Reply to: Sources of C₃₀ steroid biomarkers in Neoproterozoic–Cambrian rocks and oils

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REPLYING TO G. D. Love et al. *Nature Ecology & Evolution* <https://doi.org/10.1038/s41559-019-1048-2> (2019)

Tracing the timing of animal radiation presents an important challenge in geobiology that some geochemists have approached using putative sponge diagnostic biomarkers^{1–3}. We have recently shown that rhizarian protists contain precursors to both known ‘sponge biomarkers’ (24-isopropylcholestane (24-ipc) and 26-methylstigmastane (26-mes)), thereby negating their diagnosticity for early animals⁴. Love and colleagues, supporters of a sponge interpretation^{1,2}, criticize these findings⁵.

Love et al.⁵ argue that low absolute and relative abundances of C₃₀ sterols in extant Rhizaria preclude rhizarian sources for Neoproterozoic C₃₀ steranes. The taxonomic value of a biomarker is, however, not determined by its absolute or relative abundance in source organisms, which may vary by orders of magnitude between closely related species and under varying growth conditions^{6,7}. What counts is the unique biosynthetic capacity of a defined organism group to produce such molecules, to the exclusion of other groups. We show that precursors to 24-ipc and 26-mes are produced by demosponges and rhizarians. Thus, ecological arguments are needed to evaluate the most plausible source of these compounds in the fossil record. In this respect, rhizarians clearly win, as discussed further below and in Nettersheim et al.⁴. Moreover, an abundance of 24-*n*-propylcholestane (24-npc) precursors (up to 20%) in *Allogromia laticollaris*⁸ demonstrates that Rhizaria principally have the ability to produce C₃₀ sterols in sufficient quantities to account for geological observations. Contrary to the assertion of Love et al.⁵, many Rhizaria exceed a 24-ipc/24-npc ratio of 0.5—a threshold arbitrarily constructed to demonstrate sponge origins to the exclusion of pelagophyte algal sources¹. Thus, neither the presence and abundance of 24-ipc or 26-mes, nor elevated 24-ipc/24-npc ratios, can be used as diagnostic demosponge markers.

Notably, the critique of Love et al.⁵ equally applies to demosponges, whose vast majority biosynthesizes only traces of C₃₀ sterols. More importantly, those that do tend to produce only one type of major C₃₀ sterol. This renders it hard to explain the similar abundances of all three C₃₀ steranes in many Neoproterozoic rocks²: large portions of the Neoproterozoic seafloor—including anoxic basins—

must have been covered by similar proportions of three particular sponge groups, each producing one of the C₃₀ sterols. This scenario is not particularly likely given that it is not even known whether Neoproterozoic sponges could produce these compounds at all. In contrast, lipid hydrogenates of many early-diverging Rhizaria yield similar abundances of all three C₃₀ steranes (Fig. 1a,c,d)—a pattern closely resembling that in Neoproterozoic–Cambrian rocks (cf. Fig. 1a in ref. ²). Moreover, rhizarians can be highly abundant in most marine environments, including surface waters of anoxic basins. Thus, while sponges may have contributed minor proportions of C₃₀ steranes to Neoproterozoic sediments, Rhizaria are by far the more likely source.

Love et al.⁵ question the correct identification of compounds and imply that our C₃₀ sterane data cannot be reproduced. We provided two rhizarian lipid samples to Love and colleagues. Their independent analysis confirms the presence of the C₃₀ steranes 24-npc and 24-ipc in *Amphistegina lobifera* (Fig. 1c in ref. ⁵)—an observation that should suffice to question the sponge interpretation of C₃₀ steranes. However, presumably due to lower instrument sensitivity, they were unable to detect the three sponge steranes in the second sample (*Chlorarachnion reptans*), and claim that cercozoan C₃₀ steranes are unlikely to be genuine. Yet, the failure to detect these compounds⁵ does not imply that they are absent. Figure 1a shows that all three C₃₀ steranes are detected by our instrument in the exact sample provided to them—before it was sent and after it was returned. Our identifications are based on elution patterns compared with reference samples, co-injection experiments on two different gas chromatography columns and mass spectra (for example, a diagnostic mass spectrum of 24-ipc in Cercozoa in our Fig. 1b). Identification of cercozoan 26-mes was now additionally corroborated by co-injection with a sponge pyrolysate provided by Love et al. (Fig. 1c), confirming that the identification of C₃₀ steranes in Rhizaria extracts is robust.

Love et al.⁵ build on RNA-derived expressed sequence tag data⁹, which are unlikely to represent full enzymatic capabilities, to argue that two sterol methyltransferase (SMT) gene homologues

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Fig. 1 | C_{30} steroids in Rhizaria. **a**, The same reference sample that we provided to Love and colleagues was analysed by us before shipping and after its return. The repeated detection of all three putative sponge biomarkers before and after the analyses at UC Riverside and Massachusetts Institute of Technology (MIT) clearly confirms the presence of these analytes in the reference sample and suggests that differing mass spectrometric sensitivity is the source of the discrepant observations. Middle trace taken from Love et al. (Fig. 1a in ref. ⁵) and aligned with our analyses based on 26-mes elution positions (grey shading). Note that the sample was transferred and repeatedly analysed by Love and colleagues. Accordingly, only an unknown fraction of the original samples was returned to our laboratory—yet all steranes were still detectable, albeit in lower abundances. Signal intensities are shown on the right side above chromatographic traces. **b**, Full-scan mass spectrum of a 24-ipc chromatographic peak corroborates its identity, as previously established by co-injection experiments⁴. **c**, A cercozoan hydrogenate (grey) was repeatedly co-injected (in different relative concentrations) with a sponge pyrolysate provided by Love and colleagues, confirming the presence of 26-mes in cercozoan extracts. **d**, Full chromatographic trace (lower) of the C_{30} chromatogram shown in Fig. 2 of Nettersheim et al.⁴ (box) shows that all relevant aspects were included in the original figure. The unequivocal presence of all three putative sponge biomarkers in rhizarian hydrogenates (shown here for three different species: *C. reptans* (**a**), *Partenskyella glossopodia* (**c**) and *B. natans* (**d**)) refutes the concern of Love et al.⁵ that cercozoan C_{30} steranes reported by Nettersheim et al.⁴ could be non-genuine signals. The data negate the alleged sponge diagnosticity of 24-ipc and 26-mes as originally reported.

in *Bigelowiella natans* are insufficient for C_{30} sterol production. Yet, they acknowledge C_{30} sterol production in the foraminifera *A. laticollaris*⁵, confirming the existence of the required genetic toolkit. The presence of only two SMT gene homologues in Rhizaria indicates that the relationship between SMT copy number and sterol side chain alkylation must be inherently more complex than assumed⁵—as is, in fact, also observed in demosponges that possess promiscuous SMTs¹⁰.

While Love et al.⁵ tend to overlook ecological arguments against a sponge origin of Proterozoic steranes, they provide one pertinent observation. Three rhizarian cultures were axenic phototrophs⁴ or grown on a bacterial diet⁸ and hence incontrovertibly produce C_{30}

sterane precursors. These compounds could however have a dietary origin in other species. If correct, then common additional sources of 24-ipc and 26-mes should be discovered in the future among rhizarian food sources, such as algae. Clearly, this would further reduce the diagnostic value of these compounds. For now, rhizarian protists remain the most plausible biological source of Neoproterozoic C_{30} steranes.

Data availability

All relevant data are shown in Fig. 1. Chlorarachniophytes can be sourced from the Bigelow NCMA culture collection (<https://ncma.bigelow.org/cms/index/index/>: CCMP 239, 3166 and 2755).

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Author contributions

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Competing interests

The authors declare no competing interests.

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