BIOLOGICAL LETT. 2010, 47(1): 37–43
Available online at: http:/www.versita.com/science/lifesciences/bl/ DOI: 10.2478/v10120-009-0018-9

Microorganisms in the oribatid mite *Hermannia gibba* (C. L. Koch, 1839) (Acari: Oribatida: Hermanniidae)

MARCIN LIANA and WOJCIECH WITALIŃSKI

Department of Comparative Anatomy, Institute of Zoology, Jagiellonian University, Ingardena 6, 30-060 Kraków, Poland; phone: +48 12 6632426
Corresponding author: Marcin Liana, marcin.liana@gmail.com

(*Received on 29 March 2010; Accepted on 13 September 2010*)

Abstract: Symbiotic microorganisms associated with arthropods are known to play a significant role in the life of their hosts. Most commonly, the symbionts improve their host's food digestion and modify their meiosis/reproduction. The usual mode of parent-to-offspring transmission of the symbionts is transovarial transmission (vertical) via oocytes. Using transmission electron microscopy, we found extracellular yeast-like and bacteroid microorganisms in food boli, as well as intracellular symbiotic bacteria within cells of the digestive tract and in reproductive cells in both sexes of the oribatid mite *Hermannia gibba*. In the digestive tract, the scarce bacteria were lying individually within midgut cells. The bacteria observed in developing oocytes were numerous and formed large aggregates close to the nuclear envelope and clusters of mitochondria. In spermatocytes we found a few single bacteria located at the cell periphery. The bacteria in the digestive cells may assist in digestion of plant food, whereas the meiotic drive function of the gonad-invading microbes is uncertain. The studied mite species is biparental and its sex ratio is not biased.

Keywords: *Hermannia gibba*, endosymbionts, ovary, testis, transovarial transmission

INTRODUCTION

The study of symbiotic associations of bacteria with their arthropod hosts recently revealed rich and unsuspected relationships between the co-existing organisms. The microorganisms can be extracellular and related to digestion or other host activities, even so unusual as the host's offspring protection against mould fungi (Goettler et al. 2007) or protection of beetle-fungus mutualistic relationship (Scott et al. 2008). The gut of fungivorous arthropods, including a majority of mites belonging to the Oribatida and some Astigmata, frequently contains many bacteria with chitinolytic activity, purportedly making fungal propagules accessible for digestion (Smrž & Soukalová 2008). Some astigmatic mites are also able to utilize bacteria as a food source due to lysozyme activity (for a review, see Erban & Hubert 2008). However, antagonistic relationships are also observed, for instance in ticks, which produce and release antibacterial peptides – defensins – into the midgut lumen (NAKAJIMA et al. 2005).

In insects, at least 7 orders of Neopterygota are associated with intracellular symbiotic microorganisms (usually gamma-Proteobacteria, but occasionally also Ascomycetes), which are housed within specialized fat cells, the bacteriocytes, also called the mycetocytes (GULLAN & CRANSTON 1994). In general, such microorganisms are believed to help in better food utilization, especially in insects living on a poor or nutritionally-unbalanced diet (DOUGLAS 1989).

Intracellular microorganisms housed in the gut cells can also be involved in digestion. They improve the digestion of plant food and supply some desired nutrients to their herbivorous hosts (DOUGLAS 1989). Much more spectacular, however, is an influence of intracellular bacteria on their host's reproduction. The best-known example is *Wolbachia*, an alpha-proteobacterium, but a similar phenomenon is also reported for at least four other bacteria, the *Cytophaga*-like bacterium, *Cardinium, Baumannia*, and *Blochmannia* (WEEKS & STOUTHAMER 2004; ZCHORI-FEIN & PERLMAN 2004; CHIGIRA & MIURA 2005; GROOT & BREEUWER 2006), as well as for some microsporidia (e.g. TERRY et al. 2004). *Wolbachia* species can infect from 15% (WERREN et al. 1995) to 76% of insect species and have been found also in mites, crustaceans, and nematodes (STOUTHAMER et al. 1999). The bacteria can manipulate their host's reproduction in various ways. The best-known examples are feminization, cytoplasmic incompatibility, male killing, and induction of thelytokous parthenogenesis, whwre females are produced from unfertilized eggs (e.g. WEEKS & BREEUWER 2001; WEEKS et al. 2002; CHIGIRA & MIURA 2005; GROOT & BREEUWER 2006; KENYON & HUNTER 2007), typically leading to the absence or rarity of males in the population.

Hermannia gibba (Hermanniidae) is a bisexual mite species belonging to Desmonomata, the early-derived group of oribatid mites (TABERLY 1958), which is characterized by a large number of thelytokous species. Thelytoky was proven in 21 species belonging to various superfamilies of Desmonomata, whereas in 62 other species this mode of reproduction is suspected due to the absence or rarity of males. Thelytokous species are absent only in two families of Desmonomata: Crotoniidae and Hermanniidae (NORTON et al. 1993). Interestingly, using electron microscopy techniques, we found in both sexes of *Hermannia gibba* (Hermanniidae) bacteroid or yeast-like microorganisms not only within the food boli, passing along the gut lumen and in cells of the digestive tract, but also in gonadal tissue both in the female and in the male. These findings are reported in the present study.

MATERIALS AND METHODS

Females and males of *Hermannia gibba* (C. L. Koch, 1839) were collected from deciduous forest litter near the city of Kraków (southern Poland). The individuals were immersed in KARNOVSKY'S (1965) fixative (2.5% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer, pH 7.2). After removal of the anterior part of the body with a fine blade, to improve fixative penetration, they were fixed for 24 h at room temperature and postfixed for 12 h with 1.4% OsO_4 in 0.45 M sucrose. After routine dehydration with graded ethanol solutions followed by propylene oxide, the specimens were embedded in Epon 812. Thin sections were contrasted with uranyl acetate and lead citrate, and examined in a transmission electron microscope (TEM) JEOL JEM 100SX.

RESULTS

Extracellular and intracellular bacteria inside the digestive tract

In both sexes of *Hermannia gibba* the bacteria were found within food boli, i.e. in decomposed material enveloped by peritrophic membrane and passing via the midgut and hindgut (Fig. 1A), as well as within cells of the midgut. The intracellular bacteria are 0.4 µm in diameter and up to 1.6 µm in length. They occur singly in the cytosol, and are not covered by any host-provided membrane (Fig. 1B).

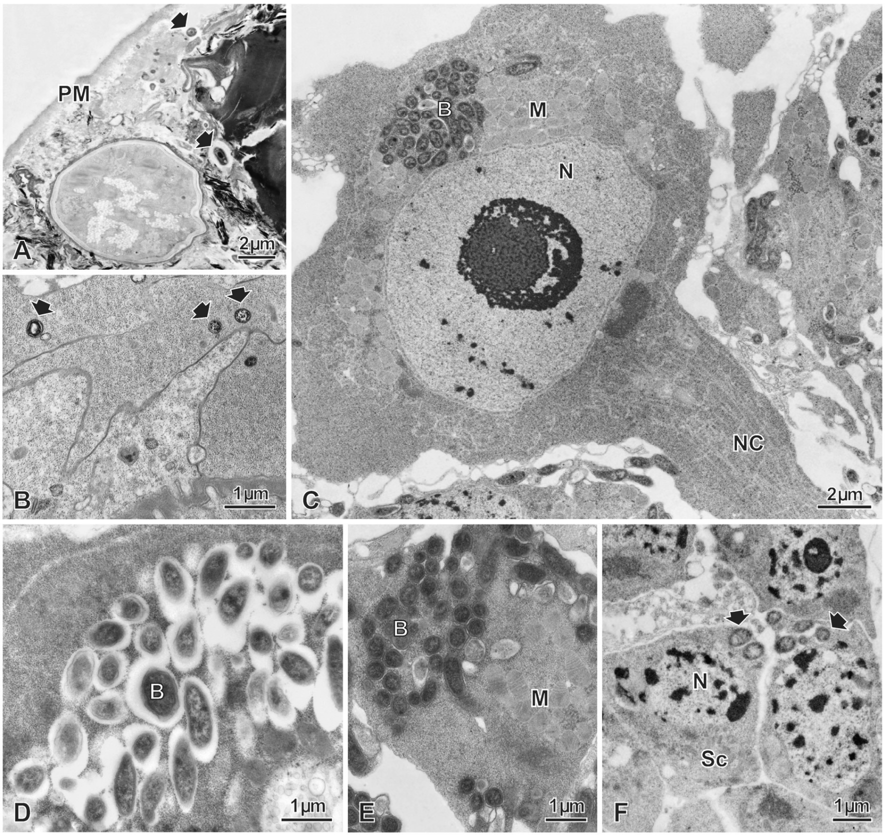

Fig. 1. (A) Bacteria in food boli; (B) bacteria inside digestive tract cells; (C–E) groups of bacteria inside developing oocytes; (F) bacteria in spermatocytes. Arrows point to bacteria. Abbreviations: B = groups of bacteria; M = groups of mitochondria; N = nucleus of an oocyte; NC = nutritive cords; PM = peritrophic membrane; Sc = spermatocyte; ScN = nucleus of a spermatocyte

Bacteria inside the gonads

The ovary in *H. gibba*, like in other representatives of Desmonomata, is unpaired, surrounded by the basal lamina and located ventrally in front of the rectum. The developing oocytes are connected in the ovary centre by radially arranged, microtubule-rich nutritive cords. The ovarian bacteria are 0.45 µm in diameter and up to 1.6 µm long, and are located inside the oocytes, nutritive cords, and in the central "rosette" of the ovary. In previtellogenic oocytes, the bacteria form large groups close to the nuclear envelope and clusters of mitochondria (Fig. 1C– E).

In contrast, in the testes we only occasionally observed a few bacteria (0.4 µm × 1.6 µm) at the periphery of early spermatocytes (Fig. 1F).

DISCUSSION

The intracellular bacteria involved in better food utilization and nutrient supply in insects are typically located either in bacteriocytes or in gut cells. Bacteriocytes share some common features: they are large cells (up to 100 µm in diameter) with the cytoplasm packed with symbiotic microorganisms, and they grow by cell enlargement, but do not divide. In relation to their large size and cytokinetic quiescence, the nuclei of many bacteriocytes are polyploid. The microorganisms inside these specialized cells are usually covered by additional, host-provided membrane (Douglas 1989).

The bacteria-containing cells in the digestive tract of *Hermannia gibba* are lacking the typical features of bacteriocytes: their size is not increased and the cytoplasm contains a moderate number of symbionts, which do not show any particular localization pattern. They seem to be similar to those found in tsetse fly (*Glossina* sp.) and a weevil (*Sitophilus* sp.), where the symbionts lie individually in the cytosol without any host-provided membrane (Douglas 1989). According to the nutritional hypothesis (Douglas 1989), such symbionts provide various nutrients that are in short supply in the diet and cannot be synthesized by the host. The studied mite species is herbivorous and/or fungivorous and inhabits the forest litter. Thus the role of endosymbionts inside the digestive cells is most probably nutritional.

The ovary in *H. gibba* is similar to that in other Desmonomata. The developing oocytes are connected in the ovary centre by the nutritive cords – radially arranged microtubule-rich tubes forming the central "rosette" (Liana 2004). Most of the microorganisms were found in oocytes, but also in the cords as well as the centre of the ovary. Thus the possible scenario is that they are present in the ovary thanks to maternal inheritance, and colonize oocytes via nutritive cords' microtubular scaffold. They occur singly or in groups, but inside oocytes they always form large clusters. We also found the bacteria in spermatocytes. The few bacteria were located in the peripheral parts of the cells. We have never found them in mature spermatozoa, so we suspect that they are removed from the cells during gamete maturation. This finding suggests that the bacteria are transmitted to the next generation only through the oocytes.

The role of the digestive tract bacteria seems to be easy to explain (see above), but the function of the microorganisms hosted in the ovary is enigmatic. If they are the commonly known *Wolbachia*, then their influence on *H. gibba* is unclear. *Wolbachia,* as a cytoplasmic incompatibility factor, often causes sex ratio bias towards females by induction of either thelytokous parthenogenesis, feminization of genetic males, or male offspring killing (DURON et al. 2008). The induction of parthenogenesis with bacteria was proven for parasitic wasps *Trichogramma* (STOUTHAMER et al. 1990; STOUTHAMER & WERREN 1993; STOUTHAMER & KAZMER 1994) and *Aphytis* (ZCHORI-FEIN et al. 1995). In those insects, the microorganisms were located inside the ovary in the nurse cells, and over the time, they entered the developing oocytes through intracellular bridges, eventually colonizing the oosome region (LIANA 2000). The pattern of spread of the bacteria in *H. gibba* seems to be similar. The microorganisms use the bridges between cells to inhabit all oocytes. The presence of the bacteria in spermatocytes is unclear. They are probably maternally delivered and colonize also the male germ cells, but mature spermatozoa seem to be bacteria-free (probably because their cytosol is scarce).

On the basis of our observations we cannot estimate how many individuals in the population are infected, but during the collection of the animals we have found a similar number of males and females (unpubl. data). Thus we assume that the 1:1 sex ratio in studied population has not been highly disturbed. We can suggest two possible explanations of this situation. The infection of this species (or of its local population) could have occurred relatively recently, and that could be why the effect of the bacteria on the structure of the *H. gibba* population is not yet fully expressed. Another possibility is that the intragonadal microorganism is not causing the sex-ratio distortion or is not *Wolbachia*. KAGEYAMA et al. (2008) reported recently that a maternally inherited (wHecCI) strain of *Wolbachia* does not cause sex-ratio distortion in the infected *Bombyx mori* population. The effect of this strain of *Wolbachia* on the host remains unknown.

In conclusion, apart from the alimentary-tract-associated microorganisms we found transovarially transmitted bacteria that probably do not influence the sex-ratio. The studied oribatid mite is obviously infected, but two questions are addressed in our ongoing research: is the ovary-infecting agent truly a *Wolbachia* species and what is its effect on the host?

REFERENCES

CHIGIRA A., MIURA K. 2005. Detection of 'Candidatus Cardinium' bacteria from the haploid host *Brevipalpus californicus* (Acari: Tenuipalpidae) and effect on the host. Exp. Appl. Acarol. 37: 107–116.

DOUGLAS A. E. 1989. Mycetocyte symbiosis in insects. Biol. Rev. Camb. Philos. Soc. 64: 409–434.

DURON O., HURST D. D. G., HORNETT A. E., JOSLING A. J., ENGELSTADTER J. 2008. High incidence of the maternally inherited bacterium *Cardinium* in spiders. Mol. Ecol. 17: 1427–1437.

ERBAN T., HUBERT J. 2008. Digestive function of lysozyme in synanthropic acaridid mites enables utilization of bacteria as a food source. Exp. Appl. Acarol. 44: 199–212.

Goettler W., Kaltenpoth M., Herzner G., Strohm E. 2007. Morphology and ultrastructure of a bacteria cultivation organ: the antennal glands of female European beewolves, *Philanthus triangulum* (Hymenoptera, Crabronidae). Arthropod Struct. Develop. 36: 1–9.

Groot T. V. M., Breeuwer J. A. J. 2006. *Cardinium* symbionts induce haploid thelytoky in most clones of three closely related *Brevipalpus* species. Exp. Appl. Acarol. 39: 257–271.

Gullan P. J., Cranston P. S. 1994. The Insects: An Outline of Entomology. Chapman and Hall, London.

Kageyama D., Narita S., Noda H. 2008. Transfection of feminizing *Wolbachia* endosymbionts of the butterfly, *Eurema hecabe*, into the cell culture and various immature stages of the silkmoth, *Bombyx mori*. Microb. Ecol. 56: 733–741.

Karnovsky M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. 27: 137A–138A.

Kenyon S. G., Hunter M. S. 2007. Manipulation of oviposition choice of the parasitoid wasp *Encarsia pergandiella*, by the endosymbiotic bacterium *Cardinium*. J. Evol. Biol. 20: 707–716.

Liana M. 2000. Morphology of the ovary and intracellular translocation of symbiotic bacteroids in hymenopterans from the genera *Muscidifurax* (Pteromalidae) and *Aphytis* (Aphelinidae). Acta Biol. Cracov. 42 (suppl. 1): 51.

Liana M. 2004. Structure of reproductive systems in mites from Astigmata and Oribatida suborders, and Astigmata phylogeny. PhD Thesis, Jagiellonian University, Kraków, Poland (in Polish).

Nakajima Y., Saido-Sakanaka H., Ogihara K., Taylor D. M., Yamakawa M. 2005. Antibacterial peptides are secreted into the midgut lumen to provide antibacterial midgut defense in the soft tick, *Ornithodoros moubata* (Acari: Argasidae). Appl. Entomol. Zool. 40: 391–397.

Norton R. A., Kethley J. B., Johnston D. E., OConnor B. M. 1993. Phylogenetic perspectives on genetic systems and reproductive modes of mites. In: Evolution and Diversity of Sex Ratio in Insects and Mites (Wrensch D., Ebbret M., Eds), pp. 8–99, Chapman and Hall, New York.

Scott J. J., Oh D-Ch., Yuceer M. C., Klepzig K. D., Clardy J., Currie C. R. 2008. Bacterial protection of beetle-fungus mutualism. Science 322: 63.

Smrž J., Soukalová H. 2008. Mycophagous mites (Acari: Oribatida and Acaridida) and their cooperation with chitinolytic bacteria. In: Integrative Acarology. Proceedings of the 6th European Congress, EURAAC, Montpellier 2008 (Bertrand M., Kreiter S., McCoy K. D., Migeon A., Navajas M., Tixier M.-S., Vial L., Eds), 491 pp. 374–377.

Stouthamer R., Kazmer D. J. 1994. Cytogenetics of microbe associated parthenogenesis and its consequences for gene flow in *Trichogramma* wasps. Heredity 73: 317–327.

Stouthamer R., Werren J. H. 1993. Microorganisms associated with parthenogenesis in wasps of the genus *Trichogramma*. J. Invert. Pathol. 61: 6–9.

Stouthamer R., Luck R. F., Hamilton W. D. 1990. Antibiotics cause parthenogenetic *Trichogramma* to revert to sex. Proc. Natl. Acad. Sci. USA. 87: 2424–2427.

Stouthamer R., Breeuwer J. A. J., Hurst G. D. D. 1999. *Wolbachia pipientis*: Microbial manipulator of arthropod reproduction. Annu. Rev. Microbiol. 53: 71–102.

Taberly G. 1958. Les nombres chromosomiques chez quelques especes d'Oribates (Acariens [The chromosome number in certain species of Oribatida (Acari)]. R. Acad. Sci. 246: 3284–3285 (in French).

Terry R. S., Smith J. E., Sharpe R. G., Rigaud T., Littlewood D. T. J., Ironside J. E., Rollinson D., Bouchon D., MacNeil C., Dick J. T. A., Dunn A. 2004. Widespread vertical transmission and associated host sex-ratio distortion within the eukaryotic phylum Microspora. Proc. R. Soc. Lond. B. 271: 1783–1789.

Weeks A. R., Breeuwer J. A. J. 2001. *Wolbachia*-induced parthenogenesis in a genus of phytophagous mites. Proc. R. Soc. Lond. B. 268: 2245–2251.

Weeks A. R., Stouthamer R. 2004. Increased fecundity associated with infection by a *Cytophaga* –like intracellular bacterium in the predatory mite, *Metaseiulus occidentalis*. Proc. R. Soc. Lond. B. (Suppl.) 271: S193–S195.

WEEKS A. R., REYNOLDS K. T., HOFFMANN A. A. 2002. *Wolbachia* dynamics and host effect: what has (and has not) been demonstrated? Trends Ecol. Evol. 17: 257–262.

WERREN J. H., WINDSOR D., GUO L. 1995. Distribution of *Wolbachia* among Neotropical arthropods. Proc. R. Soc. Lond. B. 262: 197–204.

ZCHORI-FEIN E., FAKTOR O., ZEIDAN M., GOTTLIEB Y., CZOSNEK H., ROSEN Y. 1995. Parthenogenesis-inducing microorganisms in *Aphytis* (Hymenoptera: Aphelinidae). Insect Mol. Biol. 4: 173–178.

ZCHORI-FEIN E., PERLMAN S. J. 2004. Distribution of the bacterial symbiont *Cardinium* in arthropods. Mol. Ecol. 13: 2009–2016.