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Relative symbiont input and the lichen symbiotic outcome

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The term symbiosis was first used in biology to describe the ‘living together’ of fungi and algae in lichens. For much of the 20th century, the fungal partner was assumed to be invested with the ability to produce the lichen body plan in presence of a photosynthesizing partner. However, studies of fungal evolution have uncovered discordance between lichen symbiotic outcomes and genome evolution of the fungus. At the same time, evidence has emerged that the structurally important lichen cortex contains lichen-specific, single-celled microbes, suggesting it may function like a biofilm. Together, these observations suggest we may not have a complete overview of symbiotic interactions in lichens. Understanding phenotype development and evolution in lichens will require greater insight into fungal–fungal and fungal–bacterial interplay and the physical properties of the cortex.

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Introduction

Of all the cellular multikingdom symbioses, lichens maintain a unique status. In most symbioses, a single organism acts as structural scaffold: plant roots without mycorrhizae still resemble roots; a cicada without its bacterial endosymbionts still resembles a cicada, though it may be dead; a *Euprymna* squid without *Vibrio* bacteria is still a squid. Lichens however, which contain at least a filamentous fungus and a single-celled algal or cyanobacterial partner (photobiont), have no *a priori* scaffold: neither, in isolation, forms anything resembling a lichen, nor do any of their known relatives or ancestors [1]. They self-construct and self-replicate characteristic body plans, generation over respective symbiont generation. Lichens are basically interactomes that you can roll around in your hand (Box 1).

How lichens achieve their characteristic thallus forms has been a major source of disagreement among lichen researchers since the discovery that they are a chimera [2]. At one end of the spectrum is the view, first espoused by Anton de Bary, that lichens are a mutualistic consortium with properties acquired only through cooperation of the symbionts [3]. At the other end is the view that the fungal symbiont parasitizes the photobiont and determines the lichen growth form [1,2]. According to this view, lichen traits are fungal traits, and each lichen has a distinct fungus which forms it (it was implicitly recognized early on that photobionts were promiscuous with respect to the lichens they occurred in [2]). Early experimenters sought to determine the roles of the symbionts by attempting to resynthesize the lichen *in vitro*, but found them recalcitrant to forming anything that looks like a natural lichen [4,5]. Nonetheless, incipient *in vitro* and wild symbioses always exhibit traits never produced in isolated fungal symbionts. To distinguish between symbiotic outcome and isolated symbiont, one of the most successful early experimenters began referring to the lichen and its fungal partner with separate nomenclature [6]. Systematists however pushed back. Citing contemporary practice but no actual biological evidence, they blocked the development of parallel nomenclature [7]. In 1950, the *Code of Botanical Nomenclature* was amended to anchor the name of the lichen to the fungus (Figure 1a). The move was not without criticism: the Italian mycologists Raffaele Ciferri and Ruggero Tomaselli warned that some fungi may occur in more than one lichen species [8], but struggled to find examples to prove this. Other workers similarly warned that traits such as secondary metabolite profiles occurred only in the symbiotic state [9]. Nonetheless the change to the Code and its inherent assumptions were upheld and since then whole lichens have technically remained nameless [10,11••].

It is hard to overstate the effect that the definition of lichens as fungi has had on the study of the lichen symbiosis. Because naming something is everything in a taxonomy-driven discipline, the rule change incentivized the study of the fungal partner and led to much work on its sexual reproduction and molecular evolution. Today, most lichen biologists assume a single symbiont — one fungus — is invested in its DNA with the ability to generate the lichen body plan in presence of

Box 1 The origins and diversity of lichen symbioses.

Fungi enter into several important nutritional symbioses with plants and animals. The two main kinds of symbiosis, in terms of the numbers of species involved, are lichens (approaching 20 000 species [68]) and mycorrhizal associations with vascular plants (about 50 000 species [69]). Lichens are a diverse group of fungal-algal symbioses that evolved multiple times independently within both large divisions of Fungi, the Ascomycota and Basidiomycota, together with photosynthesizing partners from a wide range of chlorophytes and cyanobacteria. Together, two or more partners form a thallus, in which one or more fungi are quantitatively dominant over the photosynthesizing partner(s).

Lichens are thought to have arisen in early terrestrial life from a biofilm-like association of fungi, cyanobacteria and other microbes [70]. A majority of lichen species, the microlichens, form crusts closely molded to the shape of their substrate. The lichen association also gave rise on multiple occasions to novel, three-dimensional architectures adapted to optimize photosynthesis, unique for a fungal-dominated organism [71••]. These 'macrolichens' include foliose lichens, which form dorsiventral structures superficially similar to a plant leaf, and fruticose lichens, which encompass both phototropic, radially symmetrical, shrub-like lichens, as well as hair lichens, which are composed of thin thallus filaments that hang from tree branches. Some lichens, such as members of the species-rich cosmopolitan genus *Cladonia*, are dimorphic, with leaf-like basal squamules bearing complex, tower-like or shrub-like structures called podetia. How lichen symbionts went from inhabiting biofilms to acquiring self-replicating architectures is one of the unsolved mysteries of lichen evolution. To achieve a specific three-dimensional motif, fungal and algal cells form differentiated meristem-like regions [71••], termed pseudomeristems [4], in which rapidly dividing fungal and algal cells are aggregated. In branching and anastomosing lichen thalli, the location and shape of these aggregations determine branching pattern, and increasing thallus size is supported in turn by diffuse or intercalary growth [71••,72]. However, as discussed in this review, it is unlikely thalli would maintain rigidity and their characteristic shapes without an extracellular polysaccharide layer that cements the fungal 'wadding' in place, with torsion and flexibility of its own.

a photobiont [12]. This is more often implicit in the annotation of fungal trees with lichen traits (something of which I am also guilty), than stated explicitly. The intensive study of fungal evolution, in particular with DNA sequencing, has provided valuable lines of evidence that now allow us to test this assumption.

Studies of the lichen species as fungal species

The first published DNA sequences of lichen fungi were used to demonstrate that two different lichens could be formed by the same fungus [13]. However, this was considered to be a curio induced by photobiont switching, and subsequent studies showed most similar lichens did indeed cluster according to their fungal DNA. As more data became available, questions shifted from broad-scale relationships to delimitation of individual lichen species. Sampling intensity and phylogenetic resolution has varied widely, but broadly speaking, three types of results have been obtained (Figure 1b). For the purpose of discussion, lichen species here refer to long-standing morphological or chemical circumscriptions, and fungal species refer to entities delimited from multi-locus molecular data.

(Result 1) Lichen phenotypes are reflected in fungal gene evolution. This kind of result has been taken as support of fungal concordance [14]. It is frequently obtained for crust lichens, which form only thin, substrate-hugging body plans and are predominantly classified according to fungal traits. However, species-level concordance has also been reported for lichens with three-dimensional architecture (macrolichens) such as *Cladonia* [15], often alongside reports of non-concordance for other species (see below).

(Result 2) More fungal genetic species can be distinguished than there are matching lichen phenotypes. In many cases, the sequence data are only part of a process of discovery of traits with which the species can later be distinguished [16–18]. In others, no distinguishing characters are found and the resulting taxa are called cryptic species [19].

(Result 3) More lichen phenotypes exist than are reflected in fungal gene evolution. This phenomenon — which I will call phantom phenotypes — is most common among macrolichens. In some cases, lichen species that have been distinguished since 1810 have been found to be formed by the same fungal species, despite differing in secondary chemistry, ecology, geographic range and thallus traits [20–25]. Sometimes the phylogenetic pattern is messy, lending no support for a connection of evolution to phenotypes [26–29].

For many lichen biologists, the third set of results has been the least intuitive, because it requires downweighting or disregarding well-documented traits, ecologies and geographic ranges. Though most recognizable at the leading edge of evolutionary differentiation — speciation — discordance with lichen phenotypes is evident throughout fungal evolution in the form of wholesale body plan changes between sister lineages [30,31]. One by one, workers have reassessed 'taxonomic value' of anatomical traits [32] and secondary metabolite chemistry [27] in favor of traits that match clades [33,34]. Not accepting that obvious phenotypes are unsupported by fungal phylogenies, at least one group has flipped the script, searching for loci that form clades matching the phenotypes [33]. In recent years, many workers have begun referring to lichen-forming fungi as opposed to lichens, but still map traits that only exist in the symbiotic state to a fungal tree [34,35]. The morphological discordance crisis is not subtle: it recently led Lumbsch and Leavitt to ask if the era of lichen morphology is over altogether, using the title 'goodbye morphology?' [36••].

Applying new tools to the lichen symbiosis

When the Code of Nomenclature was amended to define the lichen as identical with its sole fungus, the structure of