Resolving phylogenetic incongruence to articulate homology and phenotypic evolution: a case study from Nematoda

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Modern morphology-based systematics, including questions of incongruence with molecular data, emphasizes analysis over similarity criteria to assess homology. Yet detailed examination of a few key characters, using new tools and processes such as computerized, three-dimensional ultrastructural reconstruction of cell complexes, can resolve apparent incongruence by re-examining primary homologies. In nematodes of Tylenchomorpha, a parasitic feeding phenotype is thus reconciled with immediate free-living outgroups. Closer inspection of morphology reveals phenotypes congruent with molecular-based phylogeny and points to a new locus of homology in mouthparts. In nematode models, the study of individually homologous cells reveals a conserved modality of evolution among dissimilar feeding apparatus adapted to divergent lifestyles. Conservatism of cellular components, consistent with that of other body systems, allows meaningful comparative morphology in difficult groups of microscopic organisms. The advent of phylogenomics is synergistic with morphology in systematics, providing an honest test of homology in the evolution of phenotype.

Keywords: Caenorhabditis elegans; comparative morphology; evolution of novelty; congruence; plant parasitism; systematics

1. INTRODUCTION

Diverse and disjunct nematode morphology is shown to track molecular-based phylogeny, even when classically their signals appear grossly incongruent. The lesson learned from nematodes has broad implications in the face of phylogenomics, which foretells the primacy of molecular data for inferring the tree of life (e.g. Rokas et al. 2003). The role of morphology amid this overwhelming source of data has been largely debated, most notably with regard to incongruence (e.g. Hillis 1987; Donoghue & Sanderson 1992; Patterson et al. 1993; Hillis & Wiens 2000; Baker & Gatesy 2002; Scotland et al. 2003; Wiens 2004; Wortley & Scotland 2006; Springer et al. 2007). We contend that upon closer examination, superficial incongruence, or incongruence of morphology owing to inadequate character conceptualization, can be resolved. To understand the patterns and process of phenotypic evolution, such as that of parasitism, it is essential to explicitly dissect homologies of key adaptive characters. We present an example of a programme to resolve character evolution, herein between apparently disparate free-living and parasitic taxa, by addressing homology at finer scales of morphology. We propose that complexity of similarity for guiding homology proposals can be discovered at a cellular level, using precise tools such as computerized ultrastructural reconstruction. These tools can also more explicitly reject presumed homologies that are incongruent with robust molecular phylogenies.

An exemplary case of incongruence has become apparent for a well-studied group of nematodes, Tylenchomorpha, which includes most plant parasites. Classical ideas of relationships of Tylenchomorpha have been based largely on supposed transformations of feeding structures from an open stoma of bacteriovores to a piercing stomatostylet of parasites (Thorne 1961; Andrassy 1962, 1976; Goodey 1963; De Grisse 1972). Understanding this transition is central to hypotheses of the evolution of nematode parasitism. However, the advent of phylogeny based mostly on ribosomal RNA sequences has dramatically overturned traditional ideas and forced re-examination of characters used to define major lineages. Comparative ultrastructure of the stoma and pharynx in some taxa (Baldwin et al. 1997; Dolinski et al. 1998; Zhang & Baldwin 1999), as well as work on early developmental processes (Goldstein et al. 1998), have putatively supported molecular-based groupings. These studies have charted the course for a thorough re-evaluation of homologies of deep-level taxonomic characters and for discovering independent morphological evidence that has been found to be congruent with the new system. The discovery of recognizable characters across taxa is consistent with the conservation of cell components observed in other nematode organ models, also addressed herein.

2. HOMOLOGY PROPOSALS IN MORPHOLOGICAL SYSTEMATICS

The role of morphology in systematics has recently tended towards more inclusive datasets (e.g. defended
in Lee (2006), parallel to the role of molecular sequences. In terms of sheer numbers of characters for accuracy and statistical support, morphology cannot be assessed with the same quantitative rigour possible for molecular sequences (Hillis 1987; Hedges & Maxson 1996; Givnish & Symsa 1997; Hillis & Wiens 2000; Wortley & Scotland 2006). Especially in groups where morphology matrices are limited, a heavy bias towards informative, independent genomic sequence characters will probably dominate phylogenetic hypotheses. However, the study of morphology remains critical, as morphology is the primary interface of an organism with its environment. Morphological homology holds implications for development and ecology: it defines the players and results of developmental pathways and it predicts some causality for its functional consequences. A strategy that focuses on studies of primary homology (de Pinna 1991), or homology proposals grounded in character details, would strongly supplement the power of morphology in systematics. Specifically, such a strategy should explore those details that are congruent with the robust phylogeny that molecular data support.

Strictly numerical approaches to morphology reflect a general emphasis on analysis instead of character conceptualization (Rieppel & Kearney 2002; Rieppel 2007; Winther 2009). Whereas congruence provides strong tests of homology (Patterson 1982), homology proposals require solid grounding based on non-trivial similarity, as discovered through detailed, comparative anatomy (Kearney & Rieppel 2006). Because of the indefinite time requirements anticipated for deep inquiry (Rieppel 2004), this approach has been questioned as being not feasible to complete a matrix for analysis (Assis 2009). Nevertheless, detailed empirical evidence (including possibly causal foundations of homology) that defines characters remains a necessity distinct from their congruence with independent data points (Rieppel 2004; Assis 2009; Assis & Brigandt 2009). Although similarity alone is insufficient to both discover and test characters (Patterson 1982; Kluge 2003), several layers of similarity evidence construct stronger hypotheses of homology, which by being more complex and multifaceted are more severely falsifiable by independent tests (Rieppel & Kearney 2002). Through congruence, such hypotheses have greater power to iteratively corroborate, refine or challenge molecular phylogenies in their own right. Furthermore, an understanding of individual character homologies, apart from the statistical support of a broadly inclusive matrix, is necessary to properly articulate hypotheses of morphological evolution.

The value of individual characters is particularly amplified in organisms with limited cell counts and structural complexity, such as small invertebrates, which comprise the majority of metazoan phyla. Emerging hypotheses of relationships in microfauna, inferred from molecular resources, are especially challenging because previous hypotheses were largely based on characters that are barely distinguishable by light microscopy. Even in cases where we can finally be confident of molecular phylogeny, we are still compelled to understand its implications for phenotypic evolution, adaptation and processes of development. Research in all of these requires knowing where morphology has been falsely interpreted. Besides being simply limited in number, characters are subject to inadequate understanding or misinterpretation, because they can comprise multiple, independent and sometimes imperceptible components that are not all necessarily homologous (Subbotin et al. 2008). Characters relevant to taxonomy, which are also the units of hypothesized evolutionary scenarios, have often been conceived based on their utility in diagnostics, without the intention to consider the possibility of convergence.

3. NEMATODES AS MODELS FOR COMPARATIVE MORPHOLOGY

The model Caenorhabditis elegans attests the suitability of nematodes, now with a well-developed genomic and phylogenetic framework, for basic biological research, including studies of morphology at a cell-by-cell level. The constancy in cell number and patterns of cell divisions in Order Rhabditida (Sulston et al. 1983; Houthooft et al. 2003; Houthooft & Borgonie 2007) facilitates comparison of individually homologous cells across broader taxa (Fitch 1997; Félix et al. 2000). Furthermore, the limited size of nematodes makes complete, three-dimensional reconstruction of entire organ systems a feasible goal. Anatomical reconstructions based on transmission electron microscopy (TEM) serial sections in C. elegans (e.g. Ward et al. 1975; Ware et al. 1975; Albertson & Thomson 1976; Wright & Thomson 1981; White et al. 1986; Hall & Russell 1991) have stood at the foundation of a large body of basic experimental and comparative research. With digital imaging and processing capabilities, TEM reconstruction now offers an application for resolving details of multicellular complexes on a scale useful for testing hypotheses of phylogeny. As a result, precise maps of cell topography within the head regions of three distantly related nematodes are available, allowing a minimally phylogenetic approach. The application of this tool for examining the bases of homology, including for mouthpart and anterior sensory morphology, has revealed remarkable conservation across deep divergences (Bumbarger et al. 2005; Ragsdale et al. 2008, 2009, in press). The ability to identify homologues across great phylogenetic distances provides a unique opportunity for tracking the evolution of the same structures important in traditional higher-level classification. In the case of Tylenchomorpha, mouthpart homologies have additional implications for the evolution of parasitism, as they comprise the feeding equipment (i.e. a protrusible needle-like stylet) necessary for a plant parasitic lifestyle (Baldwin et al. 2004a).

4. A CASE OF GROSS PHYLOGENETIC INCONGRUENCE IN NEMATODA

An outstanding case of morphological incongruence with molecular phylogenies is in Order Rhabditida (formerly Class Secernentea), which includes C. elegans in addition to nematodes exhibiting a wide range of morphological and ecological diversity. Phylogenies based on 18S and 28S rRNA consistently support a sister-group relationship between the infraorder Tylenchomorpha (minus Aphelenchoididae) and a clade of free-living, bacteriovorous Cephalobomorpha (Aleshin et al. 1998; Blaxter et al.
Figure 1. The epidermis, muscle and cuticle of the anterior end of three representatives of Rhabditida: (a) *A. complexus*, (b) *C. elegans* and (c) *A. avenae*. Stoma/stomatostylet characters understood from three-dimensional TEM reconstruction (coloured diagrams/models) are contrasted with morphology seen by differential interference contrast (DIC) light microscopy (right of each diagram). In spite of gross divergence in feeding structures noted by several generations of taxonomists, closer examination of morphology reveals that underlying syncytia are highly conserved in number and in spatial relationships (in addition to numbers and positions of cell bodies, all not shown), allowing reliable assessments of homology. Corresponding colours represent putative homologues. The stoma lining that is falsely coloured darker grey is putatively homologous in all three taxa and is underlain, and presumably produced (Wright & Thomson 1981; Endo 1985), by the arcade syncytia and ‘e1’ in all cases, but it is expressed as a stomatostylet in the tylenchid and as an open stoma in microbivores. Epithelium ‘HypD’ between Hyp3 and Hyp4 is known only in nematodes of putative sister groups Cephalobomorpha and Tylenchomorpha; e1 is expressed as an epithelial collar cell (not muscle) only in the rhabditid. (d) Model of stomatostylet and associated cells/syncytia of *A. avenae*, showing nuances of cell morphology captured by three-dimensional TEM reconstruction, corresponding to the diagram and DIC image. The model is reconstructed directly from data presented in Ragsdale et al. (2008); the arcade syncytia and stomatostylet are shown complete; only one (dorsal) e1 muscle is shown; all other cuticle and syncytia are cut away transversely. Diagrammatic representations of stoma and associated cells/syncytia are informed by TEM reconstructions by Bumbarger et al. (2006) for *A. complexus*, by Wright & Thomson (1981), White (1988), De Ley et al. (1995) and Hall & Altun (2008) for *C. elegans*, and by Ragsdale (2008, 2009) for *A. avenae*. Abbreviations: Hyp, epidermis cell/syncytium; e1, e1 muscle/epithelial cell. Red, posterior arcade; orange, anterior arcade; yellow, Hyp1; green, Hyp2; light blue, Hyp3; dark blue, HypD; violet, Hyp4; brown, e1; light grey, cuticle; dark grey, stoma lining.

5. DIGGING DEEPER: CONGRUENCE FROM THREE-DIMENSIONAL TEM RECONSTRUCTION

A closer inspection of morphology has revealed characters that, pending further taxon sampling, are congruent with phylogeny supported by molecular data. Complete TEM reconstruction of anterior cuticular sensilla, other sensory neurons and associated cells for free-living nematodes.
representing Rhabditomorpha (C. elegans: Ward et al. 1975; White et al. 1986) and Cephalobomorpha (Acrobeles complexus: Bumberger et al. 2007) has enabled detailed comparison to a nematode from Tylenchomorpha (Aphelenchus avenae: Ragsdale et al. 2009). Similarities guiding homology proposals included spatial connectivity (Jardine 1969) to other anterior cells, relative positions (Remane 1952) with respect to the body axis, and details of dendrite terminus morphology. Correspondence of cell numbers corroborates homologies by tests of conjunction, which check for the coexistence of putative homologues within the same organism (Patterson 1982). In a comparison of three model taxa, several features are identified as shared between cephalobid and tylenchid nematodes, comprising strong morphological evidence for a clade including those taxa but exclusive of rhabditids (i.e. Rhabditomorpha). This baseline evidence can be tested in additional taxa, and relevant work is in fact under way: preliminary data from representative diplagasterids, which comprise a sister group to rhabditids, support this separation (D. J. Bumbarger 2009, personal communication). Using complete reconstructions for reference, some sensory characters can now also be identified in a broader range of ingroups based on previous, more limited ultrastructural studies. For example, the characteristic BAG termini can be identified in the cephalic region of other tylenchids studied with corresponding TEM sections, as can the presence of the second sensillum dendrites (reviewed in Ragsdale et al. 2009); the distribution of similar states across sampled ingroups supports their general conservation. Hypotheses of outgroups and character polarity are being further tested by both morphology and molecular data in additional representatives, including Myolaimus, Teratoccephalus, Plectus and other taxa surmised to be basal within Class Chromadorea (which includes Rhabditida). Limited data for Myolaimus n. sp. (Giblin-Davis et al. in press), a possible outgroup to all three fully reconstructed taxa (De Ley & Blaxter 2002; Nadler et al. 2006), corroborate hypotheses based on the three taxa; additionally, these data allow testable hypotheses of polarity for some characters.

Similarities of the internal sensory anatomy exclusive to the cephalobid and tylenchid nematodes (Ragsdale et al. 2009) are presented herein (table 1 and figure 2). Also unique to the tylenchid and cephalobid with respect to the rhabditid is the additional epithelium ‘HypD’ (possibly XXX cells in C. elegans; Bumberger et al. 2006) between the Hyp3 and Hyp4 syncytia (figure 1). HypD is expressed as a pair of cells in the tylenchid and is a weakly connected syncytium in the cephalobid; this homologue was identified on the basis of position of cell processes and connections to support (socket) cells of the amphids and the outer labial and cephalic sensilla. The conservatism of the series of all other anterior epidermal cells between the three taxa underscores the significance of an extra set of cells in two of them. Although not useful in the context of the present tree of limited taxa, several putative autapomorphies distinct for the rhabditid are also shown (table 1 and figure 2).

Neurons and other cell classes of the nematode head are remarkably similar across deep lineages, but distinctions that track these lineages are still evident. Similarity has even been sufficient in the reconstructed amphid, the most complex nematode sensory organ, to recognize cell homologies in a diversity of nematode feeding types (Ashton et al. 1995; Li et al. 2000; Bumberger et al. 2009). Reconstruction of individual anterior cells unveils a new supply of conserved phenotypic features that map cogently on phylogenies based on molecular sequence data. As expected for slowly diverging characters, several other aspects of the sensory system are conserved across all representatives, supporting homology statements but not being informative for ingroup relationships. Differences identified as ‘autapomorphic’ for each representative, such as those noted between A. avenae and other Tylenchomorpha (Ragsdale et al. 2009), may be useful for tracking the divergence of phenotypes within lineages. Given the strength of this approach, expanded representation of taxa should yield further congruence of phenotypes with phylogeny. In the cases where it does not, the same characters offer a more serious and interesting challenge to conflicting phylogenetic

<table>
<thead>
<tr>
<th>character description</th>
<th>A. avenae (tylenchid)</th>
<th>A. complexus (cephalobid)</th>
<th>C. elegans (rhabditid)</th>
<th>Myolaimus n. sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. dendrite IL1 with accessory partial loop</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>?</td>
</tr>
<tr>
<td>2. IL1 contact with Hyp2, with filaments</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>?</td>
</tr>
<tr>
<td>3. second, bare outer labial dendrite (‘OL1’)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<tr>
<td>4. OL2 termini have ‘nubbins’ in cuticle</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>?</td>
</tr>
<tr>
<td>5. CEM dendrite in female/hermaphrodite</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6. CEP termini have nubbins in cuticle</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>?</td>
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<tr>
<td>7. BAG dendrites have multilamellar termini in ventral, lateral and quadrant sectors</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>8. FLP neurons have ciliated, expanded termini</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9. URX neurons have ciliated termini</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>10. URX have branched termini</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>?</td>
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<tr>
<td>11. URX have process ending in amphid socket</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>?</td>
</tr>
<tr>
<td>12. epithelial cells/syncytium ‘HypD’ between Hyp3 and Hyp4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>?</td>
</tr>
</tbody>
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Table 1. Characters of homologous anterior sensory and associated cells in representatives of major clades of Rhabditida (Nematoda) with states unique for Rhabditomorpha or for Cephalobomorpha + Tylenchomorpha. Characters are coded as presence (1), absence (0) or missing data (?).
hypotheses. In addition to sensory morphology, carefully dissected female gonoduct characters, at least among groups of cells, have recently been shown to support a clade of Cephalobomorpha and Tylenchomorpha (Bert et al. 2008). Evidence for possible congruence in multiple organ systems underscores the potential of a focus on fine levels of morphology.

6. MORPHOLOGICAL EVOLUTION IS CONSERVED AT A CELLULAR LEVEL

The ability to recognize homologous cells in disparate taxa illustrates a conserved modality of morphological change. Corroborating this phenomenon in nematode sensory organs is conservatism in the anterior alimentary system. Despite their variety and divergence, characteristic feeding structures in reconstructed nematodes are built from the same suite of constituent cells (Ragsdale et al. 2008, in press; figure 1). The evolution of novelty in the tylenchid stomatostylet, a specialized structure used to penetrate host tissue, has elicited proposals of transformation series at a gross level (Thorner 1961; Andrus 1962, 1976; Goodey 1963; De Grisse 1972). These hypotheses have been rejected by molecular-based phylogenies because they failed with respect to postulated intermediate taxa (including generalized ‘rhabditids’). Furthermore, observation limited to light microscopy has been unable to detect specific tissues involved in the secretion of these cuticular structures, which are now hypothesized to be the locus of mouthpart homologies. Better understanding of individual component structures has also rejected hypotheses of transformations that are not based on false intermediate taxa (Poinar 1974; Siddiqi 1980). Complete, TEM-based characterization of individual cells in adults of the rhabditid C. elegans (Wright & Thomson 1981; White 1988), the cephalobid A. complexus (Bumbarger et al. 2006) and the tylenchid A. avenae (Ragsdale et al. 2008) reveals a highly similar underlying epidermis; in nematode development, epidermal tissue comprises a stack of ring-like cells functioning in cuticle deposition and morphogenesis of body shape (Priess & Hirsh 1986; Nguyen et al. 1999). The same numbers of syncytia (other than ‘HypD’) in the same relative positions, with each syncytium characterized by the same number of cell body processes, underlie the stomatal and other anterior cuticle in nematodes with either a stoma or a stomatostylet (figure 1). The same number of major anterior syncytia (presence of ‘HypD’ is unknown), with similar morphologies and connectivity, has also been identified in Myolaimus sp. (Giblin-Davis et al. in press). Individual epidermal syncytia have become reduced, stretched or expanded in nematodes with different mouthparts, but in all taxa they share the same spatial relationships to one another and have presumably conserved numbers and relative positions of nuclei.

The nematode pharynx, which operates in conjunction with mouthpart structures, has functions and morphology that differ between nematodes with stomatostyles versus those with open stomata. Yet complete reconstruction of the anterior pharynx in both the rhabditid C. elegans (Albertson & Thomson 1976) and the tylenchid A. avenae (Ragsdale et al. in press) has revealed an exact correspondence in number of cell classes and numbers of cells or nuclei per class, including pharyngeal muscle, epithelium and neurons. Topological relationships among cells and with respect to cuticular landmarks, such as the dorsal pharyngeal gland orifice (‘DGO’), are also conserved. Modalities of pharynx function are divergent between these two nematodes. In the rhabditid (and other bacteriovores), the pharynx is pumping or peristaltic for almost all of its length, functioning so that particles are ingested while water is expelled (Seymour et al. 1983; Chiang et al. 2006). Conversely, the pharynx in the tylenchid relies on an enlarged, medial pump to both draw and ingest fluid from its host (Fisher & Evans 1967). Consequently, the expression of homologous cells as either epithelial or muscular for different roles has evolved independently within these taxa. For example, the anterior two classes (e1 and e3) of pharynx radial cells, or cells lining the pharyngeal lumen, comprise a non-contractile epithelial collar that has taken on a stabilizing, mechanical function in the rhabditid; the same two cell classes in the tylenchid are muscles (e1) that protract the stomatostylet, and a long epithelial lining (e3) that comprises the non-pumping anterior part of the pharynx. Homologues of e1 and e3 in both the tylenchid and the rhabditid contrast with the dilatory, pumping muscles as expressed in other free-living Rhabditida (van de Velde et al. 1994; Baldwin &
Eddleman 1995; De Ley et al. 1995) and in Myolaimus (Giblin-Davis et al. in press). Interestingly, the e1 and e3 ‘epithelial collar’ cells in the rhabditid are also expressed in representatives of the supported sister group to Rhabditomorpha, the Diplogasteromorpha (Baldwin et al. 1997; D. J. Bumbarger 2009, personal communication). The presence of epithelial e1 and e3 in rhabditids + diplogasterids distinguishes this clade from the former groups and is thereby also congruent with independent phylogeny. Even more conserved than muscle and epithelial cells is the pharyngeal nervous system, with relative positions of cell bodies corresponding precisely between the rhabditid and tylenchid, as well as with other tylenchid representatives (Ragsdale et al. in press) and other free-living Rhabditida (Chiang et al. 2006). The co-option of the same sets of cells for divergent functions in distant nematodes, demonstrated in feeding structures, outlines a mechanism of morphological evolution that is highly adaptable yet also conservative across deep phylogenetic divergences.

The case of Tylenchomorpha illustrates that the morphological leap to produce a parasitic feeding apparatus required only expansion or reduction of individual cells along the body axis and, in two cell classes (e1 and e3), alternate expression of cell fate. Similarity in cell topology and fate between bacteriovores and tylenchids suggests the late developmental specification of plant parasitic feeding structures. This finding narrows the search for pathways of how phenotypic plasticity responds to selection, particularly in the evolution of plant parasitism. Furthermore, comparable maps of cell positions and connectivity, including long processes that separate some functional parts of cells from their cell bodies, are basic to exploring signalling pathways and roles of specific cells in development of different nematodes.

An understanding of the basic components of the stomatostylet and pharynx implies that possible parallel evolution of a similar stomatostylet in two close clades (Aphelenchoidea and a clade of all other tylenchids) may not be hopelessly irreconcilable with molecular phylogenies, which challenge the monophyly of stomatostyle-bearing nematodes (Blaxter et al. 1998; Holterman et al. 2006; Smythe et al. 2006; Meldal et al. 2007; Bert et al. 2008; van Megen et al. 2009). Reconstruction of the pharynx with comparison to corresponding cells in Aphelenchoides blastophthorus (Shepherd et al. 1980) has shown that commonly referenced pharynx similarities (e.g. posterior DGO position) in A. avenae and A. blastophthorus (traditionally both ‘Aphelenchoidea’) are based on alternate configurations of otherwise conserved cells. The cells comprising the anterior pharynx (e1, e2 and e3) differ in their relative proportions and cell body positions (Ragsdale et al. in press). Greater scrutiny of diagnostic features has explained apparent incongruence in this case by detecting evidence for convergence. Distinctions in supposed commonalities between lineages were elucidated only after complete cell enumeration and reconstruction.

7. HOMOLOGY AND PHENOTYPIC EVOLUTION IN OTHER SYSTEMS

A programme of using TEM to resolve patterns of congruence extends the utility of similar tools in two other well-studied systems, those of the nematode vulva and the male tail. These two models have also shown that homology of individual cell components can often not be interpreted by position or gross morphology alone. Sophisticated imaging tools, combined with considerable genetic and experimental research, have attested the importance of developmental positions of cells and the nuances of cell contacts in identifying homologies. These models have suggested the potential of reconstructing individual cells to address puzzling issues of taxonomically and ecologically significant head characters.

Comparative analysis of the vulva model demonstrates that component cells of the vulva apparatus are generally constant across Rhabditida, although different processes of cell signalling, patterning and fusions have arisen throughout the order to achieve this result (Kiontke et al. 2007). Thus, in spite of divergent induction pathways, the locus of homology for vulva development is considered to be among a conserved set of precursor cells (Sommer 2008). The example of the male tail, which, in contrast to the vulva (essentially a tube), is highly variable among nematodes, has revealed a similar conservatism: the many configurations of the male tail in Rhabditomorpha are formed from a consistent set of four to five epidermal cells (Hyp7–11). Knowledge of these component cells has been essential for identifying homologies of individual caudal rays (i.e. sensory papillae) anchored therein (Fitch & Emmons 1995; Fitch 1997, 2000), which were previously misunderstood based on interpretation from light microscopy observations. In both model systems, detailed TEM reconstruction in C. elegans was necessary to understand the complexity of cell-to-cell relationships in spatial topology and intercellular junctions for communication throughout development (Nguyen et al. 1999; Sharma-Kishore et al. 1999).

8. CONCLUSIONS

A detailed look at a small number of characters, such as the individual cells underlying the feeding and sensory structures of Rhabditida, reveals that, at a basic level, morphology reflects phylogeny derived from independent, molecular data. Seemingly irresolvable issues of incongruence apparent from gross morphology are understood by closer analysis of key characters. Study of the same characters has likewise provided new insight, possible only in the context of a robust phylogenetic framework, into the evolution of a parasitic phenotype. Although benefits peculiar to the study of nematodes as models may be absent in other taxonomic groups, analogous hypotheses of character change can still be tested by the same principles. For example, the lack of eutely (cell constancy) in many organisms may preclude developmental similarity in homology criteria in the study of individual cells, although cell or tissue topology regardless of ontogenetic lineage may still serve to pinpoint the locus of homology (de Beer 1971; Wagner & Misof 1993; Hall 1994; Bolker & Raff 1996; Müller 2003). Even between nematodes with divergent but determinate cell lineages, lineally non-homologous cells are in some cases locally induced to form tissue with comparable cell composition (Houthoofd et al. 2003; Houthoofd & Borgonie 2007). Similarity is still manifest as an end product.
The possibility of gathering hundreds to thousands of genes for multiple taxa offers bright prospects for phylogenetic research, but it need not be seen as precluding the importance of morphology. Molecular phylogenetics provides an honest test of classical characters. This unprecedented depth of insight is invaluable; combined with new tools, and in some cases new model organisms, it makes possible the study of characters in ways that were inconceivable in the recent past. With the benefit of an independent framework, morphology can be re-examined for real homologies and thus more clearly define processes of morphological adaptation. In such an approach, inference of phylogeny and inference of evolutionary processes are derived from independent information sources. The perceived problem of incongruence is not inherent to morphology itself, but to the interpretation and coding of characters, their historical momentum and the values idiosyncratically placed upon them. As demonstrated for nematodes of Rhhabditida, morphological systematics should not be limited to an emphasis on numerical analysis that fails to establish credible primary homologies. Given the explicit confrontation of morphology, the qualitative study of morphology will undoubtedly retain an irreplaceable role in future research programmes.

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