COMMENTARY

Taking a microscale look at symbiotic interactions–and why it matters

Thomas C. G. Bosch[a,1]

In 1881 the first significant work on earthworm behavior and ecology was published (1). It was Charles Darwin´s last scientific book and is still a fascinating and rewarding read. In PNAS, Geier et al. (2) use an earthworm taken directly from nature to present a method that allows in situ visualizations of micrometer-scale physical and chemical interactions between the worm and all its symbionts and parasites. In between the two publications lies the realization that animals evolved in a microbial world (3), that symbiosis appears as a general principle in eukaryotic evolution (4), and that animals function as metaorganisms (5–7). Microbial symbionts influence virtually all aspects of eukaryote biology (3, 8), but what maintains the balance between symbionts and their hosts? Also, where exactly in the animal body do these interactions take place? Surely the answer lies in a precise and spatially controlled cell-to-cell communication between the partners (9).Geier et al. (2) report a technique that might help to answer these questions, potentially allowing us to discover the "vocabulary of symbiosis," i.e., the metabolites exchanged between the host and the symbionts in the context of the local spatial organization of the animal. The method termed "CHEMHIST" combines microcomputed tomography (μCT) and matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI). It allows metabolite imaging in a defined part of a given host animal down to submicrometer spatial resolution.

The conventional approach for imaging an animal's three-dimensional (3D) histology is X-ray μCT. The technique allows one to obtain 3D images of a sample, including its internal structure, without the need for destructive sectioning. μCT, for example, made it possible to visualize the musculature and locomotory system in velvet worms in unprecedented detail (10). μCT datasets can be combined with light microscopic histological observations (11) and electron micrographs (12, 13). Such multiscale studies put functional and structural observations on a histological or subcellular scale into the larger spatial context provided by

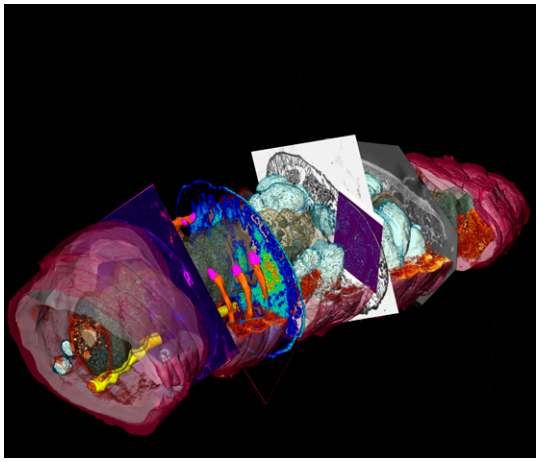

Fig. 1. A chemo-histo-tomography portrayal of an earthworm. The multimodal 3D imaging atlas of the posterior end of an earthworm was composed of five μCT datasets and four tissue sections which were each imaged with chemical, fluorescence, and bright-field microscopy and inserted between the 3D μCT datasets. Image credit: Benedikt Geier (Max Planck Institute for Marine Microbiology, Bremen, Germany).

μCT (14). With regard to symbiotic partnerships, functionally they are established and maintained as metabolic partnerships (15). Therefore, and together with other "omics" data, the investigation of the metabolites produced and exchanged in symbiotic interactions is an essential part of symbiosis research. Common bioanalytical techniques for a subcellular view of host–microbiome metabolite exchange include MALDI-MSI and nanoscale secondary ion MS techniques (16). In contrast to μCT, all MS techniques require tissue sectioning and do not allow nondestructive imaging.

Geier et al.'s (2) innovation is to combine high-resolution tomography and metabolite imaging in a single workflow. The authors used a tiny earthworm isolated directly from nature to demonstrate that the method is culture-independent and allows one to connect microhistology and localization of symbionts to

[a]Zoological Institute, Kiel University, 24118 Kiel, Germany

Author contributions: T.C.G.B. wrote the paper.

The author declares no competing interest.

See companion article, "Connecting structure and function from organisms to molecules in small-animal symbioses through chemo-histo-tomography," 10.1073/pnas.2023773118.

[1]Email: tbosch@zoologie.uni-kiel.de.

Published July 27, 2021.

the underlying metabolites. Choosing an earthworm was motivated by the ecological and evolutionary importance of these animals and the many gaps in our knowledge of them as metaorganisms. From a technical point of view, in a single workflow the technique involves snap-freezing the animal, cutting it in large pieces, recording the 3D anatomy from the tissue blocks with μCT, imaging the distribution of metabolites from corresponding tissue sections, and reconstructing a 3D model as an "atlas" to guide the high-resolution measurements. The composed atlas (Fig. 1) allows an interactive exploration of the animal's anatomy, associated microbes, tissue parasites, and the underlying metabolome of each partner in 3D.

In this pilot study (2) the authors located metabolites in individual host organs including an annelid-specific energy storage metabolite in the musculature and pigment protoporphyrin in the epidermis. Even more excitingly, the imaging technique in combination with fluorescence in situ hybridization microscopy allowed screening for metabolites that spatially correlated to colonizing bacteria. Also unexpectedly in this specimen taken fresh from nature, the authors also stumbled on very small parasitic worms encysted in the earthworm tissues. In combination with metagenomics sequencing the authors found that the small parasites were surrounded by a platelet activation factor. This phospholipid was first described by its ability to cause platelet aggregation and now is known as a potent mediator of inflammation and allergic responses. The encysted parasites, on the other hand, were found to contain a high level of the aliphatic polyamine spermidine. Spermidine is associated with a number of antiinflammatory actions, which may help to protect the parasites against reactive oxygen of the earthworm.

Although the underlying technologies (μCT and MALDI-MSI) are well established, the combination of both techniques in a single workflow in an uncultured animal is, to our knowledge, unprecedented. Geier et al.'s work (2) allows one to visualize molecules across tissues and microbial communities by combining spatial metabolomics with MSI at microscale resolution. The study represents a fine example of expanding to new areas with an interdisciplinary collaboration of ultrahigh-resolution imaging specialists, zoologists, and a marine symbioses expert.

Additional steps now need to be included in the workflow. Symbiotic partnerships are metabolic in nature but at the same time very dynamic. Different metabolites will be produced at different times of colonization and life history of the host. Analyzing and quantifying the metabolite exchange at temporal and spatial scales will enhance not only our understanding of the influence of microbes on host ecology and fitness but also the response of these symbioses to environmental perturbations. Since this imaging technology can be applied to many small model organisms across the tree of life (17, 18), it shows great promise. CHEMHIST therefore provides a key step forward in concretizing the cellular dialogue between symbionts and hosts, accelerating the path to a holistic understanding of metaorganisms and seeing symbiosis whole. The discovery also shows that we need to learn once more how to look at animals and points to yet more secrets waiting to be uncovered. How excited Charles Darwin would have been if he had found out that the earthworm's behavior might be the result of such complex multiorganismic interactions.

Acknowledgments

T.C.G.B.'s research is supported by the Deutsche Forschungsgemeinschaft, Collaborative Research Centre (CRC) 1182 "Origin and Function of Metaorganisms," and CRC 1461 "Neurotronics: Bio-Inspired Information Pathways" (Project ID 434434223 – SFB 1461). I appreciate support from the Canadian Institute for Advanced Research.

1 C. Darwin, *The Formation of Vegetable Mould Through the Action of Worms, with Observations of Their Habits* (William Clowes and Sons, Limited, London, 1881).
2 B. Geier *et al.*, Connecting structure and function from organisms to molecules in small-animal symbioses through chemo-histo-tomography. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2023773118 (2021).
3 M. McFall-Ngai *et al.*, Animals in a bacterial world, a new imperative for the life sciences. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 3229–3236 (2013).
4 A. E. Douglas, Symbiosis as a general principle in eukaryotic evolution. *Cold Spring Harb. Perspect. Biol.* **6**, a016113 (2014).
5 T. C. G. Bosch, M. J. McFall-Ngai, Metaorganisms as the new frontier. *Zoology (Jena)* **114**, 185–190 (2011).
6 D. Esser *et al.*, Functions of the microbiota for the physiology of animal metaorganisms. *J. Innate Immun.* **11**, 393–404 (2019).
7 C. Jaspers *et al.*; Consortium of Australian Academy of Science Boden Research Conference Participants, Resolving structure and function of metaorganisms through a holistic framework combining reductionist and integrative approaches. *Zoology (Jena)* **133**, 81–87 (2019).
8 S. F. Gilbert, J. Sapp, A. I. Tauber, A symbiotic view of life: We have never been individuals. *Q. Rev. Biol.* **87**, 325–341 (2012).
9 T. C. G. Bosch, M. C. Hadfield, "A dialogue that matters: Microbe–host interactions in animals and plants" in *Cellular Dialogues in the Holobiont*, T. C. G. Bosch, M. Hadfield, Eds. (CRC Press, 2020), pp. 287–290.
10 M. Müller *et al.*, Myoanatomy of the velvet worm leg revealed by laboratory-based nanofocus X-ray source tomography. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 12378–12383 (2017).
11 C. P. Geffre *et al.*, Combined micro CT and histopathology for evaluation of skeletal metastasis in live animals. *Am. J. Transl. Res.* **7**, 348–355 (2015).
12 S. Handschuh, N. Baeumler, T. Schwaha, B. Ruthensteiner, A correlative approach for combining microCT, light and transmission electron microscopy in a single 3D scenario. *Front. Zool.* **10**, 44 (2013).
13 E. A. Bushong *et al.*, X-ray microscopy as an approach to increasing accuracy and efficiency of serial block-face imaging for correlated light and electron microscopy of biological specimens. *Microsc. Microanal.* **21**, 231–238 (2015).
14 J. D. B. O'Sullivan *et al.*, X-ray micro-computed tomography (μCT): An emerging opportunity in parasite imaging. *Parasitology* **145**, 848–854 (2018).
15 M. Hamada *et al.*, Metabolic co-dependence drives the evolutionarily ancient *Hydra-Chlorella* symbiosis. *eLife* **7**, e35122 (2018).
16 M. Hudspith *et al.*, Subcellular view of host-microbiome nutrient exchange in sponges: Insights into the ecological success of an early metazoan-microbe symbiosis. *Microbiome* **9**, 44 (2021).
17 T. C. G. Bosch, K. Guillemin, M. McFall-Ngai, Evolutionary "experiments" in symbiosis: The study of model animals provides insights into the mechanisms underlying the diversity of host-microbe interactions. *BioEssays* **41**, e1800256 (2019).
18 A. E. Douglas, Simple animal models for microbiome research. *Nat. Rev. Microbiol.* **17**, 764–775 (2019).