ΝΟΤΕ

Gender- and Species-Specific Characteristics of Bacteriomes from Three Psyllid Species (Hemiptera: Psylloidea)¹

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Bacteriomes are specialized insect organs that harbor symbiotic bacteria (Baumann 2005, Annu. Rev. Microbiol. 59: 155 - 189). Bacteriomes of psyllids (Hemiptera: Psylloidea) primarily consist of 2 tissue types: bacteriocytes which house an obligate symbiont 'Candidatus Carsonella ruddii,' and multinucleate syncytia which may harbor facultative symbionts (Chang and Musgrave 1969, Tissue Cell 1: 597 - 606; Fukatsu and Nikoh 1998, Appl. Environ. Microbiol. 64: 3,599 - 3606; Subandiyah et al. 2000, Zool. Sci. 17: 983 - 989). A long-term goal of our research is to investigate the ecological interactions among agriculturally-important psyllids, their host plants, and bacteriomeassociated symbionts, and to investigate the potential role of bacteriomes in the acquisition and transmission of psyllid-vectored plant pathogens. To facilitate the control of biological variation in future studies of bacteriomes and bacteriome-associated symbionts, the objective of our study was to provide morphological descriptions of bacteriomes from 2 agriculturally-important psyllids, Bactericera cockerelli (Sulc) (Hemiptera: Triozidae) and Cacopsylla pyricola (Förster) (Hemiptera: Psyllidae), and to compare the size and appearances of bacteriomes between sexes. A third psyllid species, Aphalara calthae L. (Hemiptera: Aphalaridae), representing a family distinct from those of the 2 pest species, was included in our comparisons to provide a systematically broad description of psylloid bacteriomes. Identification of bacterial symbionts was beyond the scope of this initial study and was not included as an objective.

Bactericera cockerelli were obtained from a colony maintained on tomato plants (*S. lycopersicum*, cv. Moneymaker) at 25°C with a 16:8 (L:D) h photoperiod. The colony originated from insects collected in 2012 from fields of potato near Prosser, WA. Summerform *C. pyricola* were obtained from a colony reared on Bartlett pear seedlings in a greenhouse with supplemental light programmed to provide a 16:8 (L:D) h photoperiod. Diapausing winterform *C. pyricola* were collected from a mature pear orchard located at the USDA-ARS experimental farm near Moxee, WA, on 26 November 2012. Diapausing *A. calthae* adults were collected from conifers located near unmanaged vegetation at the USDA-ARS experimental farm on 6 November 2012.

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Insects anesthetized with CO₂ were mounted, ventral sides up, on glass microscope slides using double-sided tape. A drop of saline (0.7% NaCl [w/v]) was placed over each insect and held in place by cohesion. Dissections were performed at 32X under a dissecting microscope. Using two #5 forceps (D'Outils Dumont SA, Montignez, Switzerland), the ventral plate of the abdomen was removed taking care to minimize the displacement of the internal organs. The reproductive organs were then removed to observe the position and appearance of the bacteriome. Photographs of bacteriomes were obtained at 50X magnification using a DP25 digital camera (Olympus America Inc., Central Valley, PA) mounted to an Olympus dissecting scope (model SZX7, Olympus America Inc.). The psyllid bacteriome is typically composed of 2 lobes, extending laterally from the abdominal midline and connected by a comparatively narrow center. Therefore, the long-axis of the organ corresponds to its width. Three dimensions of each bacteriome were measured to estimate the relative size of the organ: the width of the bacteriome, and the diameters of the lobes along the anteroposterior plane of the insect. The 2 lobes of the bacteriome became separated upon dissection of a minority of insects. In those cases, width of the intact organ was estimated as the combined widths of the separated lobes. Measurements (um) were estimated using the CellSens software (Olympus America Inc.).

The bacteriome of each dissected insect was transferred to a glass microscope slide using fine forceps. Tissues were air-dried, heat fixed at 50°C for 3 - 5 min, fixed in Carnoy's solution for 20 min, and stained in Harris hematoxylin solution (BBC Biochemical Corp., Mt. Vernon, WA) and 1% eosin (Sigma-Aldrich, St. Louis, MO). Bacteriocytes and syncytia were observed at 800X using phase-contrast microscopy, and at 2000X using bright-field conditions, with a Zeiss Axiolab RE microscope (Carl Zeiss USA, Peabody, MA).

Experiments with *B. cockerelli* and *C. pyricola* bacteriomes were conducted 3 times with different cohorts of colony insects, whereas observations of *A. calthae* were made from insects obtained from a single field-collection. In total, we observed bacteriomes and bacteriocytes from 31 *B. cockerelli* (16 females and 15 males), 32 *C. pyricola* (16 per sex), and 20 *A. calthae* (10 per sex). In addition, 11 field-collected winterform *C. pyricola* (6 females and 5 males) were dissected as described above to observe bacteriome shape and color. In separate analyses, size of bacteriomes from each psyllid species was compared between sexes by multivariate analysis of variance (PROC GLM, SAS 9.2). In each analysis, the fixed effect was sex and the dependent variables were width of the bacteriome and mean diameter of bacteriome lobes. Trial and the sex by trial interaction were included as covariates in analyses comparing bacteriome size between sexes of *B. cockerelli* and *C. pyricola*. The Wilks' Lambda statistic was used to test the overall significance of the multivariate model. Multivariate analysis was followed by univariate tests for each variable when a significant multivariate effect was detected.

Bacteriomes of each psyllid species were always located dorsal to the reproductive organs within the 2nd and 3rd abdominal segments (Fig. 1). The posterior midgut appendages (caecae or diverticula) extended anteriorly and were associated with the dorsal surface of the bacteriome. Consistent with the report by Chang and Musgrave (1969, Tissue Cell 1: 597 - 606), bacteriomes were deeply penetrated by numerous tracheoles. Tracheae attached to the bacteriome along the lateral edges of the organ (Fig. 1C). The tracheoles that penetrated either side of the bacteriome generally did not extend beyond half the width of the organ. Because the tracheoles did not bridge the center of the bacteriome connecting the lateral lobes, the 2 lobes tended to easily



Fig. 1. Bacteriomes observed (50 × magnification) upon dissection of *B. cockerelli* females (A) and males (B), *C. pyricola* females (C) and males (D), and *A. calthae* females (E) and males (F).

disassociate during dissection or handling. The stained bacteriocytes and syncytia appeared similar among the 3 psyllid species.

The bacteriomes of *B. cockerelli* (Figs. 1 A-B) and *C. pyricola* (Figs. 1 C-D) were distinctly bilobed; the wide lateral lobes were connected by a comparatively narrow

center. The lateral lobes of bacteriomes in some *B. cockerelli* and *C. pyricola* were separated when observed upon dissection, appearing as separate masses. It was not clear from our observations whether the 2 lobes were separated in situ, or whether the lobes became separated during dissection. In contrast to the deeply lobed bacteriomes of *B. cockerelli* and *C. pyricola*, the bacteriome of *A. calthae* was unipartite (Figs. 1 E-F). In addition to the variation in shape, bacteriomes of *A. calthae* tended to be more darkly pigmented compared with those of *B. cockerelli* and *C. pyricola* (Fig. 1).

We also observed variations in bacteriomes between adult females and males, and these variations were consistent among the 3 species. Bacteriome color ranged from yellow to orange, but females tended to have more intensely pigmented bacteriomes than males (Fig. 1). These differences in color were more pronounced in bacteriomes of *A. calthae* females and males (Fig. 1 E-F) compared with females and males of *B. cockerelli* or *C. pyricola* (Fig. 1 A-D). Bacteriocytes from females of all 3 species also tended to stain darker in hemotoxylin/eosin than did those from males. We cannot explicate the biological significance of these gender-specific color variations based on our observations.

Multivariate analysis of bacteriome size indicated significant patterns in estimated width and lobe diameter of female and male bacteriomes (Wilks' Lambda statistic, *B. cockerelli*, *F*=6.9; df=2, 24; *P* < 0.01, *C. pyricola*, *F*=26.3; df=2, 25; *P* < 0.01, and *A. calthae*, *F*=29.4; df=2, 17; *P* < 0.01). Univariate analyses of *B. cockerelli* bacteriomes indicated that the width of female bacteriomes was greater than for males (*F*=9.5; df=1, 30; *P* < 0.01), but the average diameter of bacteriome lobes did not differ



Fig. 2. Average (±S.E.) width of bacteriomes and diameters of bacteriome lobes from *B. cockerelli*, *C. pyricola*, and *A. calthae* females and males. For each psyllid species, different upper-case letters denote significant differences between sexes in bacteriome width whereas different lowercase letters denote significant differences in mean diameter of the bacteriome lobes.

between sexes (*F*=2.0; df=1, 30; *P* = 0.18) (Fig. 2). Differences in bacteriome dimensions were more pronounced between males and females of *C. pyricola* and *A. calthae* than between sexes of *B. cockerelli*. In both *C. pyricola* and *A. calthae*, bacteriomes of females were wider (*C. pyricola*, *F*=44.8; df=1, 31; *P* < 0.01, *A. calthae*, *F*=42.5; df=1, 19; *P* < 0.01) and had lobes of greater diameter (*C. pyricola*, *F*=20.9; df=1, 31; *P* < 0.01, *A. calthae*, *F*=15.1; df=1, 19; *P* < 0.01) compared with bacteriomes of males (Fig. 2).

Our descriptions provide baseline information on species- and gender-specific variations in psyllid bacteriomes. Although the ecological consequences of the observed differences in bacteriomes from females and males are currently not known, our findings indicate that insect gender represents a source of biological variation that should be controlled in studies of psyllid bacteriomes and bacteriome-associated symbionts.

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