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Detection of *Wolbachia* Bacteria in Multiple Organs and Feces of the Triatomine Insect *Rhodnius pallescens* (Hemiptera, Reduviidae)▽

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At least two types of *Wolbachia* bacteria were detected in wild and insectarium-raised *Rhodnius pallescens*, a natural vector of *Trypanosoma cruzi* and *Trypanosoma rangeli*. *Wolbachia* was detected in all the organs and tissues studied and in the feces, and this provided a methodological advantage for determining the presence of this endosymbiont in this host, obviating the need to kill the specimens. The occurrence of trypanosomatids in wild individuals was also studied.

Wolbachia is an obligate intracellular bacterium (18) that is present in 20 to 75% of insect species (3, 14, 33, 38, 41, 42). This bacterium was first described in 1924 in mosquitoes (*Culex pipiens*) (12, 13) and was initially classified as *Rickettsia* sp. (9, 33). *Wolbachia* displays a tropism for the reproductive tissue of its hosts and is transmitted vertically from insect to insect through the ovules, while interspecific transmission appears to occur horizontally with the possible help of parasitoids (1, 3, 6, 17, 19, 33, 43). Despite the fact that infected insects show no pathological signs, the presence of *Wolbachia* can result in diverse reproductive alterations in its hosts, including parthenogenesis, feminization, male killing, and unidirectional or bidirectional cytoplasmic incompatibility (33, 35, 38, 44).

The relationship between *Wolbachia* and its arthropod hosts ranges from mutualistic to parasitic, which makes it all the more interesting and necessary to ascertain the exact nature of the interaction between particular symbionts and their hosts (40). *Wolbachia* isolates have been found in numerous disease-carrying insects, such as *Culex* (12, 13), *Aedes* (27), *Glossina* (2, 26, 39), Phlebotominae (16), *Cimex* (28, 29), *Ctenocephalides felis* (11), and *Tunga penetrans* (10). It also occurs in parasitic nematodes, such as *Onchocerca volvulus*, is responsible for the inflammatory reaction that induces blindness (22, 24), and has been detected in *Brugia malayi* (34, 36). *Wolbachia* has recently been found in *Angiostrongylus cantonensis*, a nematode that is not related to filarias (37).

The sylvatic triatomine *Rhodnius pallescens* is considered the most important vector of the trypanosomatids *Trypanosoma cruzi* and *Trypanosoma rangeli* in the neotropics. Its capacity to invade houses located near its natural habitat, the royal palm tree (*Attalea butyracea*), and to transmit *T. cruzi* and Chagas' disease to humans has been well documented in Panama (4, 23, 32). *Wolbachia* was previously found in only one individual of *R. pallescens* that was described in a list of Panamanian species (41). However, information on the occurrence of this bacterium and its distribution in the organs of its hosts is not available. In this work we examined whether there is any correlation between the presence of this endosymbiont and the presence of the parasites *T. cruzi* and *T. rangeli*.

In this study we examined a total of 72 individuals of the triatomine insect *R. pallescens*; 27 of these individuals were collected from their natural habitat, and 45 were obtained from an insectarium. Wild specimens were collected in regions of the Republic of Panama where Chagas' disease is endemic (Table 1). Insectarium specimens were obtained from the Instituto Conmemorativo Gorgas de Estudios de la Salud and from the Centro de Investigaciones Parasitarias de la Universidad de Panamá.

Each specimen was dissected, and gonads, salivary glands, and intestines were extracted under sterile conditions. The posterior intestine, rectal ampolla, and salivary glands from wild triatomines were homogenized, and any trypanosomes present were observed with a microscope or cultured in Grace medium to facilitate detection of *T. cruzi* and *T. rangeli* (20, 21). *T. cruzi* and *T. rangeli* were also detected by PCR, as previously described (7, 45, 46).

The presence of *Wolbachia* was detected in each organ by PCR using specific primers for 16S rRNA and *wsp* genes, as previously reported (42, 44). Standard reaction mixtures (final volume, 10 μl) contained 0.5 μl of the template DNA (extracted with a Qiagen DNeasy tissue kit) plus 0.08 μl of deoxynucleoside triphosphates (25 mM), 0.5 μl of the forward and reverse primers (10 μM), 0.1 μl of *Taq* polymerase (5 U/μl), 0.5 μl of $MgCl_2$ (50 mM), and 0.4 μl of dimethyl sulfoxide (5%).

The integrity of the total DNA extracted was verified by amplification of the 28S rRNA gene as previously described (5), and DNAs from *Nasonia* that was positive and negative for *Wolbachia* (kindly provided by J. Werren) were used as controls.

The results of the screening analysis of the 27 wild triatomines are shown in Table 1. This analysis revealed that 56% of the triatomines were infected with *T. cruzi* and 25% of the triatomines were infected with *T. rangeli*. Simultaneous infec-

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TABLE 1. Occurrence of trypanosomes and *Wolbachia* in *R. pallescens* individuals collected from several Panamanian districts

Stage or sex of *R. pallescens* individual ($n = 27$)[a]	Presence of trypanosomes		Presence of *Wolbachia*		Region	District
	T. cruzi[b]	*T. rangeli*[c,d]	Gonads[c,e]	Salivary glands[c,f]		
N	+[c]	−	+	ND	Viento Fronto	Chilibre
M	+[c]	+	+	+	Viento Fronto	Chilibre
M	−[c]	+	+	+	Viento Fronto	Chilibre
M	+[c]	+	+[g]	+	Viento Fronto	Chilibre
M	−[c]	−	+[g]	+	Viento Fronto	Chilibre
F	+[c]	−	+[g]	+	Viento Fronto	Chilibre
M	+[c]	−	+	+	Viento Fronto	Chilibre
M	+[c]	−	+	+	Viento Fronto	Chilibre
F	+[c]	−	+	+	Viento Fronto	Chilibre
M	+[c]	−	+	+	Viento Fronto	Chilibre
M	−[c]	−	+	+	Viento Fronto	Chilibre
M	−[c]	−	+	+	Viento Fronto	Chilibre
M	−[c]	−	+[g]	+	Viento Fronto	Chilibre
M	+[c]	−	+	+	Viento Fronto	Chilibre
M	−[c]	−	+	+	Viento Fronto	Chilibre
M	+[c]	−	+	+	Viento Fronto	Chilibre
F	+[h]	−	+	+	Loma Bonita	Arraiján
F	−[h]	−	+[g]	+	Santa Clara	Arraiján
M	+[h]	+	+[g]	+	Santa Clara	Arraiján
M	−[h]	−	+[g]	+	Santa Clara	Arraiján
M	−[h]	−	+[g]	+	Santa Clara	Arraiján
M	−[h]	+	+[g]	−	Santa Clara	Arraiján
F	−[h]	−	+	+	Loma del Río	Arraiján
F	−[h]	+	+	+	Santa Clara	Arraiján
ND	+[h,i]	ND	+	ND	Carriazo	Chepo
ND	+[h,i]	ND	+	ND	Playa Larga	Chepigana
ND	+[h]	ND	+[j]	ND	Chuzo	Chepigana

[a] N, nymph; M, male; F, female; ND, not determined. One individual was a nymph, 17 individuals were males, 6 individuals were females, and the stage or sex of 3 individuals was not determined.
[b] Fifteen individuals were positive, and 12 individuals were negative.
[c] Determined by PCR.
[d] Six individuals were positive, 18 individuals were negative, and the presence of trypanosomes was not determined for 3 individuals.
[e] All 27 individuals were positive.
[f] Twenty-two individuals were positive, 1 individual was negative, and the presence of *Wolbachia was not determined for 4 individuals.*
[g] Sample used for amplifying and sequencing *fbpA*.
[h] Determined by microscopic examination.
[i] Determined by isolation of parasites in mice.
[j] Sample used for amplifying and sequencing *wsp*.

tion with *T. cruzi* and *T. rangeli* was also detected in 12% of the specimens. The presence of *T. cruzi* in the wild insects indicates that the risk of Chagas' disease in humans was elevated in the areas where insects were captured.

PCR analysis with probes for the *wsp* gene detected the presence of *Wolbachia* in the gonads and salivary glands of 100% of the insects, while PCR analysis with the primers specific for 16S rRNA detected *Wolbachia* in 95.9% of the cases. As recommended by Duron and Gavotte (8), the two pairs of primers were used to rule out the possibility of false negatives.

The analysis of specimens from the insectarium produced very different results. Only 51.0% of the insects were positive for *Wolbachia* with both probes (Table 2). Of the positive insects, 51.0% were positive when the gonads were tested, 44.4% were positive when the salivary glands were tested, and 94.0% were positive when the intestine was tested.

Given *Wolbachia*'s presence in all of the wild triatomines collected, it seems that the presence of the endosymbiont does not influence the susceptibility of the insects to infection by the parasitic protozoan *T. cruzi*, the etiological agent of Chagas' disease, and *T. rangeli*, for which these insects are natural hosts.

Wolbachia is vertically transmitted by oocyst infection and in this way maintains a high incidence in arthropods (33). Its presence has been reported in other tissues, including nerve tissue or hemocytes (25). In order to determine the degree of *Wolbachia* infection in organs of *R. pallescens* insects other than the organs used in the screening analysis, an insectarium specimen of *R. pallescens* was dissected to extract the hemolymph, the musculature, the Malpighian tubules, and the intestine. Each organ was tested for the presence of *Wolbachia* with specific primers for 16S rRNA and *wsp* genes. All PCRs were positive, indicating that the bacterium was distributed throughout the tissues of the insect and was not restricted to the digestive tract and gonads.

The presence of *Wolbachia* both in the salivary glands and in the intestine might be explained by the coprophagous and cannibalistic habits of the insects in the early phases of their development, when they acquire the symbionts essential for their development (30, 31). This could also be the mechanism that transmits and spreads the endosymbiont among triatomi-

TABLE 2. Occurrence of *Wolbachia* in insectarium specimens of *R. pallescens*, as determined by PCR

Stage or sex of *R. pallescens* individual ($n = 45$)[a]	Presence of *Wolbachia*			Generation
	Gonads[b]	Salivary glands[c]	Intestine[d]	
F	+	+	+	ND
F	+	+	+	ND
F	+	+	+	ND
F	+	−	+	ND
M	+	+	+	ND
F	−	−	ND	4
F	−	−	ND	4
M	+	+	+	5
M	−	−	ND	6
F	−	−	ND	6
M	−	−	ND	6
M	−	−	ND	4
M	−	−	ND	4
F	+	+	+	5
F	+	+	+	5
F	+	+	−	5
F	+	+	+	5
F	+	+	+	4
F	−	−	ND	4
F	−	−	ND	4
M	−	−	ND	5
F	−	−	ND	5
F	−	−	ND	6
M	−	−	ND	6
N	−	−	ND	4
N	−	−	ND	4
F	+	+	+	4
F	+	+	+	4
M	−	−	ND	3
F	−	−	ND	4
F	−	−	ND	3
F	+	+	+	6
F	+	+	+	6
F	+	+	ND	3
F	+	−	+	3
F	+	+	ND	ND
M	+	ND	ND	3
M	+[e]	ND	ND	3
F	−	ND	ND	3
F	−	ND	ND	3
M	−	ND	ND	3
F	+[e]	ND	ND	4
F	+	ND	ND	4
M	−	ND	ND	4
M	+[e]	ND	ND	3

[a] N, nymph; M, male; F, female. Two individuals were nymphs, 14 individuals were males, and 29 individuals were females.
[b] Twenty-three individuals were positive, and 22 individuals were negative.
[c] Sixteen individuals were positive, 20 individuals were negative, and the presence of *Wolbachia* was not determined (ND) for 9 individuals.
[d] Fifteen individuals were positive, 1 individual was negative, and the presence of *Wolbachia* was not determined for 29 individuals.
[e] Sample used for amplifying and sequencing *fbpA*.

nes. To verify that *Wolbachia* is present in the digestive products of the triatomines, feces were collected after an insectarium specimen was fed, and the feces were probed with the 16S rRNA and *wsp* gene primers. Both amplification reactions were positive. The fact that *Wolbachia* can be detected in the feces of triatomines makes it unnecessary to neutralize the insects in order to determine the presence of this endosymbiont, which is an important methodological advantage.

In order to characterize the *Wolbachia* strain present in *R. pallescens*, several PCR products were sequenced. The sequence of the 16S rRNA gene obtained from feces of an insectarium specimen was 99 to 100% identical to the sequences of a *Wolbachia* endosymbiont of *Pseudolynchia canariensis* (accession no. DQ115537) and other unculturable bacteria obtained from insects, such as the cat flea *C. felis* (accession no. EF121347), the ant lion *Myrmeleon mobilis* (accession no. EF121347, DQ068883, and DQ068882), and the fruit fly *Drosophila melanogaster* (accession no. DQ981371, DQ981358, and DQ981347). The same 16S rRNA sequence was found in the feces of a wild *Rhodnius* specimen collected in Chuzo (Table 1). The *wsp* gene sequence from this wild individual was compared with the sequences in the database constructed and maintained by K. A. Jolley and L. Baldo (*Wolbachia* MLST Databases [http://pubmlst.org/wolbachia/]). The results of this comparison showed that the level of similarity with allele 92 in the database was 96.10%. Given that Jolley and Baldo consider a single difference in the nucleotide sequence an indication of different alleles, it appears that the strain of *Wolbachia* present in the triatomines is a novel strain and is not included in this database.

The *fbpA* gene was also amplified (15) from gonads of nine wild specimens positive for *Wolbachia* and sequenced (Table 1). Direct observation of the DNA chromatograms revealed superimposed peaks at 16 different positions. These results were interpreted as showing that *R. pallescens* was infected by at least two *Wolbachia* strains. Surprisingly, an analysis of the sequence of the *fbpA* gene from three insectarium specimens (Table 2) revealed only four superimposed peaks. Although insectarium and wild triatomines were collected from different areas of Panama, superimposed peaks were found at the same positions for both groups. A possible explanation for this observation is that both insect groups were infected by the same *Wolbahcia* strains and at least one of the strains was cured when triatomines were raised in laboratory colonies. This result strongly indicates that the vertical transmission of *Wolbachia* could be affected in insects raised under laboratory conditions. Some factors that alter the presence and spread of *Wolbachia* in insects have been described previously (33). In our case the exposure to high temperatures, the immune response of the vertebrate used for laboratory feeding, or genetic factors of the host could be relevant factors. It would be interesting to study the effect of removing triatomines from their natural habitat and raising them in laboratories on the development or spread of this endosymbiont.

Study of the presence of *Wolbachia* in triatomines opens up a new area of research and the possibility of using this endosymbiont to manipulate the reproduction of these insects that are responsible for the vectorial transmission of Chagas' disease.

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REFERENCES

1. **Ahrens, M., and D. Shoemaker.** 2005. Evolutionary history of *Wolbachia* infections in the fire ant *Solenopsis invicta*. BMC Evol. Biol. **5:**35.
2. **Aksoy, S., and R. V. Rio.** 2005. Interactions among multiple genomes: tsetse, its symbionts and trypanosomes. Insect Biochem. Mol. Biol. **35:**691–708.
3. **Baldo, L., N. Lo, and J. Werren.** 2005. Mosaic nature of the *Wolbachia* surface protein. J. Bacteriol. **187:**5406–5418.
4. **Calzada, J. E., V. Pineda, E. Montalvo, D. Alvarez, A. M. Santamaría, F. Samudio, V. Bayard, L. Cáceres, and A. Saldaña.** 2006. Human trypanosome infection and the presence of intradomicile *Rhodnius pallescens* in the western border of the Panama Canal, Panama. Am. J. Trop. Med. Hyg. **74:**762–765.
5. **Campbell, B. C., J. D. Steffen-Campbell, and J. H. Werren.** 1993. Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rRNA sequences. Insect Mol. Biol. **2:**225–237.
6. **Charlat, S., K. Bourtzis, and H. Merçot.** 2002. *Wolbachia*-induced cytoplasmic incompatibility, p. 621–644. *In* J. Seckbach (ed.), Symbiosis. Kluwer Academic Publisher, Dordrecht, The Netherlands.
7. **Chiurillo, M. A., G. Crisante, A. Rojas, A. Peralta, M. Dias, P. Guevara, N. Añez, and J. L. Ramírez.** 2003. Detection of *Trypanosoma cruzi* and *Trypanosoma rangeli* infection by duplex PCR assay based on telomeric sequences. Clin. Diagn. Lab. Immunol. **10:**775–779.
8. **Duron, O., and L. Gavotte.** 2007. Absence of *Wolbachia* in nonfilariid worms parasitizing arthropods. Curr. Microbiol. **55:**193–197.
9. **Fenollar, F., B. La Scola, H. Inokuma, S. Dumler, M. Taylor, and D. Raoult.** 2003. Culture and phenotypic characterization of a *Wolbachia pipiensis* isolate. J. Clin. Microbiol. **41:**5434–5441.
10. **Fischer, P., C. Schmetz, C., Bandi, I. Bonow, S. Mand, K. Fischer, and D. W. Buttner.** 2002. *Tunga penetrans*: molecular identification of *Wolbachia* endobacteria and their recognition by antibodies against proteins of endobacteria from filarial parasites. Exp. Parasitol. **102:**201–211.
11. **Gorham, C. H., Q. Q. Fang, and L. A. Durden.** 2003. *Wolbachia* endosymbionts in fleas (Siphonaptera). J. Parasitol. **89:**283–289.
12. **Hertig, M., and S. B. Wolbach.** 1924. Studies on Rickettsia-like microorganisms in insects. J. Med. Res. **44:**329–374.
13. **Hertig, M.** 1936. The rickettsia, Wolbachia pipientis (gen. et sp. n.) and associated inclusions of the mosquito, *Culex pipiens.* Parasitology **28:**453–486.
14. **Jeyaprakash, A., and M. A. Hoy.** 2000. Long PCR improves *Wolbachia* DNA amplification: *wsp* sequences found in 76% of sixty-three arthropod species. Insect Mol. Biol. **9:**393–405.
15. **Jolley, K. A., M.-S. Chan, and M. C. J. Maiden.** 2004. mlstdbNet-distributed multi-locus sequence typing (MLST) databases. BMC Bioinformatics **5:**86.
16. **Kassem, H. A., and G. Osaman.** 2007. Maternal transmission of *Wolbachia* in *Phlebotomus papatasi* (Scopoli). Ann. Trop. Med. Parasitol. **101:**435–440.
17. **Kondo, N., N. Nikoh, N. Ijichi, M. Shimada, and T. Fukatsu.** 2002. Genome fragment of *Wolbachia* endosymbiont transferred to X chromosome of host insect. Proc. Natl. Acad. Sci. USA USA **99:**14280–14285.
18. **Makepeace, B., L. Rodgers, and A. Trees.** 2006. Rate of elimination of *Wolbachia pipientis* by doxycycline in vitro increases following drug withdrawal. Antimicrob. Agents Chemother. **50:**922–927.
19. **Miller, W., and M. Riegler.** 2006. Evolutionary dynamics of wAu-like *Wolbachia* variants in neotropical *Drosophila* spp. Appl. Environ. Microbiol. **72:**826–835.
20. **Osuna, A., A. Jiménez-Ortiz, and J. Lozano-Maldonado.** 1979. Medios de cultivo para la obtención de formas metaciclicas de *Trypanosoma cruzi*. Rev. Iber. Parasitol. **39:**129–133.
21. **Osuna, A., F. J. Adroher, and J. A. Lupiañez.** 1990. Influence of electrolytes and non-electrolytes on growth and differentiation of *Trypanosoma cruzi*. Cell Differ. Dev. **30:**89–95.
22. **Pearlman, E., and I. Gillette-Ferguson.** 2007. *Onchocerca volvulus*, *Wolbachia* and river blindness. Chem. Immunol. Allergy **92:**254–265.
23. **Pineda, V., E. Montalvo, D. Alvarez, A. M. Santamaría, J. E. Calzada, and A. Saldaña.** 2008. Feeding sources and trypanosome infection index of *Rhodnius pallescens* in a Chagas disease endemic area of Amador County, Panama. Rev. Inst. Med. Trop. Sao Paulo **50:**113–116.
24. **Punkosdy, G., D. Addis, and P. Lammie.** 2003. Characterization of antibody responses to *Wolbachia* surface protein in humans with lymphatic filariasis. Infect. Immun. **71:**5104–5114.
25. **Rigaud, T., C. Souty-Grosset, R. Raimond, J. Mocquard, and P. Juchault.** 1991. Feminizing endocytobiosis in the terrestrial crustacean *Armadillium vulgare* LART (isopoda): recent acquisitions. Endocytobiosis Cell Res. **7:**259–273.
26. **Rio, R. V., Y. N. Wu, G. Filardo, and S. Aksoy.** 2006. Dynamics of multiple symbiont density regulation during host development: tsetse fly and its microbial flora. Proc. Biol. Sci. **273:**805–814.
27. **Ruang-Areerate, T., and P. Kittayapong.** 2006. *Wolbachia* transinfection in *Aedes aegypti*: a potential gene driver of dengue vectors. Proc. Natl. Acad. Sci. USA **103:**12534–12539.
28. **Sakamoto, J. M., J. Feinstein, and J. L. Rasgon.** 2006. *Wolbachia* infections in the Cimicidae: museum specimens as an untapped resource for endosymbiont surveys. Appl. Environ. Microbiol. **72:**3161–3167.
29. **Sakamoto, J. M., and J. L. Rasgon.** 2006. Geographic distribution of *Wolbachia* infections in *Cimex lectularius* (Heteroptera: Cimicidae). J. Med. Entomol. **43:**696–700.
30. **Schaub, G. A., C. A. Boker, C. Jensen, and D. Reduth.** 1989. Cannibalism and coprophagy are modes of transmission of *Blastocrithidia triatomae* (Trypanosomatidae) between triatomines. J. Protozool. **36:**171–175.
31. **Schaub, G. A., and C. Jensen.** 1990. Developmental time and mortality of the reduviid bug *Triatoma infestans* with differential exposure to coprophagic infections with *Blastocrithidia triatomae* (Trypanosomatidae). J. Invertebr. Pathol. **55:**17–27.
32. **Sousa, O. E., and C. M. Johnson.** 1973. Prevalence of *Trypanosoma cruzi* and *Trypanosoma rangeli* in triatomines (Hemiptera: Reduviidae) collected in the Republic of Panama. Am. J. Trop. Med. Hyg. **22:**18–23.
33. **Stouthamer, R., J. A. J. Breeuwer, and G. D. D. Hurts.** 1999. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. Annu. Rev. Microbiol. **53:**71–102.
34. **Suba, N., C. Shiny, M. J. Taylor, and R. B. Narayanan.** 2007. *Brugia malayi Wolbachia* hsp60 IgG antibody and isotype reactivity in different clinical groups infected or exposed to human bancroftian lymphatic filariasis. Exp. Parasitol. **116:**291–295.
35. **Sun, L., M. Riegler, and S. O'Neill.** 2003. Development of a physical and genetic map of the virulent *Wolbachia* strain wMelPop. J. Bacteriol. **185:** 7077–7084.
36. **Taylor, M. J., C. Bandi, and A. Hoerauf.** 2005. *Wolbachia* bacterial endosymbionts of filarial nematodes. Adv. Parasitol. **60:**245–284.
37. **Tsai, K. H., C. G. Huang, L. C. Wang, Y. W. Yu, W. J. Wu, and W. J. Chen.** 2007. Molecular evidence for the endosymbiont *Wolbachia* in a non-filaroid nematode, *Angiostrongylus cantonensis*. J. Biomed. Sci. **14:**607–615.
38. **Van Meer, M. M. M., J. Witteveldt, and R. Stouthamer.** 1999. Phylogeny of the arthropod endosybiont *Wolbachia* based on the *wsp* gene. Insect Mol. Biol. **83:**399–408.
39. **Weiss, B. L., R. Mouchotte, R. V. Rio, Y. N. Wu, Z. Wu, A. Heddi, and S. Aksoy.** 2006. Interspecific transfer of bacterial endosymbionts between tsetse fly species: infection establishment and effect on host fitness. Appl. Environ. Microbiol. **72:**7013–7021.
40. **Werren, J.** 1997. Biology of *Wolbahcia*. Annu. Rev. Entomol. **42:**587–609.
41. **Werren, J., D. Windsor, and L. Guo.** 1995. Distribution of Wolbachia among neotropical arthropods. Proc. R. Soc. Lond. B **262:**197–204.
42. **Werren, J. H., and D. M. Windsor.** 2000. *Wolbachia* infection frequencies in insects: evidence for a global equilibrium? Proc. R. Soc. Lond. B **267:**1277–1285.
43. **Xi, Z., and S. Dobson.** 2005. Characterization of *Wolbachia* transfection efficiency by using microinjection cytoplasm and embryo homogenate. Appl. Environ. Microbiol. **71:**3199–3204.
44. **Zhou, W., F. Rousset, and S. O′Neill.** 1998. Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences. Proc. Biol. Sci. B **265:**509–515.
45. **Zulantay, I., P. Honores, A. Solari, W. Apt, S. Ortiz, A. Osuna, A. Rojas, B. Lopez, and G. Sánchez.** 2004. Use of polymerase chain reaction (PCR) and hybridization assays to detect *Trypanosoma cruzi* in chronic chagasic patients treated with itraconazole or allopurinol. Diagn. Microbiol. Infect. Dis. **48:** 253–257.
46. **Zulantay, I., W. Apt, L. C. Gil, C. Rocha, K. Mundaca, A. Solari, G. Sánchez, C. Rodriguez, G. Martínez, L. M. de Pablos, L. Sandoval, J. Rodríguez, S. Vilchez, and A. Osuna.** 2007. The PCR-based detection of *Trypanosoma cruzi* in the faeces of *Triatoma infestans* fed on patients with chronic American trypanosomiasis gives higher sensitivity and a quicker result than routine xenodiagnosis. Ann. Trop. Med. Parasitol. **101:**673–679.