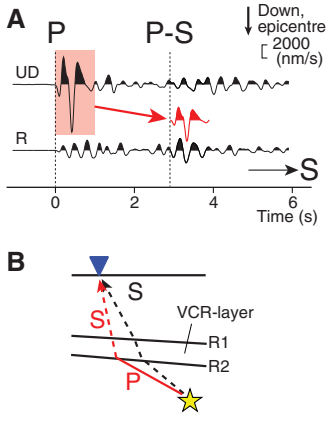

Fig. 3. (**A**) Stacked seismograms for microearthquakes in the Off-Kanto cluster. Vertical (UD) and radial (R) components are shown. A dashed vertical line indicates the arrival time of the *P-S* wave determined from cross-correlation analysis (*22*). (**B**) Schematic ray paths of direct *S* and *P-S* waves are shown. Solid and dashed lines denote *P* and *S* waves, respectively. A star and an inverted triangle denote a hypocenter and a station, respectively.

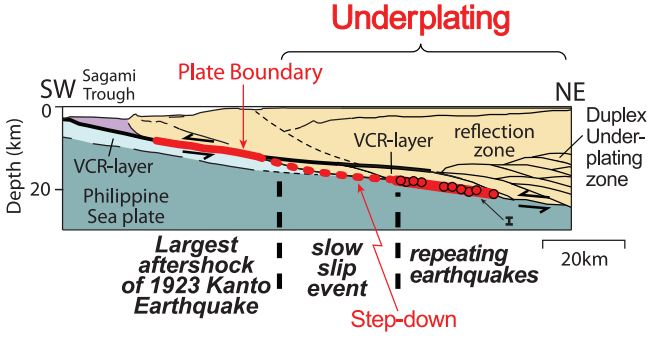

Fig. 4. Schematic illustration of subsurface structure, the plate boundary (red line), and underplating off the Kanto region of the Philippine Sea plate. The depth uncertainty of the RQs is also shown.

RQ plane is 12 ± 1°, which is about twice as large as the dip angle of the upper interface of the VCR layer (~5°) (fig. S3), which suggests that RQs are not distributed along the upper interface. The final result indicates that the plate boundary corresponds to the bottom interface of the VCR layer, showing that the zone of active slip steps down from its upper interface (Fig. 4). Such a step-down will cause accumulation of VCR. A characteristic imbricated structure called a duplex has been widely observed in association with underplating (*8*–*11*, *13*). Here, the reflection zone in P2 resembling such a structure is located northward of the active underplating region (Figs. 2 and 4).

Off Kanto, source areas of the megathrust earthquake [the largest aftershock, moment magnitude (M_w) 7.5, of the 1923 great Kanto earthquake (M_w 7.9) (*13*)], the SSE, and RQs are distributed in sequence from the trench axis to deeper regions (Fig. 4), with the region of the active step-down coinciding with the SSE. Because the SSE off Kanto repeats about every 6 years, with durations of about 10 days (*16*, *17*), underplating may also occur intermittently. In such cases, SSEs may serve as indicators of active step-down of plate boundaries and intermittent formation of duplex structures by underplating.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5988/210/DC1
Materials and Methods
Figs. S1 to S5
References

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Adaptation via Symbiosis: Recent Spread of a *Drosophila* Defensive Symbiont

John Jaenike,[1]* Robert Unckless,[1] Sarah N. Cockburn,[2] Lisa M. Boelio,[1] Steve J. Perlman[2]

Recent studies have shown that some plants and animals harbor microbial symbionts that protect them against natural enemies. Here we demonstrate that a maternally transmitted bacterium, *Spiroplasma*, protects *Drosophila neotestacea* against the sterilizing effects of a parasitic nematode, both in the laboratory and the field. This nematode parasitizes *D. neotestacea* at high frequencies in natural populations, and, until recently, almost all infections resulted in complete sterility. Several lines of evidence suggest that *Spiroplasma* is spreading in North American populations of *D. neotestacea* and that a major adaptive change to a symbiont-based mode of defense is under way. These findings demonstrate the profound and potentially rapid effects of defensive symbionts, which are increasingly recognized as major players in the ecology of species interactions.

The ancient origin (*1*–*3*) yet ongoing rapid evolution (*4*–*6*) of genes involved in defense against pathogens and parasites indicate that infective agents have been and continue to be major selective factors for virtually all organisms. In addition to the arsenal of nuclear genes encoding diverse and sophisticated mechanisms of defense, some organisms carry symbiotic microbes that provide defense against natural enemies (*7*, *8*). In insects, for example, maternally

transmitted symbionts have recently been shown to provide protection against parasitoid wasps, fungal pathogens, and RNA viruses (*9*–*13*).

Nematodes are probably the most abundant, diverse, and destructive macroparasites of plants and animals (*14*–*17*). Nematodes commonly attack *Drosophila* (*18*), and at least 10 mushroom-feeding species of *Drosophila* are parasitized by the nematode *Howardula aoronymphium* (Allantonematidae, Tylenchida) (*18*). Mated female *Howardula* infect *Drosophila* larvae, persist to the adult stage of flies, and release offspring that are passed from the fly via the gut and ovipositor into mushrooms, where the nematodes mate to renew the cycle (fig. S1). In the eastern United States, the most commonly infected *Drosophila* species is *D. neotestacea*, with a mean prevalence of parasitism of 0.23 around Rochester, New York (fig. S2) (*19*). Infections are severe, generally rendering females completely sterile, as well as reducing adult survival and male mating success (*18*). Given the high frequency and virulence of infection, there must be strong selective pressure on *D. neotestacea* to evolve defenses against these nematode parasites.

D. neotestacea is also infected with two maternally transmitted bacterial endosymbionts, *Spiroplasma* and *Wolbachia*, neither of which acts as a reproductive parasite in this species (*20*). To test whether the endosymbionts confer defense against nematode parasites, we exposed replicate iso-female lines of *D. neotestacea* to parasitism by *H. aoronymphium* in the laboratory, using lines co-infected with *Spiroplasma* and *Wolbachia* (*SW*), infected with *Spiroplasma* only (*S*), infected with *Wolbachia* only (*W*), or uninfected (*U*) (*21*). The fertility of nematode-parasitized females was greater if they were infected with *Spiroplasma*, but not *Wolbachia*, indicating that such flies have greater tolerance of parasitism [as defined in (*22*)] (Fig. 1A; nematode parasitism × *Spiroplasma* infection: $F_{1,723} = 239$, $P < 0.0001$; parasitism × *Wolbachia* interaction: $F_{1,723} = 0.04$, $P = 0.84$). We failed to detect any bacterial symbionts other than *Spiroplasma* in the *S* lines of *D. neotestacea* by cloning and sequencing 16*S* ribosomal DNA, suggesting that *Spiroplasma* alone is responsible for the fertility rescue.

To test whether *Spiroplasma* is associated with tolerance of nematode parasitism in the wild, we dissected *D. neotestacea* collected from natural populations and scored them for *Howardula* parasitism and the number of mature eggs per ovary. We then used polymerase chain reaction (PCR) to screen these flies for infection with *Spiroplasma* and *Wolbachia*. Female fertility was significantly affected by a nematode parasitism × *Spiroplasma* interaction ($F_{1,211} = 4.45$, $P = 0.036$; Fig. 1, B and C). Among nematode-parasitized flies, females infected with *Spiroplasma* had >10× greater fertility than those not infected with this symbiont (means = 11.13 ± 0.89 and 0.95 ± 0.35 eggs per ovary, respectively; $F_{1,129} = 19.34$, $P < 0.0001$). Among unparasitized flies, females harboring *Spiroplasma* carried a mean of 17.2 ± 0.8 eggs per ovary, and those without *Spiroplasma* carried 17.2 ± 1.5 ($F_{1,87} = 0$, $P = 0.99$), indicating that *Spiroplasma* has little effect on the fertility of unparasitized flies. All main effects and interactions involving *Wolbachia* were nonsignificant ($P > 0.5$), indicating that it does not play a role in defense against nematode parasitism.

To explore how *Spiroplasma* confers tolerance of nematode parasitism, we measured the sizes of motherworms (inseminated adult female worms within flies) in experimentally parasitized one-week-old *D. neotestacea* females. Using antibiotics, we selectively cured a doubly infected *SW* line of *D. neotestacea* from wild populations of either *Wolbachia* only or both *Spiroplasma* and *Wolbachia*; therefore, experimental flies had similar nuclear genetic backgrounds. At the motherworm stage, size is a good indicator of a nematode's potential reproductive output and impact on the host, as a *Howardula* motherworm is largely a sack of embryos and developing juveniles (*23*). As measured by surface area, motherworms were only half as large in flies infected with *Spiroplasma* as in those that were uninfected (means = 0.44 ± 0.04 mm^2 and 0.80 ± 0.04 mm^2, respectively; $F_{1,58} = 45.2$, $P < 0.0001$), indicating that *Spiroplasma* adversely affects motherworm growth and reproduction (Fig. 1F).

Thus, in both the wild and the laboratory, *Spiroplasma* is associated with tolerance of nematode parasites that would otherwise cause sterility in *D. neotestacea* females, and it appears to do so by impairing, through an unknown mechanism, the growth of *Howardula* within parasitized flies. To our knowledge, this is the first example of *Spiroplasma* acting as a mutualist. *Spiroplasma* are among the most widespread bacterial associates of arthropods (*24*), including

[1]Department of Biology, University of Rochester, Rochester, NY 14627, USA. [2]Department of Biology, University of Victoria, Victoria, British Columbia V8W 3N5, Canada.

*To whom correspondence should be addressed. E-mail: john.jaenike@rochester.edu

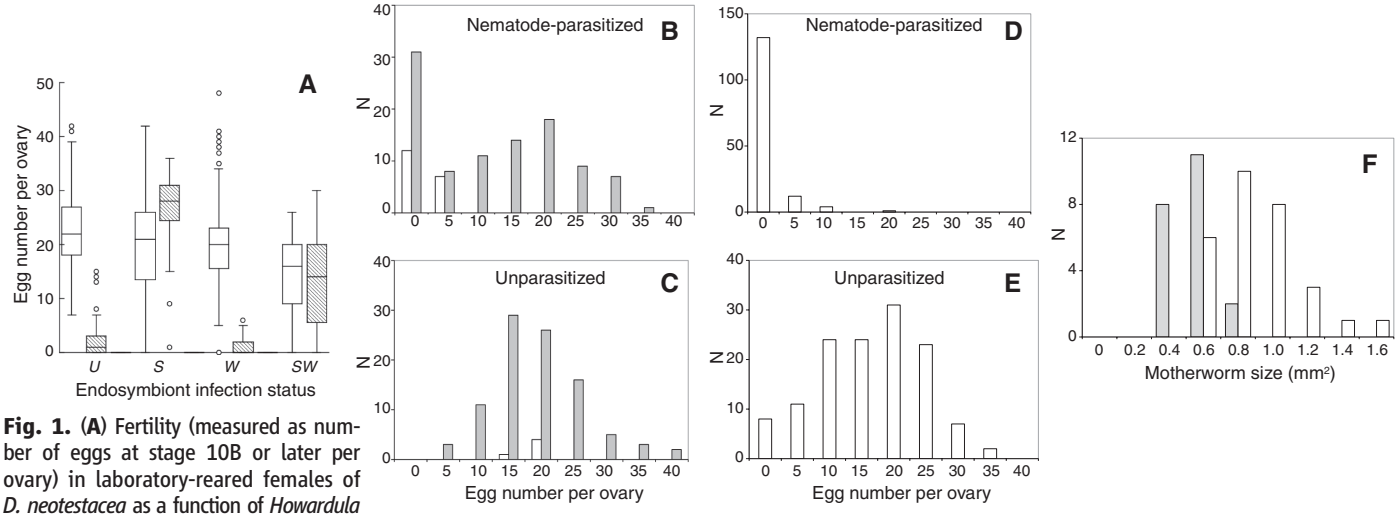

Fig. 1. (**A**) Fertility (measured as number of eggs at stage 10B or later per ovary) in laboratory-reared females of *D. neotestacea* as a function of *Howardula aoronymphium* parasitism, *Spiroplasma* infection, and *Wolbachia* infection. Hatched bars, nematode-parasitized flies; unhatched bars, unparasitized flies. *Spiroplasma* infection increases the fertility of females parasitized with *H. aoronymphium* (compare *U* to *S* and *W* to *SW*). In contrast, *Wolbachia* has no such effect (compare *U* to *W* and *S* to *SW*). (**B** and **C**) Fertility of 2008 field-collected *D. neotestacea* females parasitized (B) or not parasitized (C) by *Howardula*. Gray, infected with *Spiroplasma*; white, uninfected. The fertility of unparasitized flies was independent of *Spiroplasma* infection, whereas for parasitized flies, those carrying *Spiroplasma* were more fertile than uninfected flies. (**D** and **E**) Fertility of 1989 field-collected *D. neotestacea* females parasitized (D) or not parasitized (E) by *Howardula*. Mean number of eggs per ovary = 0.5 ± 0.2 and 14.5 ± 0.7 for parasitized and unparasitized flies, respectively. These flies were not scored for *Spiroplasma* infection, but note that the fertility of *Howardula*-parasitized flies in 1989 was similar to that of parasitized flies in 2008 that were not infected with *Spiroplasma*, suggesting a low level of *Spiroplasma* infection in 1989. (**F**) Nematode motherworms are significantly smaller in flies that harbor *Spiroplasma*. Motherworm size within one-week-old *Spiroplasma*-infected (gray) and uninfected (white) individuals of *D. neotestacea* experimentally parasitized with *Howardula*. *N*, number of flies (B to E) or motherworms (F).

Drosophila (*25*, *26*), but the role of *Spiroplasma* strains that experience solely vertical transmission has remained largely elusive. Although some *Spiroplasma* are reproductive parasites (*27*), mutualistic benefits may be responsible for the persistence of *Spiroplasma* in many host species. It is interesting to note that *Spiroplasma* occurs not only within cells of its invertebrate hosts but also within the hemocoel (*28*), where most parasitic nematodes, such as *Howardula*, reside (*29*).

Four lines of evidence independently suggest that the *Spiroplasma* infection is dynamic and spreading within natural populations of *D. neotestacea*. First, we PCR-screened museum specimens of *D. neotestacea* collected in the eastern United States in the early 1980s for *Spiroplasma*, *Wolbachia*, and, as a control for DNA quality, *Drosophila* cytochrome c oxidase subunit 1 (COI). Of the 20 flies, 18 (90%) were PCR-positive for *Wolbachia*, similar to current levels of *Wolbachia* infection in this species (*20*). In contrast, *Spiroplasma* was not detected, suggesting a prevalence in the 1980s in the range of 0 to 0.14 (the 95% confidence interval around 0 out of 20 *Spiroplasma*-infected flies). This is well below the current infection prevalence in eastern North America, which ranges from 0.5 to 0.8 at sites from Maine to Minnesota (*20*).

Second, almost all nematode-parasitized females of *D. neotestacea* collected in New York in the 1980s were sterile (*18*, *30*). The fertility distribution of nematode-parasitized flies collected in 1989 (Fig. 1D) was similar to that of nematode-parasitized flies that were uninfected with *Spiroplasma* in 2008 (Fig. 1B). A small fraction of parasitized flies in the 1980s carried 10 or more eggs, suggesting they may have been infected with *Spiroplasma*. Thus, in populations of *D. neotestacea* in Rochester, *Spiroplasma* appears to have increased from a low frequency in the 1980s to ~0.8 in less than 20 years (fig. S3 and table S6) (*20*).

Third, there is a continent-wide cline in the prevalence of *Spiroplasma* infection in *D. neotestacea* (Fig. 2A). In contrast, there is much less geographic variation in the infection prevalence of *Wolbachia*, suggesting that it is close to equilibrium across North America (Fig. 2B). *D. neotestacea* is parasitized by *Howardula* throughout its range, from Maine to British Columbia. *Howardula* infection frequencies in coastal British Columbia, where *Spiroplasma* is absent, were 0.21 in 2008 (n = 296 flies surveyed for parasitism) and 0.25 in 2009 (n = 132), similar to the long-term 0.23 prevalence of parasitism near Rochester, New York (*19*). Thus, nematode parasites probably impose selection in favor of *Spiroplasma* infection across the range of *D. neotestacea*.

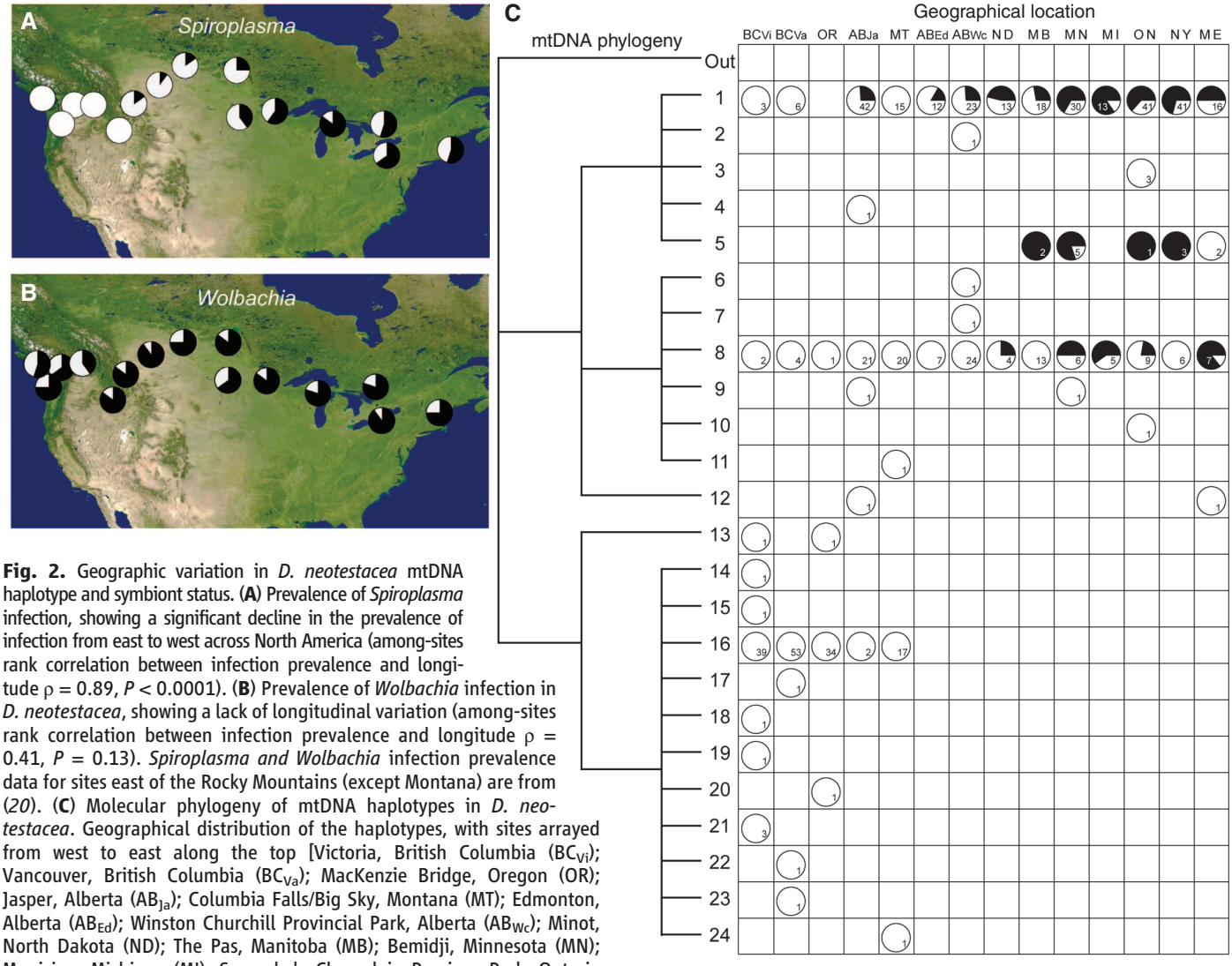

Fig. 2. Geographic variation in *D. neotestacea* mtDNA haplotype and symbiont status. (**A**) Prevalence of *Spiroplasma* infection, showing a significant decline in the prevalence of infection from east to west across North America (among-sites rank correlation between infection prevalence and longitude $\rho = 0.89$, $P < 0.0001$). (**B**) Prevalence of *Wolbachia* infection in *D. neotestacea*, showing a lack of longitudinal variation (among-sites rank correlation between infection prevalence and longitude $\rho = 0.41$, $P = 0.13$). *Spiroplasma and Wolbachia* infection prevalence data for sites east of the Rocky Mountains (except Montana) are from (*20*). (**C**) Molecular phylogeny of mtDNA haplotypes in *D. neotestacea*. Geographical distribution of the haplotypes, with sites arrayed from west to east along the top [Victoria, British Columbia (BC_{Vi}); Vancouver, British Columbia (BC_{Va}); MacKenzie Bridge, Oregon (OR); Jasper, Alberta (AB_{Ja}); Columbia Falls/Big Sky, Montana (MT); Edmonton, Alberta (AB_{Ed}); Winston Churchill Provincial Park, Alberta (AB_{Wc}); Minot, North Dakota (ND); The Pas, Manitoba (MB); Bemidji, Minnesota (MN); Munising, Michigan (MI); Samuel de Champlain Province Park, Ontario (ON); Rochester, New York (NY); and Chebeague Island, Maine (ME)]. The *Spiroplasma* infection prevalence for each site and haplotype is indicated by the proportion of black shading in the pie diagrams. Samples sizes are indicated by the numbers within each pie diagram. The clinal variation in *Spiroplasma* prevalence, the association of *Spiroplasma* infection with "eastern" mtDNA haplotypes, and the clines in *Spiroplasma* prevalence within haplotypes all suggest that *Spiroplasma* is spreading from east to west across North America. The lack of longitudinal variation in *Wolbachia* is consistent with a long-term infection and is close to equilibrium prevalence everywhere.

Finally, the *Spiroplasma* infection status of flies carrying different mitochondrial haplotypes reveals a *Spiroplasma* infection not yet at species-level equilibrium. We previously found a perfect association between *Spiroplasma* haplotype and mitochondrial DNA (mtDNA) haplotype within Rochester, New York, populations of *D. neotestacea*, indicating that horizontal transmission of *Spiroplasma* is rare or nonexistent (*20*). Consequently, mtDNA variation can be used to infer the history of *Spiroplasma* infection in *D. neotestacea*. At equilibrium between natural selection favoring *Spiroplasma* infection and imperfect maternal transmission resulting in loss of *Spiroplasma*, the prevalence of *Spiroplasma* infection should be similar among flies with different mtDNA haplotypes; flies carrying all major mtDNA haplotypes should be infected, and all individuals, whether infected or not, should be descended from infected females (*31*, *32*). This is not the case for *D. neotestacea*, as *Spiroplasma* is common in flies carrying certain mtDNA haplotypes (notably 1, 5, and 8) but absent from all flies carrying "western" haplotypes (e.g., haplotype 16; Fig. 2C). We identified 16 individuals, collected in Oregon and British Columbia, carrying the most common mitochondrial haplotypes in eastern North America, and none were infected with *Spiroplasma*, indicating that the absence of *Spiroplasma* in the west is not due to the absence of mitochondrial clades that elsewhere harbor *Spiroplasma*. Mean within-population mitochondrial diversity is greater in populations where *Spiroplasma* is absent ($\bar{\theta}$ = 0.0056 ± 0.0007) than where it is present ($\bar{\theta}$ = 0.0025 ± 0.0005; $F_{1,12}$ = 11.07, P = 0.006), consistent with theoretical expectations that *Spiroplasma* has not been present in western populations of *D. neotestacea* in the recent evolutionary past (*33*). Taken together, these four patterns suggest that *Spiroplasma* has recently increased in frequency in the eastern populations of *D. neotestacea* and may now be spreading from east to west across North America.

The equilibrium infection prevalence of maternally transmitted endosymbiont is $\hat{P} = 1 - \left(\frac{1-\beta}{s}\right)$, where β is the fidelity of maternal transmission of the symbiont, and s is the selective advantage of infected over uninfected cytoplasmic lineages (*31*). Using wild-caught females, we have estimated that β = 0.97 (*20*) and s = 0.17. The estimate of s is based on the fertility of wild females as a function of *Howardula* parasitism and *Spiroplasma* infection, weighted by the probability of nematode parasitism (*21*). The expected equilibrium prevalence, $\hat{P} \approx 0.8$, is similar to that observed in populations in eastern North America, suggesting that *Spiroplasma* prevalence is at or approaching an equilibrium based largely on a balance between imperfect maternal transmission and a selective advantage due to tolerance of nematode parasitism. Our estimates of β and s are also consistent with a hypothesized increase in *Spiroplasma* infection around Rochester from ~10% in the 1980s to ~80% today (fig. S4).

Does the apparent recent increase of *Spiroplasma* result from recent colonization of *D. neotestacea* by *Spiroplasma*, a recent favorable *Spiroplasma* mutation conferring tolerance to an existing parasite challenge, or the imposition of a new selective pressure? The occurrence of *Spiroplasma* in flies carrying three different mtDNA haplotypes (Fig. 2C) suggests that the colonization of *D. neotestacea* by *Spiroplasma* was not a recent event. We previously found a perfect match between two slightly different *Spiroplasma* variants and two closely related mtDNA haplotypes, indicating that sufficient time has elapsed since the original infection for mutations in both *Spiroplasma* and mtDNA to have accumulated in the infected cytoplasmic lineages (*20*). Thus, *Spiroplasma* was probably present within *D. neotestacea* long before its recent increase. We can also rule out a recent favorable mutation, as both of these *Spiroplasma* variants were associated with tolerance to nematode parasitism. Among nematode-parasitized flies collected in 2008 and for which *spoT* was sequenced, the mean egg numbers for flies carrying the two variants were 13.5 ± 1.0 and 17.2 ± 2.2, both of which were much greater than the 0.95 ± 0.35 eggs in parasitized flies that did not carry *Spiroplasma* ($F_{1,90}$ = 41.9 and $F_{1,29}$ = 83.2, respectively; both P values < 0.0001). Finally, we previously hypothesized that *H. aoronymphium* had recently colonized North America (*34*), based on our finding of no DNA sequence variation (mtDNA COI) among North American samples of *H. aoronymphium*, as well as sequence identity between North American and European samples of this species (*35*). Thus, the apparently rapid spread of *Spiroplasma* is most likely due to recently imposed selection on *D. neotestacea* to evolve tolerance of these sterilizing parasites. The presumed beneficial function of *Spiroplasma* in *D. neotestacea* before the arrival of *H. aoronymphium* is unknown.

Our results show that *Spiroplasma* rescues *D. neotestacea* females from the sterilizing effects of nematode parasitism and that this endosymbiont appears to have recently increased to high frequency in eastern North America and is now spreading from east to west across the continent. Thus, *D. neotestacea* is undergoing a major change to a symbiont-based mode of defense against nematode parasites. This is the first report of natural symbiont-mediated defense against nematodes, the most widespread macroparasites of plants and animals. From an applied perspective, these findings suggest novel measures for nematode control (*36*); for instance, river blindness and lymphatic filariasis are caused by nematodes that are transmitted by various species of flies (*37*). If *Spiroplasma* impaired the development of filarial nematodes within their insect vectors, this could reduce nematode transmission and, thus, incidence of disease in human populations. With respect to natural communities, this study demonstrates the profound and potentially rapid effects of defensive symbionts, which are increasingly recognized as major players in the ecology of species interactions (*7*–*13*).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/329/5988/212/DC1
Materials and Methods
SOM Text
Figs. S1 to S4
Tables S1 to S6
References

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Adaptation via Symbiosis: Recent Spread of a *Drosophila* Defensive Symbiont

John Jaenike, Robert Unckless, Sarah N. Cockburn, Lisa M. Boelio and Steve J. Perlman

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Offsetting the Cost of Parasitism

Fruit flies, like most animals, are vulnerable to infection by a range of organisms, which, in co-infections, can interact with sometimes surprising effects. **Jaenike *et al.*** (p. 212) discovered that a species of *Spiroplasma* bacterium that is sometimes found in flies, and that is transmitted from mother to offspring, protects its host from the effects of a nematode worm parasite, *Howardula aoronymphium*. The worm sterilizes the female flies and shortens their lives, but when flies were experimentally infected with *Spiroplasma*, their fertility was rescued. Similarly, in wild populations of fruit flies infected with worms, those also infected with *Spiroplasma* had more eggs in their ovaries. The bacterium inhibits the growth of the adult female worms, but such is the advantage of this bacterial infection in offsetting the burden of nematodes on reproductive fitness, *Spiroplasma* appears to be spreading rapidly through populations of fruit flies in North America.