Letter to the Editor

The Rate of Recombination in *Wolbachia* Bacteria

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Knowledge of the rate at which recombination occurs is critical for understanding the evolution and population genetics of bacteria, because it both generates novel combinations of beneficial alleles and also prevents the accumulation of deleterious mutations. Furthermore, it may influence our interpretation of evolutionary patterns, for example, during comparative analyses of phylogenies.

I have examined the presence and rate of recombination in a group of predominantly vertically (maternally) transmitted bacterial symbionts, arthropod *Wolbachia*. It is expected that vertical transmission through one sex will reduce the frequency with which different bacterial strains will come into contact and may therefore reduce the rate of recombination. I assessed this effect by comparing arthropod *Wolbachia* data with smaller data sets from related obligate horizontally and vertically transmitted bacteria.

All the three taxa studied belong to the Rickettsiaceae, a family of alpha-proteobacterial symbionts that live within the cell cytoplasm of their hosts. Our main focus was on the *Wolbachia* infections of arthropods, which mostly either distort the host sex ratio toward females (the transmitting sex) or induce cytoplasmic incompatibility (O'Neill et al. 1992; Rousset et al. 1992; Stouthamer et al. 1993; Hurst et al. 1999). I also investigated *Cowdria ruminantium*, a tick-borne parasite of ruminants, and *Wolbachia* from nematode worms, which are mutualists (Bandi et al. 1999).

The rate of horizontal transmission, and hence the hypothesized opportunity for recombination, is highest in *C. ruminantium*, the vertebrate parasite. Horizontal transmission is thought to be entirely absent in mutualistic *Wolbachia* infections because the phylogenies of the hosts and their symbionts are identical (Bandi, Anderson, and Blaxter 1998). An intermediate rate horizontal transmission is thought to occur in arthropod *Wolbachia*. Transmission is predominantly maternal because within a given host species there is typically strong linkage disequilibrium between the host mitochondria and *Wolbachia* (Montchamp-Moreau, Ferveur, and Jacques 1991; Turelli, Hoffmann, and McKechnie 1992; Grandjean et al. 1993; Ballard 2000; Schulenburg et al. 2002; but see Huigens et al. 2000 for an exception). But the bacterial and host phylogenies are largely incongruent, and distantly related *Wolbachia* strains may infect the same host, indicating that rare horizontal transmission does occur (Werren, Windsor, and Guo 1995; Schilthuizen and Stouthamer 1997; Zhou, Rousset, and O'Neill 1998).

Therefore, the hypothesis that the rate of recombination is determined by the frequency with which multiple strains coinfect a single host generates the prediction that the rate of recombination will be zero in nematode *Wolbachia*, intermediate in arthropod *Wolbachia*, and highest in *Cowdria*.

Recombination has been detected in two studies of arthropod *Wolbachia*. In the first, the phylogenies of different genes were shown to be incongruent, indicating recombination between the genes (Jiggins et al. 2001*b*). The second report described a gene sequence that was the product of recombination between bacteria that infect a parasitoid and its host (Werren and Bartos 2001). Therefore, current data provides evidence for just two recombination events. I have extended this analysis by describing both the taxonomic distribution of recombination and estimating the rate at which it occurs.

I analyzed aligned DNA sequences from two genes. The first was *wsp* from *Wolbachia* and its homologue *map1* in *Cowdria*, both of which encode surface proteins. The second was the cell cycle gene *ftsZ*, for which data is only available from *Wolbachia*. Three separate alignments of *wsp* from the nematode worm *Wolbachia* (10 sequences), the A-group arthropod *Wolbachia* (17 sequences) and B-group arthropod *Wolbachia* (35 sequences) were used. A fourth alignment was made of the *wsp* homologue *map1* from *C. ruminantium* (14 sequences). These alignments have EMBL accession numbers ranging from ALIGN_000198 to ALIGN_000200 (note that the final alignment includes both A- and B-group *Wolbachia* which I analyzed separately). The arthropod *wsp* alignment contains two regions that were omitted because they contain insertions and deletions that make the alignment of homologous sites uncertain. These regions were included in the data set as missing data, making the sequences the same length as that from strain *wRi* (accession number AF020070). Three separate alignments were made of the *ftsZ* gene from nematode *Wolbachia* and from A- and B-group arthropod *Wolbachia*. These alignments omitted the 3′-end of the gene because it contained insertions and deletions that made alignment uncertain.

Recombination can be detected by a decline in linkage disequilibrium with distance. This is because as the distance between two sites decreases, there will have been fewer recombination events to break down linkage disequilibrium. The linkage disequilibrium between pairs of sites was estimated using two different measures, r^2 and $|D'|$, and the correlation with distance was calculated using Pearson's coefficient (Awadalla, Eyre-Walker, and Maynard-Smith 1999; Jorde and Bamshad 2000). The significance of the negative correlation was

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Table 1
Recombination in the Rickettsiaceae. Analysis includes all the sites with two alleles segregating.

Taxa	Gene	Length	Number	θ_W	Correlation		Permutation Tests for Recombination			
					r^2, d	$\lvert D' \rvert$, d	P_{lk}	P_{r^2}	$P_{\lvert D' \rvert}$	$2N_er$
Arthropod *Wolbachia* (A group) . .	*wsp*	570	17	0.069	−0.110	0.021	0.00	0.00	0.79	21.94
Arthropod *Wolbachia* (B group) . .	*wsp*	570	35	0.086	−0.049	−0.028	0.00	0.00	0.12	22.65
Nematode *Wolbachia*.	*wsp*	444	10	0.222	−0.052	−0.016	0.74	0.06	0.29	0.00
Cowdria ruminantium	*map1*	870	14	0.069	−0.012	−0.034	0.00	0.16	0.05	18.18
Arthropod *Wolbachia* (A group) . .	*ftsZ*	831	29	0.015	−0.130	−0.234	0.00	0.00	0.00	24.24
Arthropod *Wolbachia* (B group) . .	*ftsZ*	831	49	0.030	−0.060	−0.028	0.00	0.00	0.13	16.16
Nematode *Wolbachia*.	*ftsZ*	903	14	0.059	0.001	0.013	0.65	0.72	0.61	5.05

calculated by a Mantel test. The position of the sites was randomized and the statistic recalculated a minimum of 1,000 times. The significance was taken as the proportion of times the correlation coefficient was the same or more negative than the observed value. The analysis only included sites with two segregating alleles and was performed using the program LDhat (McVean 2001*a*).

There was a significant decline in linkage disequilibrium with distance in both arthropod *Wolbachia* and *C. ruminantium* but not in nematode worm *Wolbachia* (table 1). In general, the r^2 measure of linkage disequilibrium detected recombination more often than the $|D'|$ measure, which is consistent with previous analyses which have shown r^2 to be more powerful (McVean 2001*b*). The analysis was then repeated including only those sites at which both alleles occurred at frequencies of over 10%. The exclusion of rare alleles is expected to make the test more powerful because common alleles tend to be older and are therefore more likely to show evidence of recombination (Awadalla, Eyre-Walker, and Maynard-Smith 1999). As expected, this increased the significance of the tests for recombination in all cases for arthropod *Wolbachia* and *Cowdria* sequences (table 2). However, the exclusion of rare alleles had no consistent effects on the analysis of the nematode sequences in which recombination was still not detected (table 2). Finally, the analysis was repeated using only those sites identified as being informative about recombination by a coalescent method (McVean 2001*b*). The exclusion of uninformative sites did not appear to consistently alter the power of the tests to detect recombination (data not shown).

A rough guide to the relative rates at which recombination occurs can be gained by comparing the correlation coefficient of linkage disequilibrium and distance. This correlation was greater in arthropod *Wolbachia* than in *Cowdria*, which suggests that $2N_er$ may be higher in *Wolbachia*.

The second approach used to test both for recombination and estimate the rate of recombination was an approximate likelihood method based on coalescent theory (McVean, Awadalla, and Fearnhead 2002). This method estimates the value of the population rate of recombination, $2N_er$, where N_e is the effective population size and r the rate of crossing over within an individual for each generation. It is this statistic that determines the effect of recombination on patterns of linkage disequilibrium. The presence or absence of recombination can be tested for using a permutation test. This method also detected recombination in arthropod *Wolbachia* and *C. ruminantium* sequences but not in nematode *Wolbachia* (tables 1 and 2).

The population rate of recombination, $2N_er$, was then estimated by this method (tables 1 and 2). The estimate in nematode worms ranged from zero to five. The rate was higher in *Cowdria* (18) and in arthropod *Wolbachia* (20–28). This again suggests that the population rate of recombination in arthropod *Wolbachia* may be similar to that in its horizontally transmitting relative. The estimated value of $2N_er$ was consistent across both the different genes and between the A- and B- group arthropod *Wolbachia* (tables 1 and 2). In all four arthropod *Wolbachia* data sets (two genes, two groups) the estimated value of $2N_er$ fell between 18 and 28. The estimates were insensitive to the exclusion of rare alleles (table 2).

Our a priori hypothesis was that the rate of recombination would be lower in taxa with lower rates of horizontal transmission. This is supported in the case of

Table 2
Recombination in the Rickettsiaceae. Analysis includes sites with two alleles segregating and frequencies >0.1.

Taxa	Gene	Length	Number	θ_W	Correlation		Permutation Tests for Recombination			
					r^2, d	$\lvert D' \rvert$, d	P_{lk}	P_{r^2}	$P_{\lvert D' \rvert}$	$2N_er$
Arthropod *Wolbachia* (A group) . . .	*wsp*	570	17	0.046	−0.153	−0.008	0.00	0.00	0.43	24.24
Arthropod *Wolbachia* (B group) . . .	*wsp*	570	35	0.036	−0.157	−0.123	0.00	0.00	0.00	20.20
Nematode *Wolbachia*.	*wsp*	444	10	0.084	−0.090	−0.032	0.65	0.03	0.20	0.00
Cowdria ruminantium	*map 1*	870	14	0.050	−0.032	−0.047	0.00	0.05	0.03	18.18
Arthropod *Wolbachia* (A group) . . .	*ftsZ*	831	29	0.006	−0.493	−0.365	0.00	0.00	0.00	28.28
Arthropod *Wolbachia* (B group) . . .	*ftsZ*	831	49	0.013	−0.140	−0.101	0.00	0.01	0.01	20.20
Nematode *Wolbachia*.	*ftsZ*	903	14	0.049	0.020	0.012	0.61	0.80	0.67	5.05

nematode *Wolbachia*, which do not recombine. But the estimate of $2N_er$ in arthropod *Wolbachia* (20–28) is similar to that of the horizontally transmitting relative *Cowdria* (18). These are typical of the rates of recombination estimated by this method for human pathogens. For example $2N_er = 41$ in the bacterium *Helicobacter pylori* and ranges from 0.84 to over 100 in various pathogenic viruses (McVean, Awadalla, and Fearnhead 2002).

The final approach used was to estimate the minimum number of recombination events R_m in the data by parsimony (Hudson and Kaplan 1985). This method uses an infinite sites model, which assumes an absence of homoplasy in the data. But some regions of these genes are highly variable and most probably include numerous homoplasies, meaning that these results must be treated with great caution. The number of recombination events that can be detected depends in part on the number of sequences, so for this analysis several alignments of 14 *wsp-map*1 sequences were used. This approach gave a slightly higher estimate of R_m in the *Cowdria map1* gene ($R_m = 51$) compared with three alignments of the same number of arthropod *Wolbachia wsp* sequences ($R_m = 26–35$).

One possible source of error (except for in the parsimony analysis) comes from the smaller number of nematode *Wolbachia* sequences analyzed. Therefore I generated 10 replicate data sets of arthropod sequences containing the same number of sequences as the nematode alignments. Recombination was only detected in nine of the 10 replicates using the likelihood and linkage disequilibrium methods.

The maximum likelihood estimate of the rate of recombination ($2N_er$) in arthropod *Wolbachia* was consistent across both *Wolbachia* groups and both genes used. This suggests that this estimate is reasonably insensitive to any biases in the sampling of sequences or factors specific to either gene such as positive selection on the surface protein gene (Jiggins, Hurst, and Yang 2002). But *Wolbachia* bacteria are likely to have complex population demographics that are not accounted for in the coalescent model. This means that the values of $2N_er$ should be treated with caution. But given the agreement across data sets and methods of analysis, I can conclude that the population rate of recombination in arthropod *Wolbachia* is similar to that in *Cowdria*.

Early arthropod *Wolbachia* research often made the unstated assumption that there was no recombination. Recently, however, there were two reports of recombination, and in each case a single recombination event could explain the data (Jiggins et al. 2001*b;* Werren and Bartos 2001). Our analysis has extended this to show that recombination occurs across both the A and B groups, across gene regions, and at a rate similar to horizontally transmitted pathogens. This surprisingly high rate of recombination may be because r is larger than expected and because different strains frequently come into contact and recombine. In particular, horizontal transmission between host species may be far more common than we realize, perhaps because most transmission events do not establish in the novel host. Alternatively, the effective population size may be very large. In this respect, it is notable that in excess of 20% of all insects may be infected with this bacterium (Werren, Windsor, and Guo 1995; West et al. 1998; Jiggins et al. 2001*a*).

An alternative explanation of these results is that the rate of recombination in arthropod *Wolbachia* is low, but natural selection favors the spread of recombinants. This could occur because of selection for evolutionary novelty in parasites (*Cowdria* and arthropod *Wolbachia*) but not mutualists (nematode *Wolbachia*). Our data do not provide any support for this hypothesis because the recombination rate in the conserved cell cycle gene *ftsZ* is similar to that in the surface protein gene *wsp*. The *wsp* gene is known to be under positive selection in parasitic but not mutualistic *Wolbachia* (Jiggins, Hurst, and Yang 2002).

The implications of such high rates of recombination in the evolution of *Wolbachia* are considerable. Recombination means that novel beneficial mutations will be able to spread across different genetic backgrounds. This may enable different bacterial strains to change the phenotypic effects they have on their host rapidly (Hurst, Jiggins, and Pomiankowski 2002). Similarly, strains that induce cytoplasmic incompatibility may rapidly combine and change their crossing type. Previous analyses have assumed that such changes required two separate mutations to occur in the same bacterium before they are favored by selection (Charlat, Calmet, and Mercot 2001). The high rate of recombination also has important consequences for biologists wanting to study these bacteria. For example, phylogenies reconstructed from gene sequence data must be treated with caution. This makes it difficult to date events in the evolution of these bacteria or reconstruct past patterns of evolution.

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