

Comparison of Host Response of *Cryphodera utahensis* with Other Heteroderidae, and a Discussion of Phylogeny

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ABSTRACT: *Cryphodera utahensis* Baldwin et al., 1983 induces a single uninucleate giant cell (SUGC) in *Rosa* sp. The giant cell is similar to those associated with certain other heteroderids, including *Meloidodera* spp., *Hylonema* sp. and *Sarisodera* sp., but contrasts with the syncytia of *Atalodera* spp. and *Heterodera* spp. sensu lato. The wall of the SUGC of *C. utahensis* is unevenly thickened with the thickest region corresponding to the area penetrated by the stylet. The remainder of the wall includes numerous pit fields with plasmodesmata. Thorough examination of the cell wall with light and transmission electron microscopy indicates that wall ingrowths or protuberances are absent. The single nucleus is deeply invaginated with at least one nucleolus, and the cytoplasm includes abundant organelles. The pattern of host responses among Heteroderidae is congruent with existing hypotheses of phylogeny, and suggests that the syncytium of *Atalodera* spp. arose independently from that of *Heterodera* spp. sensu lato.

Heteroderidae modify their hosts in various ways to sustain nutrition during development as sedentary parasites. *Heterodera* sensu lato and *Atalodera* result in a syncytium (Mundo and Baldwin, 1983a), whereas *Hylonema* (Taylor et al., 1978), *Sarisodera* (Mundo and Baldwin, 1983b), and *Meloidodera* (Mundo and Baldwin, 1983c) induce a single uninucleate giant (hyperplisphed) cell (SUGC). These two basic types of responses could be included with additional characters in a phylogenetic analysis of Heteroderidae (Mundo and Baldwin, 1983a, b, c).

The host response to *Cryphodera* has not been previously described; however, this genus shares a number of characters with *Meloidodera*, and on this basis we hypothesized that *Cryphodera* also induces a SUGC. In this paper we test the hypothesis by histological examination of roots of the type host, wild rose (*Rosa* sp. L.), infected with *Cryphodera utahensis* Baldwin et al., 1983.

**Materials and Methods**

Roots of rose infected with *C. utahensis* were collected at the type locality at Clear Creek Canyon, Sevier County, Utah (Baldwin et al., 1983). Root pieces containing mature females were prepared for histological examination including bright field and Nomarski interference light microscopy (LM), and transmission electron microscopy (TEM). Methods of processing tissue were generally as reported by Mundo and Baldwin (1983a, b, c). Roots were fixed for bright field LM in glutaraldehyde, embedded in Paraplast-Plus®, sectioned at 8 μm and stained with safranin and fast green. Additional material for examination with bright field LM was embedded in Spurr's resin, sectioned at about 2 μm and stained with methylene blue and azure II; other infected root pieces were stained with toluidine blue, which has been shown to be useful for detection of wall ingrowths. Some resin-embedded sections, which were examined by Nomarski interference LM, were not stained.

Root segments were fixed for observation with TEM in 3.0% glutaraldehyde, postfixed in 2.0% osmium tetroxide (OsO₄), embedded in Spurr's resin, thin sectioned, and stained with uranyl acetate and lead citrate.

**Results**

Rose infected with *C. utahensis* lacks external symptoms, but females partially protrude from roots (Fig. 1). The SUGC contacts cells of the vascular cylinder including phloem, vascular cambium and xylem (Figs. 1, 3). Surrounding cells were not hypertrophic, however, slight hyperplasia was often observed (Fig. 3). The SUGC varies in size and shape and averages 140 × 200 μm. Only one giant cell per nematode female was observed.

Cell walls of the SUGC induced by *C. utahensis* are unevenly thickened. The portion of wall closest to the nematode lip region is thickest (Figs. 2, 3, 11). The remaining area of wall has alternate thick and thin regions (Figs. 4, 12). However, in some areas where the SUGC wall contacts xylem elements, deposits of wall material are particularly heavy (Fig. 3). Thin portions of the wall correspond to the location of pit fields (Figs. 6, 12) which have a high frequency of plasmodesmata (Figs. 13–15). Plasmodesmata also characterize narrow portions of cells that sometimes occur adjacent to the SUGC (Fig. 13). Cell walls

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1 A portion of senior author's Ph.D. Thesis.
Figures 1–5. Transverse (unless otherwise indicated) sections of giant cells induced by *Cryphodera utahensis* in roots of *Rosa* sp. 1. Bright field light microscopy of female (Ne) in feeding position. C, cortex; GC, giant cell; VC, vascular cylinder. 2. Enlargement of the giant cell (GC) showing thick cell wall (Tk) in close proximity to nematode (Ne) lip region. 3. Giant cell (GC) showing wall thickening (Tk) in the area adjacent to the nematode head (Ne). Arrowhead indicates wall thickening in area adjacent to xylem (X). 4. Longitudinal view of a giant cell. Arrowhead indicates region of thin wall that characterizes pit field. 5. Giant cell (GC) showing nucleus (N) which includes a single nucleolus (Nu).


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Table 1. Host responses induced by Heteroderidae

<table>
<thead>
<tr>
<th>Genera</th>
<th>Host response</th>
<th>Cell wall ingrowths</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Meloidodera floridensis</em></td>
<td>SUGC* (Ruehle, 1962; Mundo and Baldwin, 1983c)</td>
<td>Absent</td>
</tr>
<tr>
<td><em>M. charis</em></td>
<td>SUGC (Heald, 1978; Mundo and Baldwin, 1983c)</td>
<td>Absent</td>
</tr>
<tr>
<td><em>M. bellii</em></td>
<td>SUGC (Mundo and Baldwin, 1983c)</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Cryphodera utahensis</em></td>
<td>SUGC (Mundo and Baldwin)</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Atalodera ucri</em></td>
<td>Syncytium (Mundo and Baldwin, 1983a)</td>
<td>Absent</td>
</tr>
<tr>
<td><em>A. lonicerae</em></td>
<td>Syncytium (Mundo and Baldwin, 1983a)</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Thecavermiculatus</em></td>
<td>Syncytium (Mundo and Baldwin, unpublished)</td>
<td>Not reported</td>
</tr>
<tr>
<td><em>Hylonema ivorense</em></td>
<td>SUGC (Taylor et al., 1978)</td>
<td>Not reported</td>
</tr>
<tr>
<td><em>Sarisodera hydrophila</em></td>
<td>SUGC (Mundo and Baldwin, 1983b)</td>
<td>Absent</td>
</tr>
<tr>
<td><em>Heterodera</em> spp.</td>
<td>Syncytium (e.g., Endo, 1964)</td>
<td>Present</td>
</tr>
<tr>
<td><em>Globodera rostochiensis</em></td>
<td>Syncytium (e.g., Feldmesser, 1953)</td>
<td>Present</td>
</tr>
<tr>
<td><em>Punctodera chalcoensis</em></td>
<td>Syncytium (Mundo and Baldwin, unpublished)</td>
<td>Not reported</td>
</tr>
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</table>

* Single uninucleate giant cell.

of the SUGC induced by C. utahensis were thoroughly examined, particularly in regions adjacent to xylem; however, wall ingrowths or protuberances were not observed.

The single nucleus is deeply invaginated and has one, or sometimes more than one, nucleolus (Figs. 5–7). Often, numerous spherical lobes and shallow invaginations occur at the surface of the nucleolus (Fig. 7). The cytoplasm is granular and organelles include mitochondria, plastids, vacuoles, and abundant endoplasmic reticulum (Figs. 6, 8–10). Plastids are irregularly shaped and contain numerous small vacuoles (Fig. 10). In older giant cells the number of organelles is generally reduced, although small cytoplasmic vacuoles increase in number (Fig. 3) and eventually coalesce.

Discussion

The SUGC induced C. utahensis apparently originated from a cell in the pericycle as in many other heteroderids (Mundo and Baldwin, 1983c). The giant cell generally resembles that reported in association with Hylonema ivorense, Sarisodera hydrophila and Meloidodera spp. (Table 1), as well as certain nonheteroderids including Rhotylechus macrodoratus (Cohn and Mordechai, 1977). The SUGC of C. utahensis is most specifically similar to that of Meloidodera floridensis with respect to the detailed morphology of the cell wall and nucleus (Mundo and Baldwin, 1983c).

The giant cell wall of C. utahensis is thickest adjacent to the nematode lip region, as is also the case for S. hydrophila, Atalodera spp., and Meloidodera spp.; this thickening probably occurs in response to penetration of the stylet (Mundo and Baldwin, 1983a, b, c). The remainder of the SUGC wall of C. utahensis is characterized by the absence of wall ingrowths and the presence of pit fields with abundant plasmodesmata. Similar morphology of the wall, in which ingrowths are absent, occurs in each case of SUGC examined among heteroderids, and of the syncytium induced by Atalodera spp. (Table 1). Although Taylor et al. (1978) did not observe a “discrete cell wall” in the SUGC of H. ivorense, we predict that examination with TEM will indicate cell walls that lack ingrowths. Wall ingrowths or abundant plasmodesmata apparently occur as alternate mechanisms for transport of large amounts of solutes in cells sustaining developing sedentary parasitic nematodes. This transport may be further increased with C. utah-
ensis by numerous plasmodesmata in certain cells adjacent to the SUGC. Among heteroderids examined, wall ingrowths are limited to syncytia produced by species of Heterodera sensu lato (Jones and Dropkin, 1975; Gommers, 1981; Jones, 1981a, b); literature on ingrowths in nematode-induced transfer cells has been summarized by Mundo and Baldwin (1983c).

The nucleus of the SUGC of C. utahensis is deeply invaginated and is similar to that of M. floridensis. Conversely, nuclei of SUGC of Meloidodera charts and M. belli are composed of a cluster of interconnected spherical nuclear units (Mundo and Baldwin, 1983c). The resulting large area of nuclear membrane would facilitate a high rate of exchange at the nucleus–cytoplasm interface. In contrast, the nuclei of SUGC of S. hydrophila and H. ivorense are only slightly ameboid or oval (Taylor et al., 1978; Mundo and Baldwin, 1983b).

The abundance of organelles in cells that sustain sedentary heteroderids suggests a similar high rate of metabolism. Yet, specific occurrence, numbers and characteristics of organelles may vary with the nematode species. For example, plastids are abundant in the SUGC of C. utahensis but they were not observed in the SUGC of Meloidodera spp. Organelles also vary among three species of Meloidodera (Mundo and Baldwin, 1983c). Our fine structural observations have, however, been primarily of mature cells. Changes with respect to organelles probably occur throughout development of the host–parasite relationship, and specifically with the stage and age of the nematode. Furthermore, detailed characteristics might vary among hosts and specific sites of infection.

Two basic types of host responses induced by heteroderids are SUGC and syncytium. We have discussed evidence that these basic responses do not change depending on the host, and probably reflect more fundamental characteristics in the digestive enzymes of the nematodes (Baldwin et al., 1983; Mundo and Baldwin, 1983a, b, c). Furthermore, we suggest that “host response” is useful as a character for testing proposed phylogenies of Heteroderidae. Ferris (1979) hypothesized a phylogeny of Heteroderidae using a cladistic analysis based on designated polarities of eight characters. Host responses of at least one representative species of the nine genera included in the cladogram are presently known (Table 1); these include C. utahensis and our preliminary observations of Thecavermiculatus gracililancea Robbins, 1978 and Punctodera chalcoensis Stone et al., 1976. The pattern of response induced by these heteroderids is generally congruent with the cladogram if we assume SUGC is plesiomorphic (primitive) and syncytium is the apomorphic (derived) character state. However, Atalodera spp. and Thecavermiculatus sp., which induce a syncytium, appear to be an exception. In this case, the cladogram requires parallel evolution of the syncytium between the common ancestor (X) of Atalodera and Thecavermiculatus as well as in the common ancestor (Y) of Heterodera sensu lato (e.g., Heterodera, and Globodera + Punctodera). This explanation is plausible because ho-
moplas y might be suggested by the fundamentally distinct walls of the two types of syncytia. That is, the host response of descendants of X lack ingrowths and have abundant plasmodesmata; conversely, syncytia of descendants of Y have wall ingrowths. This hypothesized homoplas y can be further tested by principles discussed by Rieger and Tyler (1979), including, in this case, ultrastructure of the comparative development of the two types of syncytia.

Our studies have expanded knowledge of host responses of Heteroderidae including seven species in four genera, as well as preliminary observations on two additional genera (Table 1). These results indicate that the type of response induced by a given heteroderid can be useful in phylogenetic analysis. Additional information regarding host responses of other heteroderids is needed, particularly as new species and genera are described. Such information might be useful in testing monophyly, and could be combined with conventional characters in a phylogenetic analysis of Heteroderidae.

Literature Cited


