R) showed higher preference for 'nonself'. These results support the idea that mating choice may involve testing the partner for degree of genetic or biochemical similarity with self, based on chemoreception with possible participation of immune system components.

Selective mating and the principles of mate choice are important for maintaining the genetic stability and diversity of populations, as well as for microevolutionary changes. Changes in mating preference associated with ecological adaptation may be of key importance at early stages of speciation, particularly in sympatric populations (Korol et al. 2000; Schluter 2001).

Mating choice can be based on the principle of 'good genes', when a female chooses, for example, the largest and strongest male, or on the more general principle of 'genes that are a good fit' (Trivers 1972; Mays & Hill 2004). According to the theory of optimal outbreeding, mate choice may be directed at reducing inbreeding, on the one hand, and avoiding distant crosses, including interspecies hybridization, on the other (Bateson 1982, 1983). Such choices require mechanisms, for example chemoreception, for comparison of potential partners with 'self' to estimate the degree of genetic similarity (relative versus nonrelative, similar versus different). In particular, olfactory signals, associated with gene alleles of the major histocompatibility complex (MHC), can be used in vertebrates for assessing kinship (Penn & Potts 1999; Milinski et al. 2005).

According to a recently formulated hypothesis (Markov & Kulikov 2006a, b), estimating the genetic relatedness of a potential partner via chemoreception, possibly using immunological signals and receptors, such as proteins and peptides of the MHC, may be important at the early stages of speciation. In general, this hypothesis states that individuals with an optimal (not too close and not too distant) degree of relatedness tend to be preferred as mating partners. The position of this optimum may vary depending on the environmental conditions. Under beneficial conditions, the optimum may shift towards more distant relatedness to avoid inbreeding and increase polymorphism and heterozygosity of the offspring, whereas under stress in a broad sense (Selye 1956), shifting towards closer relatives may be more advantageous, so that valuable traits and beneficial gene complexes, which have ensured the survival of the parents in a critical situation, are not disrupted by a distant cross.

The results of experiments on artificial speciation in insects suggest that in a stressful situation, for example, rearing on a poor medium or under strong directional selection for a morphological trait, the mating choice may indeed shift towards 'self', that is, to

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consanguineous mating. Consequently, strains that were subject to contrasting stressful treatment or disruptive selection may rapidly develop partial precopulatory reproductive isolation (Thoday & Gibson 1962; Kilias et al. 1980; Dodd 1989; Wilkinson & Reillo 1994). Similar phenomena have been observed in nature, for example, in *Drosophila* (Korol et al. 2000).

The rapid appearance of isolation in the above experiments cannot be explained by the reinforcement mechanism, that is, by selection of individuals that prefer genetically related mates occurring because of low competitive ability of hybrids (Dobzhansky 1951; Butlin 1989). Apparently, partial isolation (endogamy) may sometimes arise automatically, as a side effect of rapid genetic changes of the population subjected to strong selection. Individuals in such a changed population acquire genetic and biochemical differences; for instance, they may change their specific odour or, more generally, the antigen set presented to the partner for 'self/nonself' testing. It is conjectured that such changes may automatically lead to a situation in which members of a particular population perceive members of other populations of the same species as 'nonself' (Markov & Kulikov 2006a, b). A stress-induced shift in mating preference towards 'self' partners should promote isolation of the population and maintenance of its advantageous traits.

To test these views experimentally, we examined assortative mating in two genetically different strains of *Drosophila melanogaster*, depending on their infection with the intracellular bacterium *Wolbachia*. In this symbiotic system, *Wolbachia* does not induce cytoplasmic incompatibility (CI; nonviability of offspring from infected males X uninfected females) in *D. melanogaster* (Alexandrov et al. 2007). We expected the infection to change both the biochemical status of the animal and the antigen set tested during courtship. As a result, flies with different infection status should be more likely to perceive each other as 'nonself', compared to flies with the same infection status. On the other hand, infection may act as a stress or (see below), and may shift mate choice towards the preference for 'self'. Both proposed effects may be mediated by the genotype of the host fly and thus may vary among laboratory strains.

Wolbachia is a widespread endosymbiont of terrestrial arthropods and nematodes, which has strikingly diverse effects on its hosts (Stouthamer et al. 1999; Goryacheva 2004; Markov & Zakharov 2005). *Wolbachia* infection is transmitted almost exclusively vertically, transovarially and maternally. Most of its effects on host organisms are aimed at promoting the transmission of the infection. In different arthropod species, *Wolbachia* infection can lead to (1) CI; (2) parthenogenesis; (3) androcide (nonviability of male offspring); (4) feminization (transformation of genetic males into females); (5) changes in fertility and viability of infected animals. Most of these effects promote the spreading of *Wolbachia* in the host population and are thus beneficial for the parasite but usually not for the host (Goryacheva 2004).

Many wild and laboratory *D. melanogaster* populations are infected with *Wolbachia*. The symbiont–host relationships vary depending on the bacterial strain and the host genotype. Sometimes these relationships are parasitic, as, for example, in the case of the strain 'popcorn', which leads to CI and reduces the life span of *D. melanogaster* (Reynolds et al. 2003). However, more often *Wolbachia* acts as a harmless commensalist for *D. melanogaster*. In some laboratory strains of *D. melanogaster*, the infected flies benefit from increases in life span, resistance to RNA viral infections and other fitness parameters, such as fecundity and egg-to-adult viability (Olsen et al. 2001; Fry & Rand 2002; Fry et al. 2003; Teixeira et al. 2008). This is also true for the bacterial strain and for at least one of the two infected *D. melanogaster* strains used in our experiments (IW, see below). In this symbiotic system, the bacteria increase the life span of the insect, the competitive ability of the larvae and resistance to the pathogenic fungus *Bauveria bassiana* (Alexandrov et al. 2007; Panteleev et al. 2007). It seems plausible that the presence of intracellular bacteria may act as a stress factor (for instance, it may induce changes in gene expression and some kind of stress response at the biochemical level), despite the possible beneficial effects of *Wolbachia* on its hosts. This assumption is supported by numerous examples of the effects of *Wolbachia* infection on differential expression of host genes. For example, *Wolbachia* blocks the immune response of the host, controls some signal pathways, and regulates apoptosis in nurse cells within egg chambers (Siozios et al. 2008). It induces dramatic changes in expression of multiple genes in different *Drosophila* species, including *D. melanogaster* (Xi et al. 2008). Another possible link between stress and *Wolbachia* infection in *Drosophila* is that *Wolbachia* infection results in increased mRNA and protein expression of the nonmuscle myosin II gene *zipper*. This effect plays a crucial role in CI (Clark et al. 2006). Nonmuscle myosin II is responsible for maintaining the integrity of stress fibres, bundles of actin filaments that appear and disappear in response to mechanical stimuli and are thought to be one of the major components that sustain mechanical stresses in cells (Goeckeler et al. 2008). It is also known that *Wolbachia* strain wMel, which infects many strains of *D. melanogaster* including our experimental strains, is potentially harmful to its hosts. The transfer of seemingly harmless *Wolbachia* strains from *D. melanogaster* into *D. simulans* induces high levels of CI in the latter (Poinsot et al. 1998). CI in *D. simulans* is typically very strong, whereas it is weak or absent in *D. melanogaster* (Hoffmann et al. 1996). However, *Wolbachia* strains from *D. simulans* failed to induce strong CI when transferred into *D. melanogaster* (Boyle et al. 1993). These findings imply that the absence of deleterious effects in *D. melanogaster* most probably results from the active resistance of the host to attempted manipulations by the parasite. Therefore our assumption that *Wolbachia* acts as a stressor in the *Wolbachia*–*D. melanogaster* system appears plausible. Here we use it as a convenient framework for interpreting our results, although we recognize that the evidence is indirect and the question requires further clarification.

In a number of studies, the effect of *Wolbachia* on assortative mating of the host has been investigated. Most of these studies used *Drosophila* species as the host. Some of them failed to prove an association between assortative mating and *Wolbachia* infection (Jenkins et al. 1996; Sullivan & Jaenike 2006). Champion de Crespigny & Wedell (2007) showed male mating preference of females with the same infection status of *Wolbachia* in *D. simulans* at least in one series of experiments. Robinson (2006) examined mating choice in two partially isolated *Drosophila* species, one of which was totally infected (*D. recens*), and the other totally free of *Wolbachia* (*D. subquinaria*). Complete loss of the offspring induced by the endosymbiont was observed only in one crossing direction (infected *D. recens* male*uninfected *D. subquinaria* female). In this direction, marked assortative mating was observed. In some spider mites, uninfected females preferentially mate with uninfected males. This selective mating avoids the negative consequences of CI (loss of offspring from crosses with infected males) and thus increases fitness (Vala et al. 2004). In both cases (in spider mites and *Drosophila recens*/*D. subquinaria*), mating of infected males with uninfected females occurs at a lower rate, which may be interpreted as host adaptation (defence against the CI effect).

According to Markov & Kulikov's (2006a, b) hypothesis, *Wolbachia* or other bacterial infection may automatically change mating preference, regardless of the parasite's effects on the host. Moreover, these changes in assortative mating will not necessarily be adaptive. Mating selectivity may change simply because animals with different infection status differ from one another

biochemically, they may have different individual odours, and are more likely to be perceived as 'nonself' by potential mates with different infection status. Moreover, intracellular infection, like any other stressor, may hypothetically shift mate choice towards the preference for 'self'.

Mathematical models that account for low CI and fitness cost have postulated existence of control mechanisms, which determine the preferential mating of the host (Champion de Crespigny et al. 2005; Telschow et al. 2007). According to Markov & Kulikov's (2006a, b) hypothesis, these mechanisms may be associated with automatic changes in mate preferences induced by *Wolbachia* infection. Our main aim in the current study was to check for the effects postulated by the hypothesis.

METHODS

Strains of D. melanogaster

We used six *D. melanogaster* lines provided by I.D. Alexandrov (Institute of Nuclear Physics, Siberian Division, Russian Academy of Sciences).

We derived the strains as follows. As the stock, we used a laboratory strain: females, *C(1)RM*, *y v f*/Y^{bb-}, and males, *w sn*/Y^{bb-} (in both sexes: Y-bobbed, partial deletion of the RNA cluster in the Y chromosome; in females: attached X chromosomes; *yellow*, yellow body; *vermillion*, vermillion eyes; *forked*, 'burnt' bristles; in males: *white*, white eyes (the mutation causes blindness); *singed*, curly bristles). *Wolbachia* infection has been found in this strain (Alexandrov et al. 2007). Here, we refer to this strain as IW (infected white-eyed).

From the IW strain, we derived another *Wolbachia*-infected strain, which differed from the former in the male X chromosome and autosomes. The strain with genotype *C(1)RM*, *y v f*/Y^{bb-}/*w- sn-* was generated by crossing IW females with wild-type males, and designated IR (infected red-eyed).

Based on strains IW and IR, strains of the same genotypes but cured of *Wolbachia* infection by tetracycline treatment were derived (Alexandrov et al. 2007). Each of the strains (IW and IR) gave rise to two isofemale 'cured' strains (CW1, CW2, CR1 and CR2). Before the experiment started, 10 or more generations had passed since the tetracycline treatment. The results of the experiments involving CW1 and CW2 did not show significant differences according to a Pearson chi-square test; neither did those with CR1 and CR2. Consequently, these data were pooled, and the corresponding strain pairs denoted CW and CR.

All strains were maintained at 25 ± 0.5 °C in vials of 25 mm diameter containing 5–7 ml of a standard semolina–yeast medium. To avoid possible effects of overpopulation on behaviour in the mating tests, we used *Drosophila* from cultures containing no more than 50–80 flies per vial.

Assessment of Mating Preference

In these tests, we used 6–8-day-old males and 8–10-day-old virgin females. Flies were separated by sex within the first 12 h of eclosion by means of an aspirator without the use of ether. All behavioural tests were conducted in standard vials, 18 mm in diameter, with standard nutrition medium (3 ml per vial). Females and males were put in test vials with the help of an aspirator. Testing began at 1400 hours and the experiment lasted up to 5 h.

We did three experiments. The design of the first allowed us to determine the copulating male by eye colour (red or white). The design of the second eliminated the effect of the *white* mutation on courtship behaviour. Finally, in the last experiment male (rather than female) mating preferences were tested.

(1) We first tested mating competition of males with different *Wolbachia* infection status and different alleles at the white locus. A female from one of the two strains and two males, one with red eyes (IR or CR) and the other with white eyes (IW or CW), were placed in a vial. All 16 possible combinations were tested. Observation was conducted until successful copulation. We scored the genotype of the copulating male visually, by eye colour. To check repeatability, tests in six randomly selected combinations out of 16 were conducted twice: in spring 2005 and autumn 2005. The results of the repeated tests did not differ significantly from the previous results. In what follows, we present the combined results of the spring and the autumn tests. The reason for this design was to obtain all possible combinations of the parameters (infection status of the female and two males and their genetic background), so that the results could be corrected for the evident effect of the *white* mutation on the competitive ability of males.

(2) We then tested mating competition of males with different *Wolbachia* infection status, but with the same allele at the white locus. Two males with the same eye colour but with different *Wolbachia* infection status and a female from the same strain as one of the two males were placed in a vial. All four possible combinations were tested: (1) ♀ IW, ♂ IW, ♂ CW; (2) ♀ CW, ♂ IW, ♂ CW; (3) ♀ IR, ♂ IR, ♂ CR; (4) ♀ CR, ♂ IR, ♂ CR. The males were distinguished by small punctures in the wings, which were made by a sharp needle in the submarginal wing cell. The right wing of the infected male and the left wing of the cured male were punctured. Punctures were made under ether anaesthesia on the first day after eclosion, which was at least 6 days prior to testing. Such tiny punctures, either in the left or in the right wing, as well as early anaesthesia with ether (not less than 3 days before testing) do not affect the competitive mating ability of males (Kulikov & Mitrofanov 1990). Observation was conducted until successful copulation. The unsuccessful male was removed from a vial immediately after the start of copulation and checked for which of its wings had a puncture.

(3) Finally, we tested mating competition of females with different *Wolbachia* infection status. Twenty virgin females of the strain *W (10 IW and 10 CW) or from strain *R (10 IR and 10 CR) and a male from the same strain as half of the females were placed in a vial 24 mm in diameter with 6 ml of medium. All four possible combinations were tested: (1) ♀♀ IW, ♀♀ CW, ♂ IW; (2) ♀♀ IW, ♀♀ CW, ♂ CW; (3) ♀♀ IR, ♀♀ CR, ♂ IR; (4) ♀♀ IR, ♀♀ CR, ♂ CR. After 24 h, the females were transferred to individual 18 mm vials with 5 ml of medium. The females were observed for 5 days. The absence of larvae by the end of this time interval was considered to indicate that this female had not been fertilized. The *Wolbachia* infection status in fertilized females was determined by means of PCR with primers wsp81F/wsp691R for the *Wolbachia wsp* gene (Braig et al. 1998).

We used a chi-square test to compare the observed distribution with the expected one, a *G* test for comparisons of two observed distributions, and a *t* test to compare average numbers of fertilized females from different replications in the third experiment. The exact Fisher's test was used in a few cases when the number of observations was small. We used a Mann–Whitney test and sign test to assess the differences in mating preferences of infected and cured males in experiment 3. *P* values were Bonferroni corrected in cases of multiple comparisons in experiments 1 and 3. Statistical tests were two tailed.

RESULTS

Red-eyed males were more successful in mating than white-eyed ones. Of 722 successful (ending in copulation) tests, a *R male copulated in 617 (85.46%) and a *W male only in 105 (14.54%;

$\chi^2_1 = 363.1$, $P = 9.6 \times 10^{-80}$; Table 1). Judging from our observations of the mating behaviour in these tests, the superior competitive ability of red-eyed males was largely determined by their greater locomotor activity than in white-eyed males. White-eyed males had a chance for success only with the females that had previously rejected the more active red-eyed competitor.

Wolbachia infection increased the competitive ability of *W males. For males of strain IW, mean mating ability (average N_w) for eight combinations with their participation was 19.54% (60 matings in 307 tests); the corresponding value for CW males was 10.84% (45 matings in 415 tests). Infected and uninfected W males differed significantly in mating success ($G_1 = 10.57$, $P < 0.001$; Table 1).

Wolbachia infection had no effect on the mating ability of *R males. The mean mating ability was 84.06% (290 matings in 345 tests) for IR males and 86.74% (327 matings in 377 tests) for CR males. ($G_1 = 1.03$, $P = 0.309$; Table 1). Thus, *Wolbachia* infection affected the mating ability of white-eyed and red-eyed males differently: in the former, it significantly enhanced this parameter, while in the latter it had no effect.

IW females mated with *W males more often, compared to CW females. Thus, *Wolbachia* infection promoted preference for genotypically 'self' in W females. The mean mating ability of *W males in crosses with IW and CW females was 24.55% (41 matings in 167 tests) and 7.66% (16 matings in 209 tests), respectively ($G_1 = 20.63$, $P < 0.001$; Table 1).

*W females more often mated with *W males with the same infection status, than *W males with a different infection status (preference for 'self' with respect to infection status), but the difference was not significant. The mean mating ability of male *W with female *W with the same or different infection status was 17.65% (42 matings in 238 tests) and 10.87% (15 matings in 138 tests), respectively (Fisher's exact test: $P = 0.051$; Table 1). *W females did not show preference for *R males with regard to infection status. The mean mating ability of male *R with female *W having the same or different infection status was 85.94% (165 matings in 192 tests) and 83.70% (154 matings in 184 tests), respectively (Fisher's exact test: $P = 0.322$; Table 1).

CR females more often mated with *W males, compared to CW females (preference for 'nonself' with regard to genotype status). The mean mating ability of *W males with female C* having the same or different genotype status was 7.66% (16 matings in 209 tests) and 18.29% (32 matings in 175 tests), respectively (Fisher's exact test: $P = 0.003$; Table 1). IW females more often mated with *W males, compared to IR females (preference of 'self' with regard to genotype status). The mean mating ability of *W males with female I* having the same or different genotype status was 24.55% (41 matings in 167 tests) and 9.36% (16 matings in 171 tests), respectively (Fisher's exact test: $P < 0.001$; Table 1). On the whole, this result demonstrated that *Wolbachia* infection shifted female mate choice towards preference for males with similar genotype (preference for 'self' with regard to genotype status).

*R females more often mated with the *R males with a different infection status (preference for 'nonself' with regard to infection status). The mean mating ability of male *R with female *R having the same or different infection status was 82.98% (195 matings in 235 tests) and 92.79% (103 matings in 111 tests), respectively (Fisher's exact test: $P = 0.013$; Table 1). *R females also tended to prefer *W males with different infection status, compared to *W males with the same infection status, but this effect was not statistically significant. The mean mating ability of male *W with female *R with the same or different infection status was, respectively, 12.77% (18 matings in 141 tests) and 14.63% (30 matings in 205 tests; Fisher's exact test; $P = 0.371$; Table 1).

CR females more often mated with *W males than IR females (the absence of infection in R females led to the preference for 'nonself' with respect to the potential partner's genotype). The mean mating ability of male *W with female CR and IR was 18.28% (32 matings in 175 tests) and 9.36% (16 matings in 171 tests), respectively (Fisher's exact test: $P = 0.013$; Table 1).

The results imply that two internal factors, namely *Wolbachia* infection and attribution to strain *W (mutations *white* and *singed* in males), enhanced mating preference for 'self' (partners with similar genotype and/or infection status). In the absence of these factors (i.e. absence of infection and attribution to strain *R which is closer to the wild type), the flies tended to prefer 'nonself' (partners with different genotype and/or infection status). For instance, *W females preferred males with the same infection status; IW females preferred males with both the same infection status and similar genotype; *R females preferred partners with a different infection status; CR females preferred partners with both a different infection status and different genotype. Generally, both *Wolbachia* infection and the presence of genetic load shifted female mate choice from preferring 'nonself' to preferring 'self'.

The second experiment confirmed that *Wolbachia* infection increased mating ability in W males (infected females: $\chi^2_1 = 6.76$, $P = 0.009$; cured females: $\chi^2_1 = 7.22$, $P = 0.007$) and did not significantly affect mating ability in R males (infected females: $\chi^2_1 = 0.16$, $P = 0.692$; cured females: $\chi^2_1 = 0.62$, $P = 0.431$; Table 2).

The third experiment confirmed that *W females prefer *W males with the same infection status and tended to support the result that *R females prefer *R males with different infection status. The analysis of the pooled data from all the males used (Table 3, N_{wolb+}, N_{wolb-}) showed, for instance, that male *W fertilized $100 + 82 = 182$ females with the same infection status and $58 + 70 = 138$ females with a different infection status ($\chi^2_1 = 6.05$,

Table 1
Results of competitive mating in groups of one female–two males from strains differing in eye colour

♀	♂1 (*R)	♂2 (*W)	N_r	N_w	N_r (%)
IW	IR	IW	29	14	67.44
IW	IR	CW	27	7	79.41
IW	CR	IW	42	15	73.68
IW	CR	CW	28	5	84.85
CW	IR	IW	23	2	92.00
CW	IR	CW	61	8	88.41
CW	CR	IW	45	1	97.83
CW	CR	CW	64	5	92.75
IR	IR	IW	24	7	77.42
IR	IR	CW	77	9	89.53
IR	CR	IW	20	0	100.00
IR	CR	CW	34	0	100.00
CR	IR	IW	24	5	82.76
CR	IR	CW	25	3	89.29
CR	CR	IW	40	16	71.43
CR	CR	CW	54	8	87.10

N_r: the number of tests in which the red-eyed male (*R) copulated with the female; N_w: the number of tests in which the white-eyed male (*W) copulated with the female; N_r(%): the N_r: value divided by $N_r + N_w$ (competitive ability of red-eyed males *R).

Table 2
The results of competitive matings in groups of one female + two males from strains differing in *Wolbachia* infection status

♀	♂1 (I*)	♂2 (C*)	N_{wolb+}	N_{wolb-}	χ^2_1	P
IW	IW	CW	63	37	6.76	0.009
CW	IW	CW	64	37	7.22	0.007
IR	IR	CR	53	49	0.16	0.692
CR	IR	CR	26	32	0.62	0.431

N_{wolb+}: the number of tests in which the infected male (I*) mated with the female; N_{wolb-}, the number of tests in which the cured male (I*) mated with the female.

Table 3
Results of competitive mating with 20 females (10 females from each of the two strains differing in infection status) and one male in each test

♂	♀1 (I*)	♀2 (C*)	N_{wolb+}	N_{wolb-}	$N_{wolb?}$	χ^2	P	N (N_i,N_c,N_e,N_{st})	$q_{♀I}$	N_f	SD_{Nf}
IW	IW	CW	100	70	18	6.76	0.021	21 (13; 5; 3; 4)	0.600	8.95	3.01
CW	IW	CW	58	82	10	5.29	0.043	21 (4; 14; 3; 2)	0.438	7.14	3.07
IR	IR	CR	76	89	2	1.04	0.312	17 (4; 9; 4; 3)	0.418	10.44	3.08
CR	IR	CR	68	50	4	2.75	0.098	13 (6; 5; 2; 0)	0.567	9.38	2.02

N_{wolb+}: the number of fertilized infected females (I*); N_{wolb-}: the number of fertilized cured females (I*); $N_{wolb?}$: the number of fertilized females with unknown infection status; N: the number of tests in which at least one female was fertilized; N_i: the number of tests in which the male fertilized more infected females than cured ones; N_c: the number of tests in which the male fertilized more cured females than infected ones; N_e: the number of tests in which the male fertilized equal numbers of infected and cured females; N_{st}: the number of tests in which the females were not fertilized (the male was sterile); $q_{♀I}$: ratio of infected females fertilized, averaged across males; N_f: the mean number of females fertilized by one male (excluding sterile males); SD_{Nf}, standard deviation of N_f. χ^2 and P values correspond to N_{wolb+}/N_{wolb-} ratio.

$P = 0.014$). Male *R fertilized $50 + 76 = 126$ females with the same infection status and $68 + 89 = 157$ females with a different infection status ($\chi^2_1 = 3.40$, $P = 0.065$).

The analysis of the pooled data (Table 3, N_{wolb+}, N_{wolb-}) reveals selectivity of mate choice at the population level rather than individual variability of mating preferences in males. To assess the latter, we analysed the ratio of infected females fertilized by each male. These data, averaged across males in each of the four series of tests, are shown in column $q_{♀I}$ in Table 3. We found that mating selectivity in male IW differed significantly from that in male CW (Mann–Whitney U test: $U = 105.0$, $N_1 = N_2 = 21$, $P = 0.01$). Mating selectivity in male IR did not differ significantly from that in male CR ($U = 73.5$, $N_1 = 17$, $N_2 = 13$, $P = 0.123$.). We also found that male *W preferentially mated with females with the same infection status (sign test: $Z = 2.92$, $P = 0.005$). Although male *R preferred, on average, to mate with females with different infection status, this trend is below the level of statistical significance ($Z = 1.02$, $P = 0.307$).

In addition, in the third experiment, *Wolbachia* infection probably increased mating activity of *W males. Each IW male fertilized on average 8.95 females, each CW male, 7.14 (student's t test: $t_{40} = 1.904$, $P = 0.060$). *Wolbachia* infection seemed to have no effect on the mating activity of *R males ($t_{27} = 1.077$, $P = 0.290$). Mating activity of CW males was significantly lower than that of CR males: the former fertilized on average 7.14 each, the latter, 9.38 ($t_{32} = -2.361$, $P = 0.024$). Male IW and IR did not differ in mating activity ($t_{35} = -1.451$, $P = 0.156$).

DISCUSSION

Our results on the effects of the symbiotic bacterium *Wolbachia* on mating preference in *D. melanogaster* correspond to previous results obtained for other species (*D. recens* and *D. subquinaria* Jaenike et al. 2006; Robinson 2006) and spider mites (Vala et al. 2004), and show that such effects are widespread, as predicted in theoretical works (Champion de Crespigny et al. 2005; Telschow et al. 2007).

The experiments reveal a complex pattern of assortative mating in *D. melanogaster* depending on the strain and on the *Wolbachia* infection status. To interpret the results, genetic differences between strains *R and *W should be taken into account. The former strain is less inbred, because its construction involved crosses with wild-type flies. This resulted not only in the absence of deleterious mutations *w* and *sn* in *R males (the *w* mutation leads, among other effects, to weak eyesight in males), but also in the presence of a number of different autosomal alleles in the genetic background of these strains, which probably decreased their homozygosity.

The hypothesis that individuals test the mating partner as 'self' or 'nonself' (Markov & Kulikov 2006a, b) implies that in a 'stressed' population subjected to adverse environmental conditions and/or strong directional selection, a shift in mate choice towards preference for 'self' may occur, because such a reproductive strategy reduces the probability of diluting beneficial properties that enhanced survival in critical conditions. Moreover, in a stressed population, the level of inbreeding and homozygosity should increase because of high selective mortality, that is, irrespective of the mate choice strategy.

In view of the properties of the *W strains described above, we suggest that they may be regarded as model analogues of stressed populations that are under strong selection. The *R strains, which are closer to the wild type, may be considered analogous to populations under normal conditions. Accordingly, one would expect that in *W strains the preference for similar mating partners would be more prominent, while in *R strains the opposite trend would prevail. In view of the results obtained, *Wolbachia* is likely to be an additional stress factor, which would shift mating choice towards 'self'. It is possible that the presence of intracellular bacteria may induce some kind of stress response even if the bacteria are actually beneficial to their host (see above). (Note that the definition of stress is interpreted in the present study in a broad sense in accordance with Selye 1956.) The results of the present study support these assumptions.

Strain IW, which presumably experienced the strongest stress, had a significantly higher mating rate with *W flies ('self' with regard to genotype), compared to strain CW. In *W strains, we obtained no support for the preference for 'self' with regard to infection status owing to high conservatism of the Bonferroni correction. The second experiment also did not support the preference for 'self' by *W females, but it clearly showed an increase in male mating ability. However, a significant result was obtained in the third experiment. This was probably because in this experiment a single male had an opportunity to choose among many females, that is, there was no need to hurry and compete with other males. Thus, CW males were able to demonstrate higher preference for 'self' (i.e. females with the same infection status). In experiments 1 and 2, the main factors affecting the results were female receptivity and male competitiveness and locomotor activity, while male mating preferences probably played a lesser role. In experiment 3, male mating preferences were of primary importance, while their locomotor activity did not matter that much. We suggest that in some situations the differences in general locomotor and sexual activity may obscure the subtle effects of mate preference based on 'self/nonself' recognition. This was probably the reason for our failure to detect the latter effects in experiment 2, as well as in somewhat similar experiments performed by Sullivan & Jaenike (2006) with wild-type flies.

The opposite pattern (preference for 'nonself') was observed in *R strains. In these strains, the flies with the opposite infection status were preferred as mating partners: infected flies preferred uninfected partners, and vice versa. The least stressed strain CR had a significantly higher mating rate with flies of *W strains, that is with partners having the 'nonself' genetic status, compared to strain IR.

Wolbachia infection clearly switched mating preference of flies between 'nonself' and 'self'. Thus, our results are adequately explained in the framework of Markov & Kulikov's (2006a, b) hypothesis and may be regarded as supporting evidence for this hypothesis.

Note that, according to this hypothesis, the pattern of assortative mating is not necessarily adaptive in each particular case. The hypothesis assumes that 'self/nonself' testing of the partner prior to mating is a basic and widespread behavioural pattern. The probability of mating depends on the results of comparison of biochemical and genetic characteristics of the potential partner with the corresponding parameters of the individual that performs the testing. There is an optimal level of similarity at which the attractiveness of the partner is highest; very high or very low similarity reduces attractiveness. As noted above, the position of the optimum presumably may shift towards preference for 'self' under stressful conditions. If a mechanism underlying this shift exists, stress should automatically switch it on, even if the stressor is novel and unknown. Besides, any change in biochemical status of an animal, for example, infection by *Wolbachia*, may change its individual odour, and can automatically change the level of its biochemical similarity to other individuals. Thus it can alter its sexual attractiveness, differently for different individuals. It is important that such changes in attractiveness occur instantaneously, automatically, without many generations of selection for preference for particular partners. Such changes are adaptive but only generally. They promote adaptation to adverse, stressful environments by reducing outbreeding. However, such automatic changes in mutual attractiveness of the mating partners, caused by changes in their genetic and biochemical status, are not necessarily adaptive in each particular situation. This aspect of the proposed hypothesis can be verified experimentally, which we tried to do in the present work.

Can the assortative mating found in our experiments be explained on the basis of its adaptive character? Does it provide any benefits either to *Wolbachia* or to its host? Apparently, this is true for some, but not all, of the results.

For CI-causing *Wolbachia*, it would be beneficial to increase the mating ability of infected males. However, the absence of a positive effect (or weak negative effect) of *Wolbachia* on the mating ability of R males cannot be thus explained and requires an ad hoc hypothesis. For instance, we can conjecture that drastically weakened *W males may be more sensitive to regulatory factors released by *Wolbachia* than more sexually successful, closer to wild-type *R males.

Preference for 'self' in *W strains in principle can be regarded as an adaptation of the host aimed at reducing the harm caused by CI. Such adaptation may not be relevant for this particular *Wolbachia* strain, but could be for other *Wolbachia* strains that cause CI. By mating preferentially with partners with the same *Wolbachia* infection status, the host insect reduces the probability of an infected male mating with an uninfected female, which may result in loss of the offspring. This explanation, however, cannot be applied to our results since preference for 'nonself' mating partners is not advantageous for insects infected with a CI-causing *Wolbachia* strain.

Alternatively, the results may be partially explained by the fact that the IW strain was originally infected by *Wolbachia*; hence it may have developed adaptations to minimize the detrimental effects of *Wolbachia* or even to benefit from it. The *R strains were derived from IW by crossing with uninfected wild-type flies, so that the influx of alien genes may have eliminated or weakened these adaptations. More experiments are needed to test this possibility.

In the symbiotic system examined, *Wolbachia* improve some fitness characteristics (longevity, larval survival, resistance against pathogens). Therefore, mating with infected females is advantageous for males. However, such preferences were found only in IW and CR males, whereas CW and IR males preferred to mate with uninfected females.

Thus, to interpret all the results, we need either to advance several complex ad hoc hypotheses, or use the explanation formulated above (based on the hypothesis of 'self/nonself' testing of mating partners and stress-induced shift of mating behaviour towards the preference for 'self', Markov & Kulikov 2006a, b). Here, the presumed stress factors are *Wolbachia* infection and belonging to *W strains. In our view, this explanation is more plausible and consistent, because it helps to explain most of our results as manifestations of one general trend, without special assumptions for each individual case.

We acknowledge that our results do not provide an ultimate proof of the hypothesis. Only six strains of *D. melanogaster* were used in the tests (IW, IR, CW1, CW2, CR1 and CR2); hence there is a possibility that chance differences between the strains might explain the results. More experiments are needed to verify the hypothesis and to define the limits of its applicability.

Conclusions

(1) In crosses between laboratory strains of *D. melanogaster* differing in genotype and *Wolbachia* infection status, marked mating assortativeness was observed (crosses of flies from different strains were not equiprobable).

(2) The probability of mating success of a male in competition tests depended both on the genotype and on the infection status. Males from the more inbred *W strains, carrying deleterious mutations *w* and *sn*, were less likely to mate than males from the *R strains, which were genetically closer to the wild type. The effect of *Wolbachia* infection on male mating success and mating activity depended on its genotype: in *W strains, this effect was pronounced and positive, in *R strains, it was absent or slightly negative.

(3) Assortative mating, observed in all strains examined, was in agreement with the assumption that in *D. melanogaster*, 'self/nonself' testing of the partner occurs before mating. The degree of similarity (both in genotype and infection status) between the potential partners, measured during this testing, affected the mating probability in a way that also depended upon both genotype and infection status. Generally, *W strains showed positive assortative mating (preference for 'self'), while *R strains showed negative assortative mating (preference for 'nonself'). *Wolbachia* infection in *W strains induced positive assortative mating with regard to genotype (IW females more often mated with *W males than CW females). In *R strains, *Wolbachia* infection reduced negative assortative mating (IR females less frequently mated with *W males than CR females).

(4) The results support the hypothesis that there are mechanisms shifting mating behaviour towards preference for 'self' under conditions of stress or internal imbalance. In our study, the presumed stress factors were *Wolbachia* infection and belonging to *W strains, which were more inbred and carried deleterious mutations. Accordingly, the most stressed flies (strain IW) showed positive assortativeness by both the infection status and the genotype. The least stressed flies (strain CR) showed negative assortativeness by both parameters. In flies that were under moderate stress, assortative mating was expressed to a lesser degree and confined only to the infection status.

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