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Report

Competing Selfish Genetic Elements in the Butterfly *Hypolimnas bolina*

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Summary

Maternally inherited selfish genetic elements are common in animals [1]. Whereas host genetics and ecology are recognized as factors that may limit the incidence of these parasites [2, 3], theory suggests one further factor—interference with other selfish elements—that could affect their prevalence [4, 5]. In this paper, we show that spatial heterogeneity in the occurrence of the male-killing *Wolbachia* *w*Bol1 in the tropical butterfly *Hypolimnas bolina* [6] is caused by a second infection that can exclude the male-killer. We first provide evidence of a second *Wolbachia* strain, *w*Bol2, present in most populations that do not carry the male-killer but rare or absent when the male-killer is present. Crossing data indicate that *w*Bol2 in males induces cytoplasmic incompatibility to both uninfected and *w*Bol1-infected females. The *w*Bol2 infection can therefore not only spread through uninfected populations but also resist invasion by *w*Bol1. Thus, we provide empirical support for the hypothesis that the incidence of particular selfish genetic elements can limit the presence of competing types.

Results and Discussion

Cytoplasmic elements, such as the intracellular bacterium *Wolbachia*, have evolved a number of different ways of manipulating host reproduction [3, 7]. Through cytoplasmic incompatibility (CI) or male-killing (MK), they increase the survival rate of infected females above that of uninfected females, and by feminization or induction of parthenogenesis, they increase the rate of production of female offspring from infected female hosts compared to that of uninfected ones. Asymmetry in the transmission of the cytoplasmic element (through females only) makes males evolutionary dead ends and underlies the adaptive nature of these manipulation phenotypes. Their dramatic effects at the individual level, coupled with their widespread occurrence and their ability to reach high frequencies in natural populations, make them important agents, affecting both the ecology and evolution of their host [8].

Variation in the frequency of these elements between populations is known to depend largely on factors affecting the fitness of the "parasitized" cytoplasmic lines: the strength of the phenotypes induced, direct or indirect effect on host fitness, and efficiency of the transmission through the egg [3, 7]. However, theoretical models suggest an alternative category of factors that may affect their distribution in natural populations: interference with other selfish genetic elements [4, 5, 9]. Considering the two most commonly observed *Wolbachia* effects (MK and CI), theoretical models predict that the presence of one type of infection in a population may prevent the spread of the other type [5].

In a structured habitat (e.g., islands), interference of this kind would translate into a spatially heterogeneous, yet stable, distribution of the two infection types. A striking case of spatial heterogeneity of *Wolbachia* distribution is found in the tropical butterfly *Hypolimnas bolina* [6]. In this species, a MK *Wolbachia* (called *w*Bol1) is found in many, but not all, South Pacific islands. Moreover, uninfected islands can be found in the vicinity of infected ones. The present study is based on samples from 25 natural populations across the South Pacific (Figure 1). In addition to *w*Bol1, a second infection (here termed *w*Bol2) was found in 14 populations. Among the 898 wild caught individuals tested, coinfection (presence of *w*Bol1 and *w*Bol2 in a single individual) was never observed. Sequencing of the *wsp* gene from 30 infected individuals from 14 island populations revealed no variability among *w*Bol2 populations and placed *w*Bol2 in the A *Wolbachia* clade, consistent with five *Wolbachia* MLST genes [10] that were also sequenced. Table 1 and Figure 1 show the prevalence of *w*Bol1 and *w*Bol2 infections across the 25 populations sampled. As shown in an earlier survey of 16 populations [6], *w*Bol1 prevalence is highly variable over space and is without any detectable geographic trend; the same appears to be true for *w*Bol2.

We observed *w*Bol2 at equal prevalence in males and females, suggesting it is not a sex-ratio distorter (Table 1). To confirm this conjecture, we measured egg-hatch rates and sex ratio in laboratory-reared lines from the islands of Tubuai and Raivavae. As expected, wild *w*Bol2-infected females from both islands produced a high

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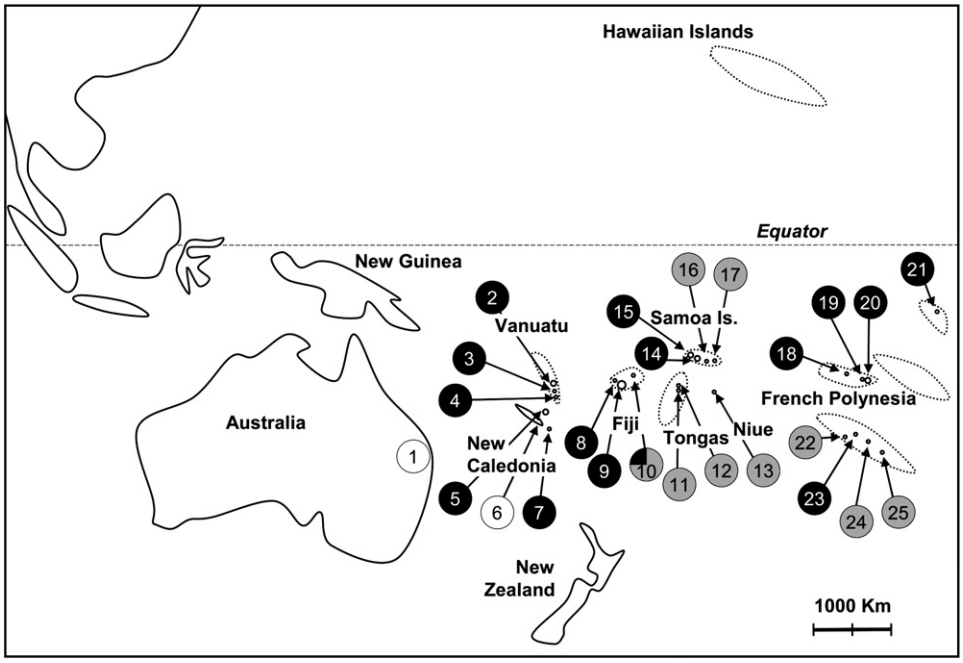

Figure 1. Geographic Distribution and Schematic Presentation of Prevalence Variation across the South Pacific

The color in the circle indicates in which of the three following categories a population falls under: uninfected (empty circle), *w*Bol1 (black circle), and *w*Bol2 (gray circle); refer to Table 1 for detailed data on prevalence and sample size; for clarity, rare infection ($\leq$7%) was considered as absent in this figure.

egg-hatch rate (median hatch rate = 100%, n = 15 wild females from Tubuai; median hatch rate = 100%, n = 13 wild females from Raivavae; total number of eggs observed, 896 and 545, respectively) and an even adult sex ratio (proportion of males produced across seven wild *w*Bol2-infected females from Tubuai = 0.51, n = 262 total adult progeny; proportion of males across six wild *w*Bol2-infected females from Raivavae = 0.55, n = 118 total adult progeny).

We then tested the hypothesis that *w*Bol2 maintains itself in the host through CI. In its simplest form, CI results in embryonic mortality in crosses between infected males and uninfected females. To test this hypothesis, we thus raised a Tubuai line on antibiotics for two generations to cure it of *w*Bol2 infection. In order to prevent reduction in hatch rates associated with inbreeding depression, we went back to the island of Tubuai to collect a fresh *w*Bol2 matriline and performed the four possible combinations of crosses between cured and *w*Bol2-infected individuals, the results of which are shown in Table 2 (crosses 1–4). A comparison between crosses 1 (uninfected females and infected males) and 2 (uninfected females and uninfected males) shows that *w*Bol2 induces strong CI (Wilcoxon test, p value < 0.0001), as indicated by the very low median hatch rate in cross 1. We note hatch rates in cross 2 (between cured females and cured males) are lower than expected. Although this most likely results from two generations of inbred crosses that were necessary for ensuring efficient curing by antibiotics (see the Experimental Procedures), alternative interpretations (including the hypothesis that the removal of *w*Bol2 decreases fertility) cannot be ruled out. Notwithstanding the cause, this unexpected reduction in egg-hatch rate does not affect the conclusion that *w*Bol2 induces CI: Crosses between infected males and infected females (cross 3) are fully fertile, whereas crosses between infected males and uninfected females (cross 1) are fully sterile. The other control in this experiment (cross 4: infected females and uninfected males) shows high egg-hatch rates, as expected from a typical CI phenotype.

Past work has demonstrated that CI can also be found in crosses between males and females carrying different strains of *Wolbachia* [11]. Having established that *w*Bol2 can induce CI in crosses with uninfected females, we therefore examined whether it could induce CI against *w*Bol1, the MK strain. If *w*Bol2 can induce CI against *w*Bol1-infected females, then *w*Bol1 would be unable to invade a *w*Bol2-infected population. To this end, crosses were performed between F1 progeny of wild collected females from the islands of Tubuai (natural condition: *w*Bol2) as well as Rurutu and Moorea (natural condition of females: *w*Bol1 or uninfected). The results are presented in Table 2 (crosses 5–10). A comparison between crosses 5 and 6 shows that *w*Bol2-infected males from Tubuai induce CI in crosses against uninfected females from the other islands with a reduced egg-hatch rate in crosses between *w*Bol2-infected males from Tubuai and uninfected females from Rurutu and Moorea (Wilcoxon test, p value < 0.0001). We then examined whether *w*Bol2 also induced CI in crosses with females from Moorea and Rurutu infected with *w*Bol1. A comparison between crosses 7 and 8 demonstrates that this is the case: Embryonic mortality is almost complete after mating with *w*Bol2-infected males compared to 50% (because of male-killing) after mating with uninfected males (Wilcoxon test, p value < 0.0001). Cross 10 allows us to

Table 1. Prevalence of *w*Bol1 and *w*Bol2 across the South Pacific

Map	Location (country)	Sex	Infection Status			Sample Size	Reference
			*w*Bol1	*w*Bol2	U		
1	Australia	f	0%	0%	100%	8	This study
		m	0%	0%	100%	2	
2	Efate (Vanuatu)	f	43%	0%	57%	7	This study
		m	0%	0%	100%	7	
3	Tanna (Vanuatu)	f	25%	0%	75%	4	This study
		m	0%	0%	100%	6	
4	Aneityum (Vanuatu)	f	24%	0%	76%	21	This study
		m	0%	5%	95%	22	
5	Lifou (New Caledonia)	f	31%	0%	69%	16	This study
		m	0%	0%	100%	15	
6	Grande Terre (New Caledonia)	f	0%	0%	100%	10	This study
		m	0%	0%	100%	42	
7	Ile des pins (New Caledonia)	f	83%	0%	17%	23	This study
		m	0%	7%	93%	15	
8	Waya Lailai (Fiji)	f	54%	0%	46%	76	[18]
		m	0%	0%	100%	8	
9	Viti Levu (Fiji)	f	59%	3%	38%	34	[18]
		m	0%	0%	100%	9	
10	Taveuni (Fiji)	f	25%	75%	0%	24	[18]
		m	—	—	—	0	
11	Kapa (Tonga)	f	0%	67%	33%	12	This study
		m	0%	42%	58%	12	
12	Neiafu (Tonga)	f	0%	67%	33%	3	This study
		m	0%	100%	0%	3	
13	Niue	f	0%	80%	20%	10	This study
		m	0%	100%	0%	2	
14	Savaii (Independent Samoa)	f	100%	0%	0%	35	[17]
		m	50%	0%	50%	2	
15	Upolu (Independent Samoa)	f	99%	0%	1%	257	[17]
		m	33%	0%	67%	3	
16	Tutuila (American Samoa)	f	0%	67%	33%	6	[6]
		m	0%	70%	30%	10	
17	Olosega (American Samoa)	f	0%	100%	0%	23	[6]
		m	0%	96%	4%	25	
18	Huahine (French Polynesia)	f	100%	0%	0%	1	This study
		m	—	—	—	0	
19	Moorea (French Polynesia)	f	83%	0%	17%	48	[6], this study
		m	0%	2%	98%	46	
20	Tahiti (French Polynesia)	f	96%	0%	4%	28	[6], this study
		m	9%	0%	91%	11	
21	Ua Huka (French Polynesia)	f	86%	0%	14%	43	[6]
		m	13%	0%	88%	24	
22	Rimatara (French Polynesia)	f	—	—	—	0	This study
		m	0%	93%	7%	15	
23	Rurutu (French Polynesia)	f	69%	2%	29%	246	[6], this study
		m	0%	1%	99%	81	
24	Tubuai (French Polynesia)	f	0%	98%	2%	49	[6], this study
		m	0%	96%	4%	48	
25	Raivavae (French Polynesia)	f	0%	100%	0%	25	This study
		m	0%	100%	0%	30	

Abbreviations are as follows: U, uninfected. Map numbers correspond with Figure 1. The reference indicates the publication where the sample was first used.

rule out the hypothesis of nuclear incompatibility between butterflies of different islands causing embryo death. This cross, between Tubuai females and Moorea or Rurutu males, demonstrates normal egg viability, indicating there is no reduction in the survival of hybrid eggs if males do not carry *w*Bol2. The levels of incompatibility observed in crosses between *w*Bol2-infected males and *w*Bol1-infected females leave little possibility of maintenance to *w*Bol1 lineages in a *w*Bol2-infected population: The *w*Bol2-infected males that mate with *w*Bol1-infected females will render *w*Bol1-infected progeny inviable. Consistent with this observation, all but one population harbouring *w*Bol2 at high prevalence are completely devoid of *w*Bol1 infection (Table 1).

Theoretical models predict that MK infection can also inhibit invasion by CI-inducing *Wolbachia* [5]. Testing this hypothesis with our field prevalence data is not trivial. Because the frequencies of *w*Bol1, *w*Bol2 and uninfected lineages must sum up to one, we expect a negative correlation between *w*Bol1 and *w*Bol2 prevalence even in the absence of an active mutual exclusion. In addition, drift alone would be sufficient to produce a geographic partitioning between the two strains. To circumvent this problem, we used a slightly different approach, by asking whether *w*Bol1 appears more efficient

Table 2. Compatibility Relationships between *w*Bol1, *w*Bol2, and Uninfected Individuals

Cross Number	Female: Island (infection)	Male: Island (infection)	Median HR (IQR)	N	Ne
1	Tubuai (U)	Tubuai (*w*Bol2)	0.00 (0.00)	17	1335
2	Tubuai (U)	Tubuai (U)	0.59 (0.52)	8	712
3	Tubuai (*w*Bol2)	Tubuai (*w*Bol2)	0.95 (0.06)	14	1411
4	Tubuai (*w*Bol2)	Tubuai (U)	0.90 (0.13)	10	748
5	Moorea and Rurutu (U)	Moorea and Rurutu (U)	0.98 (0.06)	36	7281
6	Moorea and Rurutu (U)	Tubuai (*w*Bol2)	0.09 (0.19)	14	1749
7	Moorea and Rurutu (*w*Bol1)	Tubuai (*w*Bol2)	0.07 (0.19)	27	5236
8	Moorea and Rurutu (*w*Bol1)	Moorea and Rurutu (U)	0.49 (0.08)	77	14079
9	Tubuai (*w*Bol2)	Tubuai (*w*Bol2)	0.95 (0.20)	44	12995
10	Tubuai (*w*Bol2)	Moorea & Rurutu (U)	0.97 (0.04)	24	6675

Abbreviations are as follows: N, number of crosses; Ne, total number of eggs obtained (clutches with less than 20 eggs were discarded); median HR, median of egg hatch rates, with interquartile range in parentheses.

than uninfected lineages at keeping the prevalence of *w*Bol2 at low levels. To this end, we defined two groups of islands: (1) group 1, which includes islands where the *w*Bol1 infection is found and (2) group 2, which includes islands where the *w*Bol1 infection is not found. For each population, we then calculated the proportion of *w*Bol2 females among non-*w*Bol1 individuals and compared this value among groups 1 and 2. In other words, we asked whether the proportion of *w*Bol2-infected versus uninfected females is higher in populations where *w*Bol1 is absent than in populations where *w*Bol1 is present. Consistent with our prediction, we observed that the *w*Bol2/(*w*Bol2 + uninfected) ratio is significantly lower in populations where *w*Bol1 is present (Wilcoxon test, p value = 0.016). Thus, the MK *w*Bol1 appears efficient at impeding the spread of *w*Bol2, the CI-inducing strain.

According to previous modelling, MK and CI *Wolbachia* cannot coexist in a single panmictic population [5]. Although the majority of the populations surveyed are in accord with this prediction (with only one strain observed within a sample), the co-occurrence of *w*Bol1 and *w*Bol2, with one variant present at much higher prevalence than the other, was also observed (Table 1). A possible explanation is that such populations represent a dynamic equilibrium between introduction of the rare strain through migration and elimination through selection. We have explored this hypothesis by using a simulation approach based on an earlier model [5]. By using this model, we have estimated migration rates that would result in equilibrium frequencies of the two strains similar to the prevalence data we obtained empirically (see the Experimental Procedures). As can be seen in Figure 2, the simulation results suggest that *w*Bol2 (the CI strain) can be maintained at frequencies below ~1.1% in a population with *w*Bol1 at high prevalence under a reasonable range of migration rates. In other words, low migration rates (as expected between island populations) are sufficient to explain persistence of a CI strain at low frequency in an MK population in a migration-selection equilibrium.

In some of our samples, the frequency of *w*Bol2 was found to be higher than the threshold frequency above which *w*Bol2 should invade and exclude *w*Bol1. A first possible explanation for this pattern is that these populations are not at equilibrium but, instead, are on their way to fixation of the *w*Bol2 strain. Alternatively, it is possible that the parameters used in our simulations do not accurately reflect the field situation (as observed in comparisons of *w*Ri-infected *D. simulans* between laboratory and field [11]) or that the respective values for these parameters vary among populations. We have explored this question by repeating the above analysis with various values of CI levels, transmission rates, and fitness costs. In Figure 2, we show that with CI levels lower than what we have measured in the laboratory, the balance between migration and selection can lead to substantially higher equilibrium prevalence of the *w*Bol2 strain. Lower transmission rates and fecundity reduction induced by *w*Bol2 similarly broaden the range of equilibrium frequencies for which coexistence of *w*Bol1 and *w*Bol2 is possible under migration (see Figure S1 in the Supplemental Data available with this article online).

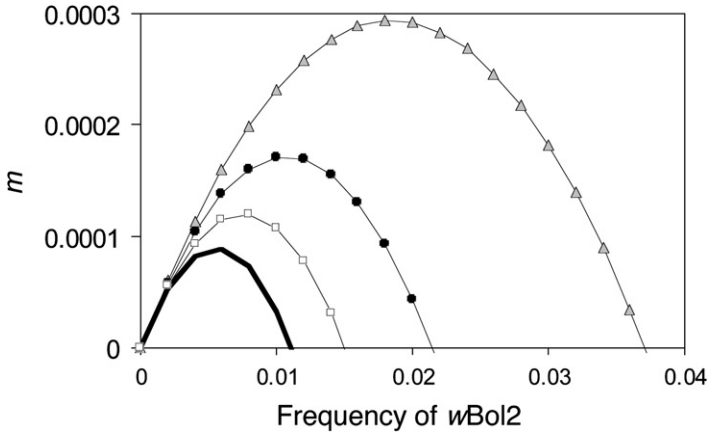

Figure 2. Estimated Migration Rates from a Population Fixed for *w*Bol2 Required for Maintaining a Given Fraction of Females with *w*Bol2 Infection Polymorphic with *w*Bol1

The x axis shows the equilibrium frequency of *w*Bol2-infected females among all females, and the y axis shows the migration rate that is required for obtaining this equilibrium frequency. Intersections of the curves with the x axis signify maximum equilibrium frequencies of *w*Bol2 with stable *w*Bol1 infection. The bold line shows migration rates for empirically obtained parameters (see the Experimental Procedures). The other curves are based on the same parameter values, except CI levels of I = 0.7 (white squares), I = 0.5 (black circles), and I = 0.3 (gray triangles).

Only long-term surveys will allow us to determine whether such populations are indeed at equilibrium or whether the prevalence of *w*Bol2 is currently increasing.

Although populations carrying the CI strain at low prevalence together with the MK strain at high prevalence can be interpreted with the above analysis, the case of Taveuni, where *w*Bol2 was found in 75% of the females and *w*Bol1 in the remaining 25%, cannot be explained under reasonably low migration rates. Even with lower CI levels than what we have estimated in the laboratory, unrealistically strong unidirectional migration must be assumed for the maintenance of *w*Bol1 in this population at such a high prevalence to be explained. Assuming the levels of CI are as high in this population as was measured with lines from the island of Tubuai, simulations predict that, in the absence of migration, the MK strain should be lost from the population (frequency below 0.1%) in only three generations. From this, we conclude that the Taveuni case must represent an extremely transient situation, on its way to fixation of the *w*Bol2 infection.

The present study provides empirical support for the theoretical prediction that MK and CI-inducing symbionts are mutually inhibiting and that the incidence of *Wolbachia* strains of a given phenotype can be limited by the presence of a strain with a different phenotype. It is notable that this conclusion has been made possible only because of the highly structured habitat of the host species. Thorough investigations of field prevalence in other island species will provide the material for testing the generality of this finding.

Experimental Procedures

Sampling, DNA Extraction, and PCR

This study is based on samples from 25 populations across the South Pacific (Table 1). Three samples from Southeast Asia, although available [6], were excluded from the present analysis because *w*Bol1 does not kill males in these area as a result of host suppression [12]. The wings of collected individuals were detached, and their bodies were stored in 95% ethanol. For previously published samples, DNA was prepared as detailed in [6]. For recently collected samples, DNA was extracted from a small abdominal tissue sections (2–5 mm^3) with Qiagen DNeasy tissue kits. DNA extracts were diluted 10× and assayed for the presence of A or B clade *Wolbachia* in a single duplex-PCR reaction, where A clade infection was screened with specific 16S primers [13], and B clade infection was screened with specific *wsp* primers (wsp81f and wsp522r) [14].

Because the estimation of prevalence relied on PCR assays in some of our samples, we took special care to eliminate false negatives. To this end, we first assessed the quality of DNA by using a general "metazoan" PCR of the COI mitochondrial gene with the primer pair LCO/HCO [15]; nonamplifiable material was discarded from the analysis. We combined several approaches to test whether this method was ensuring an accurate estimate of prevalence. First, we compared the assayed infection status of wild uninfected, wBol1-, and wBol2-infected females and 60 laboratory-reared F1 individuals from these females (20 produced by wild uninfected females, 20 by wild *w*Bol1 females, and 20 by wild *w*Bol2 females). Maternal and F1 infection status were in each case concordant (incidentally this experiment also provides a crude estimate of the transmission efficiency of *w*Bol1 and *w*Bol2; the transmission efficiency appears to be perfect or nearly perfect). In addition, we compared the sensitivity of the mtDNA, *w*Bol1, and *w*Bol2 PCR assays after dilution of the DNA template; the three PCR assays showed exactly the same pattern: PCR products were detected after 1/10, $1/10^2$, $1/10^3$ and $1/10^4$ dilution of the template but not after $1/10^5$ dilutions. Finally, for the *w*Bol1 infection, we crosschecked the PCR and F1 sex-ratio data in populations where the later was available: All the females found uninfected by PCR produced both males and females in F1 in proportions compatible with a 1/1 sex ratio (n = 25 broods, n = 519 F1 adults); by contrast, none of the *w*Bol1-infected females produced any male (n = 42 broods, n = 547 F1 adults). From these experiments, we conclude that the rate of false negatives in our *Wolbachia* PCR assays must be very low, if anything above zero.

Affiliation of Strains Detected

Partial sequences of the *wsp* (*Wolbachia* Surface Protein) gene were obtained from A-infected individuals from the following populations: Aneityum (n = 1), Iles des pins (n = 1), Viti Levu (n = 1), Taveuni (n = 1), Kapa (n = 4), Neiafu (n = 3), Niue (n = 4), Upolu (n = 1), Tutuila (n = 2), Olosega (n = 2), Moorea (n = 1), Rimatara (n = 4), Tubuai (n = 4), and Raivavae (n = 2). The sequences were attained directly from PCR product with primer 81F [14] after amplification with the 81F/691R primer pair. In addition to the *wsp* locus, the five *Wolbachia* MLST genes [10] were sequenced. For each of these loci, at least two sequences were obtained, from individuals of different geographic origin, and no variation was found.

Phenotype of *w*Bol2

We first examined the presence of sex-ratio distortion in lines infected with *w*Bol2. Wild female butterflies carrying *w*Bol2 from the islands of Tubuai and Raivavae were allowed to oviposit. Egg-hatch rates and F1 sex ratio were measured as detailed in [6]. We then assessed the ability of *w*Bol2-infected males to induce CI. This involved the classical four possible combinations of crosses between infected and cured individuals with one cured line and one fresh *w*Bol2-infected line from the island of Tubuai. CI is characterized by low egg-hatch rates in crosses between uninfected females and infected males. Furthermore, we investigated whether the CI induced by *w*Bol2 could prevent the spread of *w*Bol1, the MK strain. This involved crosses between F1 progeny of wild collected females originating from the islands of Tubuai (ten *w*Bol2 matrilines), Rurutu (ten *w*Bol1 matrilines and nine uninfected matrilines), and Moorea (four *w*Bol1 matrilines and two uninfected matrilines).

Butterflies were reared to adulthood as detailed in [6]. Upon emergence, adults were labeled individually with Tough Tags (USA Scientific) stuck on the basal-front part of their front wings. Individual labels allow us to retrieve the pedigree of any individual. After labeling, adults were placed in an outdoor cage (1.80 m × 1.80 m × 3.60 m, Bioquip model 1412A) exposed to sunlight, where a bright yellow synthetic sponge impregnated with 15% w/v sugar solution was available for feeding. Water was sprayed on the cage at the end of sunny days so that risks of dehydration were reduced. The cage was split in two parts so that males were isolated from females. When at least half of the emerged females had reached sexual maturity (circa 4 days after emergence [16]), the mating experiment was initiated on sunny days by mixing of males and females. Mating pairs were checked for every 15 min and isolated in a small cage. Mating duration (±15 min) was recorded. After mating, mated males were returned to the mating cage, whereas mated females were kept apart and induced to oviposit 12 hr later for ensuring that sperm had enough time to reach the spermathecae. Egg-hatch rate was recorded as follows. Five days after oviposition, freshly hatched larvae were counted and removed from laying boxes. Unhatched eggs were also counted. On the following day, additional hatched larvae were counted. This method ensures (1) that all eggs have time to hatch before estimating hatch rates and (2) that larvae do not feed on the leaves used for oviposition and thus ensures an accurate counting of unhatched eggs. Hatch rate was calculated as the total number of larvae among all the eggs hatched. Females that laid less than 20 eggs were excluded from the analysis.

Antibiotic Treatments

Tetracycline treatment was used for eliminating the *w*Bol2 infection from Tubuai individuals. Larvae from one Tubuai matriline collected in September 2004 were fed on *Asystasia gangetica* covered with a 1‰ Tetracycline w/v solution. Approximately 5 ml of solution was spread on leaves on each occasion the food was renewed (that is, every 5 days for 10 days after hatching, then every 2 days for 10 more days, until pupation). After this larval treatment, infection was still detected by PCR assay in some adults. The treatment was

therefore applied for a second generation, after which all adults tested (including mothers of the individuals used in CI assays), were found uninfected. Owing to the shortage of Tubuai lines in the course of this curing experiment, inbreeding could not be avoided. Specifically, we calculated that the average inbreeding coefficient of the zygotes produced in the crosses between cured male and cured females (Table 2, cross 2) is F = 0.25. All other crosses in this assay were noninbred with at least one parent from a freshly collected line. This makes the test conservative with respect to the hypothesis of low egg hatch being caused by CI.

Simulations

The computer simulations we performed are based on the model in [5]. To assess the likelihood that coexistence of *w*Bol1 at high prevalence with *w*Bol2 at low prevalence is due to recurrent migration of *w*Bol2-infected individuals, we assume that in each generation, a fraction m of the population is replaced by individuals infected with *w*Bol2 (equal numbers of males and females). This corresponds to a situation of unidirectional migration at a rate m from a population fixed for *w*Bol2 infection. To obtain estimates for the migration rate m (see Figure 2 and Figure S1), we initiated the population with *w*Bol1 at equilibrium prevalence and introduced a fraction of males and females infected with *w*Bol2. We then simulated the dynamical system for a single generation. For the frequencies to be in equilibrium, the decline in *w*Bol2 frequency within one generation due to selection must be balanced by subsequent influx of *w*Bol2-infected immigrants. Thus, denoting by p the initial *w*Bol2 frequency among females and by p^+ the *w*Bol2 frequency after one generation of reproduction and selection, we have $p = m + (1 - m)p^+$. This gives $m = (p - p^+)/(1 - p^+)$ as an estimate for the migration rate.

The simulation results presented in Figure 2 and Figure S1 are based on the following parameter values: (1) CI level: $I = 0.93$ (as inferred from Table 2, cross 7); (2) MK penetrance: $k = 0.99$ (as inferred from breeding experiments and field prevalence in males [6, 17, 18]); (3) transmission efficiency: $t_{CI} = t_{MK} = 0.99$ (consistent with our laboratory estimate, see Sampling, DNA Extraction, and PCR); (4) fitness compensation: $b = 0.024$ (a value that would explain, according to classic MK-dynamics theory [19], an equilibrium prevalence of 56%, that is, the average of the prevalence observed in all our samples); (5) direct effect on host fitness: $f_{CI} = f_{MK} = 1$ (no fitness effect assumed).

Supplemental Data

Supplemental Data include one figure and can be found with this article online at http://www.current-biology.com/cgi/content/full/16/24/2453/DC1/.

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Accession Numbers

Sequence data was deposited in Genbank under accession numbers EF025179-EF025183 and EF078895 and in the Wolbachia MLST database (http://pubmlst.org/wolbachia/) under ID 38.

Supplemental Data

Competing Selfish Genetic Elements in the Butterfly *Hypolimnas bolina*

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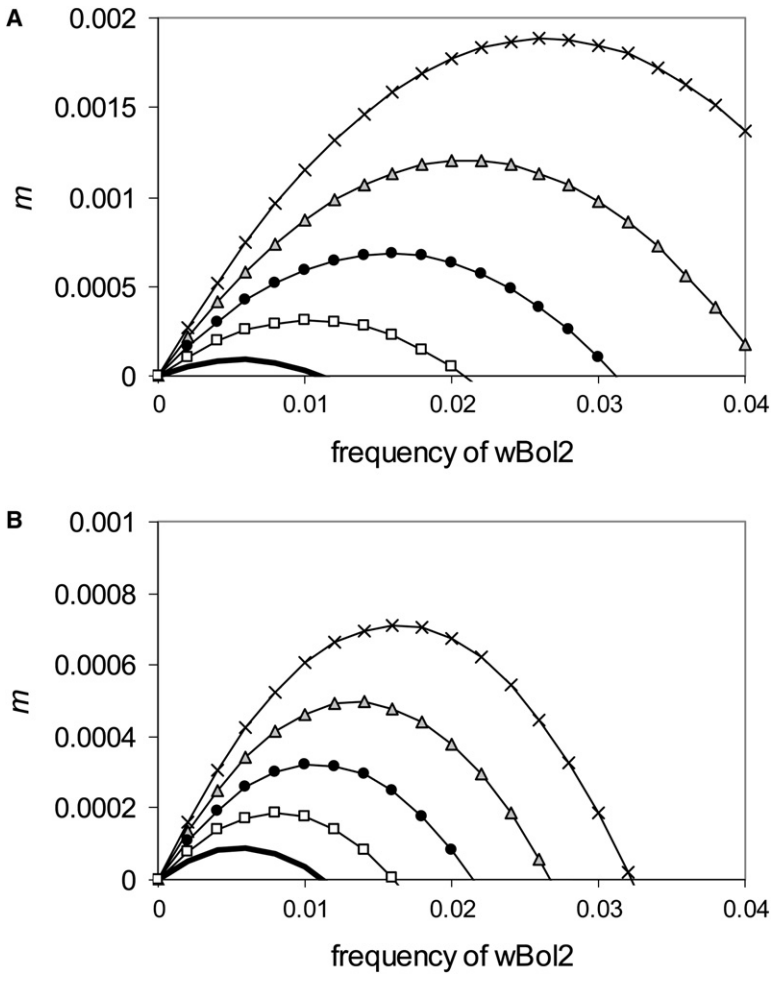

Figure S1. Estimated Migration Rates as Shown in Figure 2

In both figures, the bold lines give migration rates for empirically obtained parameters (see Simulations in the Experimental Procedures). In (A), fecundity reduction of *w*Bol2 is varied to $f_{CI} = 0.98$ (white squares), $f_{CI} = 0.96$ (black circles), $f_{CI} = 0.94$ (gray triangles), and $f_{CI} = 0.92$ (crosses). In (B), the transmission rate of *w*Bol2 is varied to $t_{CI} = 0.98$ (white squares), $t_{CI} = 0.97$ (black circles), $t_{CI} = 0.96$ (gray triangles), and $t_{CI} = 0.95$ (crosses). Note the different scale of the y axis in plots (A) and (B).