

SHORT COMMUNICATION

Widespread infections by the bacterial endosymbiont *Cardinium* in arachnids

Oliver Y. Martin: Experimental Ecology, Institute for Integrative Biology, ETH Zürich, CH-8092 Zürich, Switzerland.
E-mail: oliver.martin@env.ethz.ch

Sara L. Goodacre: School of Biology, University of Nottingham, Queen’s Medical Centre, Nottingham, NG7 2UH, United Kingdom

Abstract. Maternally inherited bacterial endosymbionts such as *Wolbachia* can potentially have a major impact on the reproduction of their arthropod hosts. Most previous studies have focused on the effects on insects, but recent evidence demonstrates that the endosymbionts *Wolbachia*, *Rickettsia*, and *Spiroplasma* are also common in spiders. Such infections potentially explain observed characteristics of reproduction in this group such as skewed sex ratios or reported cases of parthenogenesis. Here we test spiders and a range of other arachnids for infection with another, more recently described maternally acquired endosymbiont, *Cardinium*. We present data from a survey of spiders and other arachnids collected in the field and obtained from museum collections. Infections with *Cardinium* are found to be very widespread, perhaps more so than in other arthropod groups. The consequences of this and directions for future research on endosymbiont-arachnid interactions are discussed.

Keywords: *Rickettsia*, *Spiroplasma*, *Wolbachia*, spider, cytoplasmic incompatibility

Maternally inherited endosymbionts such as *Wolbachia*, *Rickettsia*, and *Spiroplasma* species are known to affect reproductive and behavioral traits of their predominantly arthropod hosts (see Charlat et al. 2003). Such infections are relatively widespread in insects and

mites and recent evidence indicates that they are also found in both haplogyne and entelegyne spiders (Oh et al. 2000; Cordaux et al. 2001; Rowley et al. 2004; Goodacre et al. 2006). Incidences of multiple infections were also found to be common (Goodacre et al. 2006).

Table 1.—Examples of potential hallmarks of endosymbiont infection in arachnid species;* denotes where the same species was tested by PCR and found to harbor *Cardinium* (Table 2).

Hallmark	Family	Species
<u>Acarina</u>		
Parthenogenesis	Argasidae	<i>Ornithodoros</i> spp.
Parthenogenesis	Ixodidae	<i>Amblyomma agamum</i> Aragão 1912, <i>Amblyomma dissimile</i> Koch 1844*, <i>Boophilus microplus</i> Canestrini 1887, <i>Haemaphysalis bispinosa</i> Neumann 1897, <i>Hyalomma</i> spp, <i>Rhipicephalus bursa</i> Canestrini and Fanzago 1877.
Parthenogenesis	Trombiculidae	<i>Leptotrombidium arenicola</i> Traub, 1960
<u>Scorpiones</u>		
Parthenogenesis	Buthidae	<i>Ananteris coinaui</i> Lourenço 1982, <i>Hottentota hottentota</i> Fabricius 1787, <i>Tityus columbianus</i> Thorell 1876, <i>Tityus metuendus</i> Pocock 1897, <i>Tityus stigmurus</i> Thorell 1876, <i>Tityus trivittatus</i> Kraepelin 1898, <i>Tityus uruguayensis</i> Borelli, 1901
Parthenogenesis	Hemiscorpiidae	<i>Liocheles australasiae</i> Fabricius, 1775*
<u>Opiliones</u>		
Parthenogenesis	Caddidae	<i>Acropsopilio chomulae</i> Goodnight & Goodnight 1948
Parthenogenesis	Phalangiidae	<i>Leiobunum globosum</i> Suzuki 1953, <i>Leiobunum manubriatum</i> Karsch 1881
<u>Araneae</u>		
Absence of males or strongly biased sex-ratio	Symphytognathidae	<i>Anapistula caecula</i> Baert & Jocqué 1993
Absence of males or strongly biased sex-ratio	Araneidae	<i>Hypognatha</i> spp.
Reduced viability of offspring from inter-population crosses	Salticidae	<i>Habronattus pugillus</i> Griswold 1987
Primary SR distortion	Linyphiidae	<i>Pityohyphantes phrygianus</i> Koch 1836
Primary SR distortion	Eresidae	<i>Stegodyphus dumicola</i> Pocock 1898
Primary SR distortion	Theridiidae	<i>Anelosimus domingo</i> Levi 1963
Primary SR distortion	Thomisidae	<i>Diaea socialis</i> Main 1988
Parthenogenesis	Dysderidae	<i>Dysdera hungarica</i> Kulczyn'ski 1897
Parthenogenesis	Ochyroceratidae	<i>Theotima minutissima</i> Petrunkevitch 1929
Parthenogenesis (suggested)	Amaurobiidae	<i>Coelotes</i> spp.

Table 2.—Overview of results of PCR screens for *Cardinium* in various arachnid species. Key to symbols: *¹Sources - abbreviations: FM: Field Museum Chicago; GS: Gioia Schwarzenbach, University of Zurich, mites collected from Fehraltorf, Switzerland; MV: collection by Marija Vugdelic, unknown thomisid collected in the Balkans; OM: collected by O. Y. Martin in Norwich, United Kingdom; *² i.e. specimens tested; f = females, m = males, j = juveniles, e = egg sacs, of unknown sex if not specified; *³ i.e. specimens found to harbor *Cardinium*; *⁴ 1 = a DNA preparation of 3 individuals of unknown sex, *⁵ parthenogenetic.

	Genus	Species		* ¹	Number tested* ²	Number of positives* ³	
<u>Acarina</u>							
Ixodidae	<i>Amblyomma</i>	<i>cajennense</i>	Fabricius 1787	FM	3	2	
Ixodidae	<i>Amblyomma</i>	<i>dissimile</i>	Koch 1844	FM	2f 1m	1f 1m	
Nothridae	<i>Nothrus</i>	<i>sp.</i>		FM	1* ⁴	1	
Siteroptidae	<i>Pediculoides</i>	<i>mesembrinae</i>	Canestrini 1880	GS	2	2	
<u>Scorpiones</u>							
Liochelidae	<i>Liocheles</i>	<i>australasiae</i>	Fabricius 1775	FM	2f* ⁵	2f	
<u>Opiliones</u>							
Phalangidae	<i>Phalangium</i>	<i>opilio</i>	Linnaeus 1761	FM	3f	3f	
Phalangidae	unknown			OM	1f	1f	
<u>Araneae</u>							
Agelenidae	<i>Tegenaria</i>	<i>duellica</i>	Simon 1875	OM	1f 1m 2j	1f 1m 2j	
Araneidae	<i>Araneus</i>	<i>diadematus</i>	Clerck 1757	OM	4f1m	2f	
	<i>Zygiella</i>	<i>x-notata</i>	Clerck 1757	OM	4f	4f	
Linyphiidae	<i>Lepthyphantes</i>	<i>minutus</i>	Blackwall 1833	OM	1	1	
Liocranidae	unknown			OM	1f	1f	
Pholcidae	<i>Pholcus</i>	<i>phalangioides</i>	Fuesslin 1775	OM	1m	1m	
Salticidae	<i>Ghelnia</i>	<i>canadensis</i>	Banks 1897	FM	2f 1m	2f 1m	
	<i>Habrocestum</i>	<i>pulex</i>	Walckenaer 1837	FM	1f 1m	1f 1m	
	<i>Maevia</i>	<i>inclemens</i>	Koch 1846	FM	2f 1m	2f	
	<i>Marpissa</i>	<i>lineata</i>	Hentz 1846	FM	2f	1f	
	<i>Neon</i>	<i>neli</i>	Peckham & Peckham 1888	FM	2f	2f	
	<i>Pelegrina</i>	<i>proterva</i>	Walckenaer 1837	FM	1f 1m	1f	
	<i>Phidippus</i>	<i>audax</i>	Hentz 1845	FM	1f	1f	
	<i>Salticus</i>	<i>scenicus</i>	Clerck 1757	OM	3f	3f	
	Tetragnathidae	<i>Meta</i>	<i>mengeri</i>	Blackwall 1870	OM	4f 2m 1e	2f 2m
		<i>Tetragnatha</i>	<i>montana</i>	Simon 1874	OM	3j	2
Theridiidae	<i>Enoplognatha</i>	<i>ovata</i>	Clerck 1757	OM	1f 1m	1f 1m	
Thomisidae	unknown			MV	1	1	

Another endosymbiont, *Cardinium*, has been described more recently (Zchori-Fein et al. 2004). *Cardinium* is known to infect nematodes (Noel & Atibalentja 2006), insects (Provencher et al. 2005; Bigliardi et al. 2006; Marzorati et al. 2006) and mites (Chigira & Miura 2005; Groot & Breeuwer 2006). A recent study shows that it is also found in spiders (Duron et al. 2008), although its occurrence in other groups of arachnids has not yet been established.

The effects of endosymbionts such as *Wolbachia* or *Cardinium* on their hosts can include skewing the sex ratio (SR) towards females (due to an increased male mortality rate, a feminization of males, or a resulting parthenogenesis) or cytoplasmic incompatibility (Engelstädter et al. 2006) where uninfected individuals are at a reproductive disadvantage. The consequence of such infection has been studied in mites, but little research has focused on the arachnids as a whole despite the fact that various species or population traits such as distorted SR, parthenogenesis, and potential CI could potentially be explained by the presence of such microbes (see Table 1 for a summary of examples compiled from the literature; references supplied on request; see also Goodacre et al. 2006). These phenomena may have other well-founded explanations, but the possibility that they are the consequence of endosymbiont infections cannot be evaluated without testing for infections in such “hallmark” species.

Various arachnids (Acari, Opiliones, Scorpiones, and Araneae) were assembled for this survey and assessed for infection with *Cardinium*. A major portion of the species included were borrowed from the collection of the Field Museum Chicago, including species known to exhibit potential hallmarks of infection (see Table 1) or

closely related species. These samples were supplemented with an additional subset of arachnid samples from the authors' collections held at the University of East Anglia. DNA was extracted from a combined sample comprising both leg and abdominal tissue for females and leg and palp (where possible) for males. DNA was extracted using QIAGEN DNEasy™ kits and eluted in 100 µl distilled water (see Goodacre et al. 2006 for further information). The localization of *Cardinium* in particular hosts is not yet established and it may not be equally distributed through all host tissue types. However, it is always likely to be closely-associated with reproductive tissue given its vertical method of transmission. DNA was therefore extracted from tissue that included reproductive organs (ovary containing abdominal tissue and male palps respectively) in order to maximize the chances of detecting any bacteria present, while avoiding areas such as the mouthparts, which are potentially contaminated with non-spider tissue. All samples were tested for *Cardinium* using PCR methods described by Zchori-Fein & Perlman (2004), which involves amplifying a section of the *Cardinium* 16S rRNA gene and visualizing the amplified DNA using gel electrophoresis. A summary of the specimens tested and the results of the PCR tests are given in Table 2.

Our study shows that *Cardinium* is present in a range of diverse arachnid groups and appears to be much more common than in the insects that had been assessed previously (Table 2). The infection was found in at least 1 representative of all of the species tested (100% infected, $n = 25$) vs. 6% of insect species in Zchori-Fein & Perlman 2004 (of which 24% harbored *Wolbachia*). This finding echoes that of

another recent study of spiders, which also found *Cardinium* to be more common than in insects (22% of spider species infected, Duron et al. 2008). Furthermore, *Cardinium* seems to be more common in spiders than are the other three endosymbionts that have been isolated in this group of hosts (Goodacre et al. 2006). The small sample sizes in our study do not allow us to test for differences in infection between the sexes in individual species. We note, however, that there was no significant difference overall between the males and females in their likelihood of carrying the infection (84% females ($n = 37$) and 73% males ($n = 11$) infected, Exact Test $P = 0.49$).

Further research will pinpoint more precisely how widespread this microbe is within and among arachnid groups and will allow us to understand the effects on individual arachnid hosts. Future work will be directed towards identifying the phenotypes caused by individual bacteria, and in determining the selective advantage that the phenotype confers, and studying the consequence of co-infection with more than one endosymbiont. Spiders are a particularly well-suited group (see Goodacre et al. 2006) for testing theoretical predictions regarding evolutionary relationships between endosymbiont infection and host traits, particularly reproductive traits under sexual selection. Endosymbionts such as *Cardinium* can specifically impact such traits, being potentially involved in both interspecific conflict between host and symbiont, as well as intraspecific sexual conflict between the sexes (see Martin & Gage 2007). A powerful means of illuminating the separate and combined action of these evolutionary conflicts would be to apply an artificial selection approach similar to previous experiments focusing on sexual conflict (e.g., Martin & Hosken 2003).

ACKNOWLEDGMENTS

The authors would like to thank Dr. Petra Sierwald (Field Museum, Chicago), Dr. G. Schwarzenbach (University of Zurich) and Dr. M. Vugdelic for providing specimens and the University of East Anglia for supporting this work. OYM was supported by the Swiss National Science Foundation and NERC, SLG by a BBSRC grant to Prof. Godfrey Hewitt. Additional funding was provided by an ASAB small research grant to OYM and SLG.

LITERATURE CITED

- Bigliardi, E., L. Sacchi, M. Genchi, A. Alma, M. Pajoro, D. Daffonchio, M. Marzorati & A.M. Avanzati. 2006. Ultrastructure of a novel *Cardinium* sp. symbiont in *Scaphoideus titanus* (Hemiptera: Cicadellidae). *Tissue & Cell* 38:257–261.
- Charlat, S., G.D.D. Hurst & H. Mercot. 2003. Evolutionary consequences of *Wolbachia* infections. *Trends in Genetics* 19:217–223.
- Chigira, A. & K. Miura. 2005. Detection of ‘*Candidatus Cardinium*’ bacteria from the haploid host *Brevipalpus californicus* (Acari: Tenuipalpidae) and effect on the host. *Experimental and Applied Acarology* 37:107–116.
- Cordaux, R., A. Michel-Salzat & D. Bouchon. 2001. *Wolbachia* infection in crustaceans: novel hosts and potential routes for horizontal transmission. *Journal of Evolutionary Biology* 14:237–243.
- Duron, O., G.D.D. Hurst, E.A. Hornett, J.A. Jostling & J. Engelstädter. 2008. High incidence of the maternally inherited bacterium *Cardinium* in spiders. *Molecular Ecology* 17:1427–1437.
- Engelstädter, J., S. Charlat, A. Pomiankowski & G.D.D. Hurst. 2006. The evolution of cytoplasmic incompatibility types: integrating segregation inbreeding and outbreeding. *Genetics* 172:2601–2611.
- Goodacre, S.L., O.Y. Martin, C.F.G. Thomas & G.M. Hewitt. 2006. *Wolbachia* and other endosymbiont infections in spiders. *Molecular Ecology* 15:517–527.
- Groot, T.V.M. & J.A.J. Breeuwer. 2006. *Cardinium* symbionts induce haploid thelytoky in most clones of three closely related *Brevipalpus* species. *Experimental and Applied Acarology* 39:257–271.
- Martin, O. & M.J.G. Gage. 2007. Evolutionary conflicts: rapid suppression of a male-killer. *Current Biology* 17:R849–851.
- Martin, O.Y. & D.J. Hosken. 2003. The evolution of reproductive isolation through sexual conflict. *Nature* 423:979–982.
- Marzorati, M., A. Alma, L. Sacchi, M. Pajoro, S. Palermo, L. Brusetti, N. Raddadi, A. Balloi, R. Tedeschi, E. Clementi, S. Corona, F. Quaglino, P.A. Bianco, T. Beninati, C. Bandi & D. Daffonchio. 2006. A novel bacteroidetes symbiont is localized in *Scaphoideus titanus*, the insect vector of flavescence doree in *Vitis vinifera*. *Applied and Environmental Microbiology* 72:1467–1475.
- Noel, G.R. & N. Atibalentja. 2006. ‘*Candidatus Paenicardinium endonii*’, an endosymbiont of the plant-parasitic nematode *Heterodera glycines* (Nemata: Tylenchida), affiliated to the phylum Bacteroidetes. *International Journal of Systematic and Evolutionary Microbiology* 56:1697–1702.
- Oh, H.W., M.I.G. Kim, S.W. Shin, K.S. Bae, Y.J. Ahn & H.Y. Park. 2000. Ultrastructural and molecular identification of a *Wolbachia* endosymbiont in a spider, *Nephila clavata*. *Insect Molecular Biology* 9:539–543.
- Provencher, L.M., G.E. Morse, A.R. Weeks & B.B. Normark. 2005. Parthenogenesis in the *Aspidiotus nerii* complex (Hemiptera: Diaspididae): A single origin of a worldwide, polyphagous lineage associated with *Cardinium* bacteria. *Annals of the Entomological Society of America* 98:629–635.
- Rowley, S.M., R.J. Raven & E.A. McGraw. 2004. *Wolbachia pipientis* in Australian spiders. *Current Microbiology* 49:208–214.
- Zchori-Fein, E. & S.J. Perlman. 2004. Distribution of the bacterial symbiont *Cardinium* in arthropods. *Molecular Ecology* 13:2009–2016.
- Zchori-Fein, E., S.J. Perlman, S.E. Kelly, N. Katzir & M.S. Hunter. 2004. Characterization of a ‘Bacteroidetes’ symbiont in *Encarsia* wasps (Hymenoptera: Aphelinidae): proposal of ‘*Candidatus Cardinium hertigii*’. *International Journal of Systematic and Evolutionary Microbiology* 54:961–968.

Manuscript received 8 January 2008, revised 15 August 2008.