Rapid Spread of a Bacterial Symbiont in an Invasive Whitefly Is Driven by Fitness Benefits and Female Bias

Anna G. Himler, *et al.*

Science **332**, 254 (2011);
DOI: 10.1126/science.1199410

Rapid Spread of a Bacterial Symbiont in an Invasive Whitefly Is Driven by Fitness Benefits and Female Bias

Anna G. Himler,[1,2] Tetsuya Adachi-Hagimori,[2,4] Jacqueline E. Bergen,[2] Amaranta Kozuch,[2] Suzanne E. Kelly,[2] Bruce E. Tabashnik,[2] Elad Chiel,[2,3] Victoria E. Duckworth,[2] Timothy J. Dennehy,[2,5] Einat Zchori-Fein,[3] Martha S. Hunter[2]*

Maternally inherited bacterial symbionts of arthropods are common, yet symbiont invasions of host populations have rarely been observed. Here, we show that *Rickettsia* sp. nr. *bellii* swept into a population of an invasive agricultural pest, the sweet potato whitefly, *Bemisia tabaci*, in just 6 years. Compared with uninfected whiteflies, *Rickettsia*-infected whiteflies produced more offspring, had higher survival to adulthood, developed faster, and produced a higher proportion of daughters. The symbiont thus functions as both mutualist and reproductive manipulator. The observed increased performance and sex-ratio bias of infected whiteflies are sufficient to explain the spread of *Rickettsia* across the southwestern United States. Symbiont invasions such as this represent a sudden evolutionary shift for the host, with potentially large impacts on its ecology and invasiveness.

The life history traits of multicellular organisms, generally thought of as fundamental properties of the organism themselves, are often influenced by interactions with symbiotic species (*1*–*4*). These symbiont associations may form enduring evolutionary relationships with their hosts but can also be dynamic, entering and leaving populations in spans of a few years to thousands of years (*5*, *6*). The maternally inherited, endosymbiotic bacteria of insects comprise some of the most intimate of these associates. These symbionts spread in host populations either by increasing the fitness of all infected hosts or by manipulating host reproduction in ways that increase maternal transmission of the symbiont. Although the former is clearly beneficial to the host, the female-biased sex ratios or reproductive incompatibility caused by the latter are not in the host's genetic interests and are considered reproductive parasitism (*7*). Generally symbionts fall clearly into one of these categories and/or symbiont spread can be clearly assigned to one or the other function [e.g., (*8*)]. Here we describe the rapid spread of a bacterial symbiont, *Rickettsia* sp. nr. *bellii*, in a population of an invasive whitefly, *Bemisia tabaci*. *Rickettsia* infection increases whitefly performance; infected whiteflies produce more offspring that survive to adulthood at greater rates and develop more quickly. Symbiont infection also causes a strong female bias. Our results provide evidence of a rapid symbiont invasion in nature driven by the simultaneous expression of two host phenotypes, both contributing to symbiont spread.

Intracellular bacterial symbionts are common in insects. For example, *Wolbachia* is estimated to infect 66% of all insects (*9*), and ~10% of insect species have obligate nutritional symbionts that provide essential nutrients to their insect hosts (*10*). The endosymbiotic α-proteobacteria in the genus *Rickettsia* are best known for causing arthropod-vectored human diseases such as typhus and Rocky Mountain spotted fever (*11*). However, recent surveys have uncovered a diversity of *Rickettsia* in arthropod hosts that are not blood feeders (*12*, *13*). In the majority of these cases, the role of *Rickettsia* is unknown [e.g., (*14*)], but they may cause plant disease or manipulate arthropod host reproduction in ways that enhance transmission. For example, different *Rickettsia* species cause male-killing in a coccinellid beetle and parthenogenesis in parasitoid wasps (*12*, *15*).

The host of *Rickettsia* sp. nr. *bellii* in the current study is the sweet potato whitefly, *B. tabaci* (Hemiptera: Aleyrodidae). This sap-feeding insect is cosmopolitan and one of the worst pests of tropical and warm-temperate agriculture worldwide (*16*). *B. tabaci* is a species complex composed of several populations or biotypes that are genetically distinguishable, are more or less reproductively isolated, and differ in a wide range of biological characteristics, including invasiveness (*17*). These "biotypes" house distinct symbiont complements (*18*, *19*). In addition to *Portiera aleyrodidarum*, the obligate nutritional symbiont that is thought to supplement the amino acid–poor diet (*20*), populations of *B. tabaci* contain different suites of facultative symbionts, including species of *Wolbachia, Cardinium, Hamiltonella, Fritschea, Arsenophonus,* and *Rickettsia* (*19*). The B biotype, the dominant *B. tabaci* biotype in the United States and the subject of this study, is fixed for both *P. aleyrodidarum* and *Hamiltonella defensa* (*18*, *21*). The roles of these facultative symbionts are largely unknown but likely contribute to the biological differences observed among whitefly biotypes (*19*).

Here, we report the rapid spread of *Rickettsia* sp. nr. *bellii* (referred to as *Rickettsia* hereafter) in *B. tabaci* in the southwestern United States. Preserved samples of whiteflies from several sites in Arizona, New Mexico, and California show that the frequency of infection rose from 1% in 2000 to 51% in 2003 and to 97% in 2006 (Fig. 1) (*22*). Samples taken in 2008 and 2009 indicate that infection rates continue at near fixation. Given that *B. tabaci* may complete as many as 13 generations per year in the desert southwest (*23*), the spread from low *Rickettsia* frequency to near fixation occurred in less than 80 generations. This speed of increase is comparable to, or faster than, known examples such as the *Wolbachia* invasion

[1]Center for Insect Science, The University of Arizona, Post Office Box 210106, Tucson, AZ 85721–0106, USA. [2]Department of Entomology, The University of Arizona, Forbes 410, Tucson, AZ 85721–0036, USA. [3]Entomology Lab, Newe Ya'ar Research Center, Agricultural Research Organization, Post Office Box 1021, Ramat Yishay, 30095, Israel. [4]Research Institute of Environment, Agriculture and Fisheries, Osaka Prefectural Government, 442 Shakudo, Habikino, Osaka 583-0862, Japan. [5]Global Insecticide Resistance Management, Bayer CropScience Limited Partnership, 2 T.W. Alexander Drive, Research Triangle Park, NC 27709, USA.

*To whom correspondence should be addressed. E-mail: mhunter@ag.arizona.edu

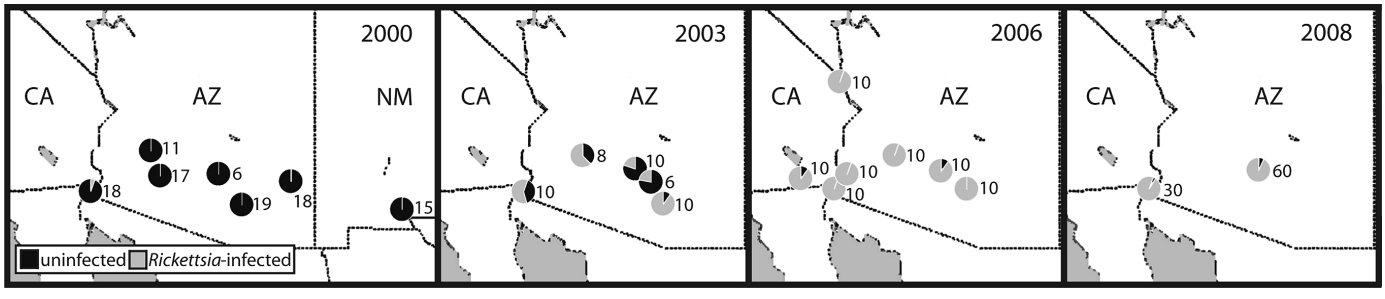

Fig. 1. Spread of *Rickettsia* in *B. tabaci.* The black proportion of circles represents uninfected whiteflies, and gray represents *Rickettsia*-infected whiteflies. Numbers indicate sample size. Infection rate spread from 0.01 in 2000 (N = 103), to 0.51 in 2003 (N = 47), to 0.97 in 2006 (N = 70), and to 0.94 in 2008 (N = 90).

of *Drosophila simulans* documented in California by Turelli and Hoffmann (*6*).

To determine whether *Rickettsia* also spreads in the laboratory, the frequencies of *Rickettsia* were followed over five whitefly generations on three host plants (cotton, melon, and cowpea). The infection spread quickly when initiated at 14% of hosts infected (Fig. 2) (Wald's $\chi^2_{\text{time,2 df}} = 59.48$, $P < 0.0001$; Wald's $\chi^2_{\text{time} \times \text{host plant,4 df}} = 31.22$, $P < 0.0001$). These data show that spread can occur on whitefly populations reared on at least three different host plants in the laboratory and is not limited by factors that occur only in the field, such as a particular host plant or natural enemy.

How did *Rickettsia* spread? After an introgression series of crosses to homogenize genetic background (*22*), we examined the rates of both vertical and horizontal transmission of *Rickettsia* in whiteflies. Experiments showed near perfect vertical transmission of *Rickettsia* from infected mothers to daughters (99.17% infected; $n = 120$ daughters from 18 mothers). In contrast, the rate of horizontal transmission from infected males to uninfected females on the same plant did not differ significantly from 0% (median = 0% infection for 8 replicates) (*22*). Therefore, subsequent experiments compared the performance of *Rickettsia*-infected (R^+) and uninfected (R^-) whiteflies.

Rickettsia-infected whiteflies on cowpea leaf disks produced almost double the number of adult progeny over their lifetimes (Fig. 3A) ($t_{47\ \text{df}} = 4.55$, $P < 0.0001$), and their offspring had greater survival to adulthood relative to uninfected whiteflies (Fig. 3B) (Wald's $\chi^2_{,1\ \text{df}} = 14.37$, $P < 0.001$). Moreover, R^+ females produced a higher proportion of daughters than R^- whiteflies, which would also contribute to *Rickettsia* spread (Fig. 3C) (Wald's $\chi^2_{,1\ \text{df}} = 9.52$, $P < 0.002$). An experiment with cohorts of whiteflies on whole cowpea plants produced similar results (Fig. 3, D to F). *Rickettsia*-infected females produced more juvenile progeny (Fig. 3D) ($F_{1,6} = 53.37$, $P < 0.0003$), had greater survival to adulthood (Fig. 3E) (Wald's $\chi^2_{,1\ \text{df}} = 93.52$, $P < 0.0001$), and had more female-biased sex ratios (Fig. 3F) ($\chi^2_{,1\ \text{df}} = 9.64$, $P < 0.002$) than uninfected females. In addition, the latter experiment showed R^+ females and males develop 1 to 2 days faster than R^- whiteflies (Fig. 4) ($t_{\text{females,14 df}} = 5.82$, $P < 0.0001$; $t_{\text{males,14 df}} = 2.37$, $P < 0.05$).

To determine whether the benefits of infection observed in the lab were sufficient to explain the rate of spread measured in the field, we used a simple growth model that incorporated the effects of infection on whitefly fecundity, survival, and sex ratio, as measured in two separate laboratory experiments (*22*). Using the data from either of the two experiments, the results from the model indicate that the observed effects of infection were sufficient to explain the rate of spread in the field (*22*). The rate of spread predicted from the model was higher than that seen in the field, suggesting that the spread in the field was slowed by one or more factors not included in the model (*22*).

How *Rickettsia* influences performance and sex ratio of whiteflies is unknown. Among possible mechanisms are manipulation of plant quality by the *Rickettsia*-infected whiteflies; plant quality can dramatically change whitefly performance (*24*). Alternatively, *Rickettsia* could serve as a nutritional mutualist or a defensive mutualist against cryptic pathogens present in the field and laboratory [e.g., (*8*, *25*)].

Our results contrast with previous work from Israel that showed slight performance benefits (R^+ whiteflies developed faster than R^-) but no reproductive manipulation associated with *Rickettsia* infection in the B biotype of *B. tabaci* (*26*). The reason for this disparity is unclear, but whiteflies in Israel also show lower *Rickettsia* infection frequencies in the field (*18*), suggesting a fundamental difference between the Israeli and U.S. whitefly-*Rickettsia* interactions.

The results of this study show both host reproductive manipulation (strong female bias) and large fitness benefits associated with a single symbiont lineage. Multiple roles have recently been discovered for a few symbionts. A *Wolbachia* strain previously shown to cause weak reproductive incompatibility in *Drosophila melanogaster* (*27*) was shown to confer strong virus resistance (*8*). More commonly, reproductive manipulator symbionts have been associated with modest increases in performance relative to uninfected individuals, likely as a result of coevolution of host and symbiont lineages [e.g., (*28*, *29*)].

The sex-ratio bias and performance benefits associated with *Rickettsia* infection in the current study both provide an explanation for the sweep of *Rickettsia* in the field and in the laboratory.

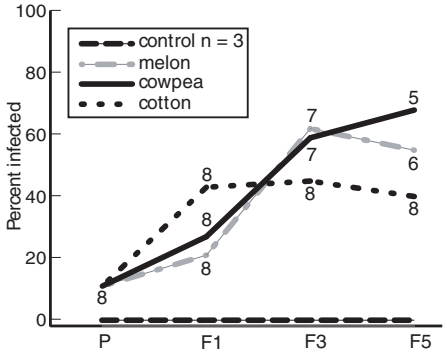

Fig. 2. Spread of *Rickettsia* infection in population cages of whiteflies over five generations on three host plants from 14% starting frequency. Numbers indicate the number of replicate cages per plant type at each generation. Control cages were inoculated only with R^- whiteflies and served as a check to detect possible contamination. A general linear model analysis showed that frequencies of infection changed significantly over time ($P < 0.0001$) and that the host plant influenced frequencies in interaction with time ($P < 0.0001$).

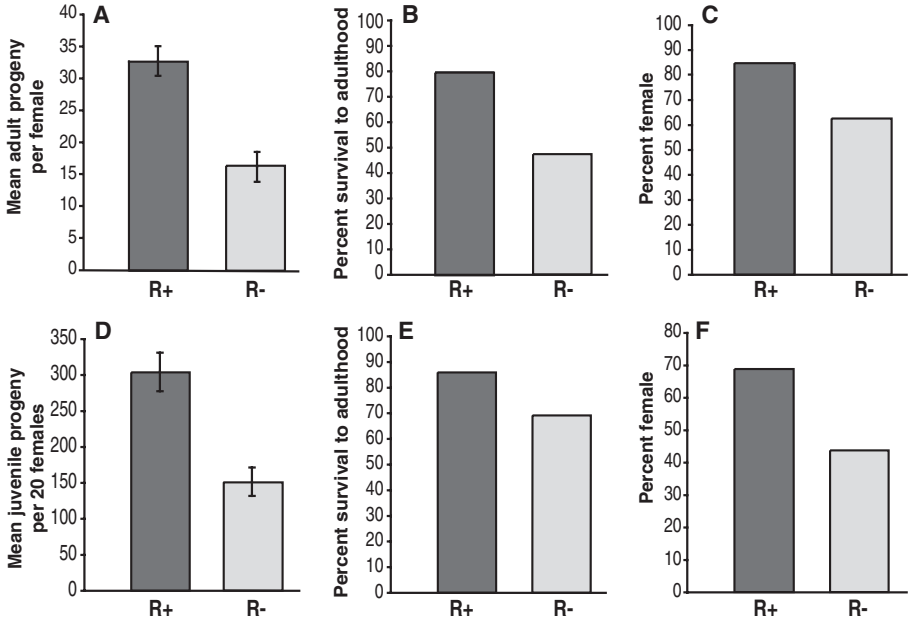

Fig. 3. (**A** to **C**) Life history parameters of R^+ and R^- whiteflies on cowpea leaf disks. (A) Mean adult progeny produced per female after a 3-day oviposition period ± SEM ($P < 0.0001$). (B) Percentage survival to adulthood ($P < 0.001$). (C) Sex ratio (percentage female, $P < 0.002$). (**D** to **F**) Life history parameters of R^+ and R^- whiteflies on whole cowpea plants. (D) Mean juvenile progeny produced per cohort of 20 females after a 5-day oviposition period ± SEM ($P < 0.0003$). (E) Percentage survival to adulthood ($P < 0.0001$). (F) Sex ratio (percentage female, $P < 0.002$). Sample sizes were 21 to 29 individual females per treatment for (A) to (C) and eight replicates per treatment of 20 females each for (D) to (F) (*22*).

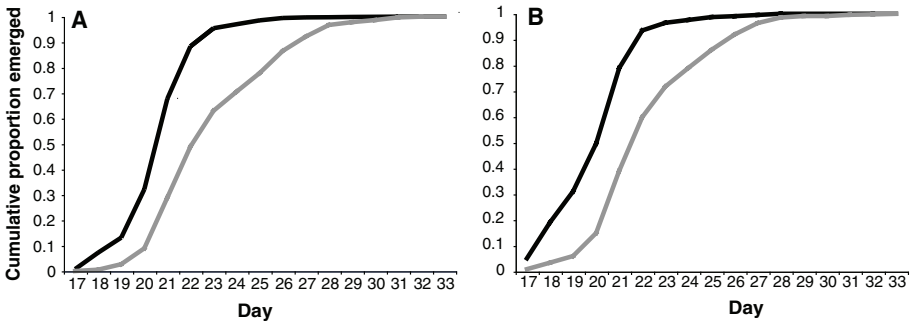

Fig. 4. Development time of R^+ (black) and R^- (light gray) progeny, expressed as the cumulative proportion emerged. (**A**) On average, R^+ females emerged 2.26 days earlier than R^- females ($P < 0.0001$). (**B**) On average, R^+ males emerged 1.32 days earlier than R^- males ($P < 0.03$).

The speed at which *Rickettsia* moved through the whitefly population and the apparent differences in the phenotype of the association in Israel and the United States suggest an especially dynamic interaction between *Rickettsia* and their whitefly hosts.

In general, new associations of inherited symbionts and hosts may result in rapid evolution of both partners and phenotypic shifts that optimize the symbiosis (*29*). Both the transformation of a host population with a new symbiont infection and the subsequent coevolution of the symbiotic partners are important sources of evolutionary novelty and adaptation for insect hosts. In hosts that are invasive agricultural pests or disease vectors, symbiont infection may both increase the speed at which they adapt to their new environment and exacerbate their influence (*1*). Clarifying the role of symbiotic microbes may offer insights for ameliorating pest invasiveness or impact (*30*, *31*).

References and Notes

1. D. M. Richardson, N. Allsopp, C. M. D'Antonio, S. J. Milton, M. Rejmanek, *Biol. Rev. Camb. Philos. Soc.* **75**, 65 (2000).
2. R. J. Rodriguez *et al.*, *ISME J.* **2**, 404 (2008).
3. K. M. Oliver, J. A. Russell, N. A. Moran, M. S. Hunter, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 1803 (2003).
4. S. L. Goodacre *et al.*, *BMC Biol.* **7**, 32 (2009).
5. J. H. G. V. Schulenburg *et al.*, *Genetics* **160**, 1075 (2002).
6. M. Turelli, A. A. Hoffmann, *Nature* **353**, 440 (1991).
7. J. Engelstadter, G. D. D. Hurst, *Annu. Rev. Ecol. Evol. Syst.* **40**, 127 (2009).
8. L. M. Hedges, J. C. Brownlie, S. L. O'Neill, K. N. Johnson, *Science* **322**, 702 (2008).
9. K. Hilgenboecker, P. Hammerstein, P. Schlattmann, A. Telschow, J. H. Werren, *FEMS Microbiol. Lett.* **281**, 215 (2008).
10. A. Douglas, *Biol. Rev. Camb. Philos. Soc.* **64**, 409 (1989).
11. D. H. Walker, N. Ismail, *Nat. Rev. Microbiol.* **6**, 375 (2008).
12. S. J. Perlman, M. S. Hunter, E. Zchori-Fein, *Proc. Biol. Sci.* **273**, 2097 (2006).
13. L. A. Weinert, J. H. Werren, A. Aebi, G. N. Stone, F. M. Jiggins, *BMC Biol.* **7**, 6 (2009).
14. A. Behar, L. J. McCormick, S. J. Perlman, *Appl. Environ. Microbiol.* **76**, 2280 (2010).
15. M. Giorgini, U. Bernardo, M. M. Monti, A. G. Nappo, M. Gebiola, *Appl. Environ. Microbiol.* **76**, 2589 (2010).
16. M. R. V. Oliveira, T. J. Henneberry, P. Anderson, *Crop Prot.* **20**, 709 (2001).
17. A. Dinsdale, L. Cook, C. Riginos, Y. M. Buckley, P. De Barro, *Ann. Entomol. Soc. Am.* **103**, 196 (2010).
18. E. Chiel *et al.*, *Bull. Entomol. Res.* **97**, 407 (2007).
19. G. Gueguen *et al.*, *Mol. Ecol.* **19**, 4365 (2010).
20. M. L. Thao, P. Baumann, *Appl. Environ. Microbiol.* **70**, 3401 (2004).
21. E. Zchori-Fein, J. K. Brown, *Ann. Entomol. Soc. Am.* **95**, 711 (2002).
22. Materials and methods are available as supporting material on *Science* Online.
23. J. C. Palumbo, A. R. Horowitz, N. Prabhaker, *Crop Prot.* **20**, 739 (2001).
24. M. Jiu *et al.*, *PLoS ONE* **2**, e182 (2007).
25. L. A. Moreira *et al.*, *Cell* **139**, 1268 (2009).
26. E. Chiel *et al.*, *Ann. Entomol. Soc. Am.* **102**, 413 (2009).
27. A. A. Hoffmann, D. J. Clancy, E. Merton, *Genetics* **136**, 993 (1994).
28. S. L. Dobson, E. J. Marsland, W. Rattanadechakul, *Genetics* **160**, 1087 (2002).
29. A. R. Weeks, M. Turelli, W. R. Harcombe, K. T. Reynolds, A. A. Hoffmann, *PLoS Biol.* **5**, 997 (2007).
30. S. Aksoy, B. Weiss, G. Attardo, in *Transgenesis and the Management of Vector-Borne Disease*, S. Aksoy, Ed. (Springer, New York, 2008), vol. 627, pp. 35–48.
31. C. J. McMeniman, S. L. O'Neill, C.-C. Chen, *PLoS Negl. Trop. Dis.* **4**, e748 (2010).

32. We thank H. Kim, N. Doidge, N. Dowdy, and J. Garcia for technical help and V. Corby-Harris and M. Torabi for advice on analyses. We are grateful to K. Oliver for helpful comments on an earlier version of the manuscript. A.G.H. was funded by NIH training grant 1K 12 GM00708, and E.C. was funded in part by the United States–Israel Binational Agricultural Research and Development fellowship (GS-1-2007). This research was supported by Binational (US-Israel) Science Foundation grant 2004416 and U.S. Department of Agriculture National Research Initiative Cooperative State Research, Education, and Extension Service grant 2006-35302-17165 to M.S.H. and E.Z.-F. and NSF grant DEB-1020460 to M.S.H. and A.G.H. Sequences have been deposited in GenBank under accession numbers HQ439024 to HQ439027, HQ997365, and JF288560.

Supporting Online Material

www.sciencemag.org/cgi/content/full/332/6026/254/DC1
Materials and Methods
SOM Text
Table S1
References

22 October 2010; accepted 10 February 2011
10.1126/science.1199410