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The gonads of *Zyginidia pullula* males feminized by *Wolbachia pipientis*

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Abstract

Zyginidia pullula (Boheman) (Hemiptera Cicadellidae) is a widespread central European leafhopper about 3 mm long. It is a multivoltine species, with a bisexual reproduction. Some females collected in Northern Italy, when mated with males, give origin to an exclusively female brood. These lineages are infected by *Wolbachia pipientis* (Rickettsiales), an α-proteobacterium known to induce several reproductive manipulations in its arthropod host. In *Z. pullula*, the infection by *W. pipientis* induces feminization of genetical males. These feminized males are characterised by intersexual phenotypes, i.e. they are females harbouring upper pygofer appendages, a typical male secondary sexual feature. In this study we report preliminary results on the ovary histological structure and the immunohistochemical localization of *W. pipientis* in the gonads of these aberrant females (intersexes).

Key words: Feminization, gonads, Hemiptera, *Wolbachia pipientis*, *Zyginidia pullula*.

Introduction

Zyginidia pullula (Boheman) (Hemiptera Cicadellidae) is a widespread central European leafhopper about 3 mm long. It is a multivoltine species, able to produce up to four generations a year in Northern Italy, and feeds on grasses and maize crops as a mesophyll sucker. This species is characterized by a bisexual reproduction mode, but some females collected in Northern Italy, when mated with males, give origin to an exclusively female brood (Verzé and Mazzoglio, 1994). These lineages are infected by *Wolbachia pipientis*, a Gram-negative α-proteobacterium belonging to Rickettsiales (Negri *et al.*, 2002). *W. pipentis* is known to be a "reproductive manipulator" of a wide range of arthropods, where it causes cytoplasmic incompatibility between gametes, thelytokous parthenogenesis, male-killing and feminization of genetic males (Stouthamer *et al*., 1999). In *Z. pullula*, *W. pipentis* is able to feminize males (Negri *et al.*, 2006), a not well understood phenomenon, until now described only in Crustacea Isopoda and in the moth *Eurema hecabe* (L.) (LeGrand *et al*., 1987; Hiroki *et al.*, 2002). The feminizing effect induced by *W. pipientis* in *Z. pullula* leads to the production of females having a male genotype and typical male secondary sexual characteristics localized in the last abdominal segments, i.e. the upper pygofer appendages.

In the present study we report preliminary results on the ovary histological structure and the immunohistochemical localization of *W. pipientis* in the gonads of these aberrant females (intersexes).

Materials and methods

Gonads from uninfected females and males, and *W. pipientis* infected intersexes were dissected, fixed in Bouin's mixture and embedded in agar/paraffin wax as described by Franchini *et al*. (2003).

Hematoxylin/eosin histological stain and gallocyanin-chrome alum histochemical reaction were performed on 7 μm serial sections in order to study gonad's structural modifications. The immunocytochemical procedure was also performed using a rabbit polyclonal antiserum against the *Wolbachia* surface protein WSP (Kramer *et al.*, 2005). Sections were incubated 1 h at RT in the primary antibody diluted 1:300 and the labelling sites were revealed by an immunoperoxidase technique using the avidin-biotin peroxidase complex (Hsu *et al.,* 1981). Controls of the immunohistochemical reactions were performed by substituting the primary antibody with non-immune sera. Nuclei were counterstained with hematoxylin.

Results and discussion

Preliminary histological examination of sections from feminized male ovarioles, compared with uninfected females, showed modifications in the structural organization that are related to the extent of development of upper pygofer appendages, the conserved male secondary sexual characteristics. Feminized males with upper pygofer appendages reduced to a stump were characterized by ovaries morphologically similar to those of uninfected females. The *Z. pullula* female gonad was composed of typical telotrophic meroistic ovarioles containing nurse cells and young oocytes located in a spindle-shaped germarium. The nurse cells, generated by mitotic divisions in the most anterior region, progressively enlarged and became located in the periphery of germarium central zone surrounding a fibrous basophilic region, the trophic core. Pre-vitellogenic oocytes moved into the vitellarium where they developed and remained connected to the trophic core by nutritive cords. Feminized males with well developed upper pygofer appendages were characterized by an altered morphology of the germarium that appeared irregularly shaped with

a not clearly polarized organization and reduced in size. Moreover, a large number of nurse cells lost the syncytial architecture around the trophic core, decreased the adhesion with neighbouring cells and became round. The trophic core region shrunk and the nutritive cords were often hardly distinguishable. In some ovarioles a lot of apoptotic pictures and degenerating cells were seen. The oocytes growing in the vitellarium showed a reduced and less compact cytoplasm that sometimes was seen to contain different sized vacuola with heterogeneous granula inside. They also were less adherent to the surrounding follicular epithelium. The observed morphological modifications of intersex ovariole cells and in particular of the nurse cells, known to provide macromolecules and organelles for oocyte development, suggest that the *Wolbachia* infection could lead to an altered gonad function and a reduction in oocyte quality.

As far as immunoreactivity (IR) to anti-WSP antibody is concerned, it was detected in all ovariol cells including the oocytes. Indeed IR was seen in the cytoplasm of fully differentiated nurse cells, in central fibrous trophic core and in the cytoplasm, surrounding the nucleus, of young oocytes housed in the trophic chamber. IR was also present in the inner cytoplasm of growing oocytes entered in the vitellarium and in the follicular epithelium.

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