

# Sex in the extremes: lichen-forming fungi

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Lichens are characteristically found in environments subject to extremes of temperature, desiccation and low nutrient status. Despite this sexual structures are often formed in abundance. The underlying mechanisms of sex in lichen-forming fungi are discussed, together with possible ecological reasons for the persistence of sexuality. Special features of lichen sex are highlighted including sex at the limits of life on earth in Antarctica, re-lichenization following sex and dispersal, and the perennial nature of lichen fruiting bodies.

**Keywords:** lichen, fungi, sex, breeding system, symbiosis, extreme environments, Antarctica

## **Lichens - living together in a long-term relationship**

Lichens are seen as a textbook example of a successful mutualistic symbiosis. They consist of at least two organisms: a fungus (the 'mycobiont'), and an intimately associated photosynthetic partner (the 'photobiont'). The photobiont can be either a green alga or a cyanobacterium, and some lichens contain both. The symbiosis is usually between specific mycobiont and photobiont partner species. The mycobiont typically forms the bulk of the lichen and encloses, or even penetrates, the photobiont cells (Dobson, 2003; Purvis, 2000). The physiological interactions that occur between the partners result in a new distinct entity termed the lichen 'thallus', the morphology of which is determined, in the majority of cases, by the mycobiont (Büdel & Scheidegger, 1996). Therefore, by convention the name given to the lichen 'species' is that of the fungal component. The symbiotic relationship is obligatory for the lichen-forming fungus to complete its life-cycle, whereas the algal and cyanobacterial partners are often able to exist independently (Ingold & Hudson, 1993). Lichens are long-lived and extremely slow-growing with some thalli sustaining growth for hundreds or even possibly in excess of thousands of years (Honegger, 1996).

Lichen-forming fungi constitute almost one fifth (19%) of all known fungal species with the majority

(98%) belonging to the Ascomycotina (Kirk *et al.*, 2001). They display a variety of morphologies, from flattened crust (crustose) or leafy (foliose) forms to shrubby or pendulous fruticose types (Honegger, 2001) (Figs 3, 4, 7, 8).

## **Life in extreme environments**

A key characteristic of lichens is that they have a remarkable ability to tolerate extreme environmental conditions and sustain growth despite frequent cycles of desiccation and rehydration, low nutrient availability and large daily and annual fluctuations in temperature (e.g. Øvstedal & Lewis-Smith, 2001; Nash, 1996). They thrive on soil-less substrata such as the surfaces of rocks or tree bark, and on poorly developed soils such as those of heathlands, peatlands, sand dunes and toxic spoil heaps (e.g. Gilbert, 2000; Dobson, 2003). Lichens are found globally and it has been estimated that they comprise the dominant components of vegetation on 8% of the Earth's terrestrial surface (Larson, 1987).

The resilience of lichens is emphasized by their success in some of the most extreme environments on Earth: from the freezing boreal-Arctic, alpine and Antarctic regions, to hot arid deserts (e.g. Purvis, 2000). For example, lichens dominate the terrestrial 'vegetation' of ice-free areas of Antarctica, mostly in coastal regions (Figs 1-4) (Øvstedal & Lewis-Smith, 2001). Here thalli can be exposed to a diurnal amplitude in temperature in excess of 40°C, and an annual difference of perhaps 60-70°C (Øvstedal & Lewis-Smith, 2001; Purvis, 2000). Lichens have even

been found in the heart of continental Antarctica as far as 86° South on exposed rocky nunataks and in dry valleys; precipitation in the latter areas is light, falling exclusively as snow (c. 150 mm y<sup>-1</sup> rainfall equivalent) and much of it is lost by sublimation due to low relative humidity so that little free liquid water becomes available (Longton, 1988; Purvis, 2000; Øvstedal & Lewis-Smith, 2001). At the other extreme, lichen dominated communities are found in some of the world's hottest desert environments where daytime temperatures soar to 55°C and night temperatures may fall below freezing (Nash, 1996) e.g. rich lichen 'fields' occur in the coastal Namib Desert in south west Africa, the most spectacular of which are dominated by the yellow fruticose *Teloschistes capensis* (Fig 5). Even in temperate habitats, lichens may experience temperatures in excess of 70°C, considerably above the ambient air temperature.

What is more remarkable is that even in these hostile environments many lichens produce sexual structures, often in abundance (Figs 3, 4, 8). This is perhaps surprising, as there are metabolic costs to sex and many of these environments are subject to low biotic pressure, which might be predicted to favour vegetative/asexual reproduction. Examples of sexual reproduction include communities of *Acarospora*, *Buellia* and *Lecidia* species in the Antarctic dry valleys which grow *within* rocks (termed cryptoendolithic lichens), the only visible structures being apothecia produced on the rock surface (Purvis, 2000), and most species in hot desert lichen communities, including *Teloschistes capensis*. Thus lichen-forming fungi provide fascinating examples of 'extreme sex'! Of course it must be remembered that lichen thalli are invariably desiccated and physiologically inactive when exposed to high temperatures and that growth and physiological activity, including reproduction, occur in hydrated thalli at usually moderate to low temperatures. Nonetheless, the preponderance of sexual reproduction in lichens raises questions such as how do they have sex, what are the reasons for sexuality in harsh environments, and does 'lichen sex' have any special characteristics?

### Reproduction in Lichens

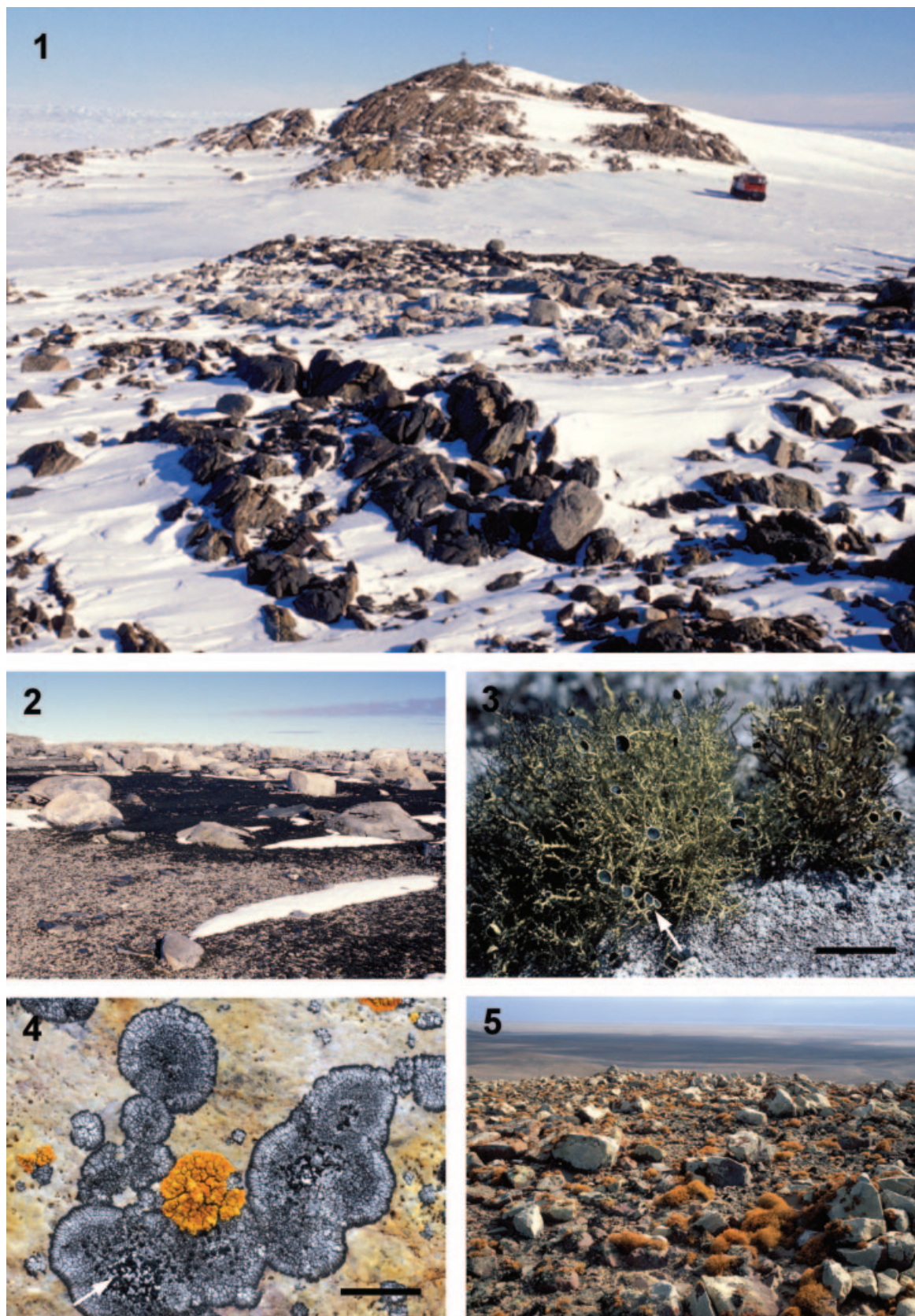
Being dual organisms, reproduction and dispersal of lichens present challenges since both partners have to be present for the successful development of a new lichen thallus (Dobson, 2003). Evolution has provided one solution in the form of vegetative propagules such as 'isidia' and 'soredia', which contain both the fungal and photosynthetic partner. Soredia typically consist of

photobiont cells enclosed in a loose network of hyphae; these structures can develop diffusely over the surface of the thallus, or in specialized areas called soralia. Isidia are small, smooth, cylindrical peg-like structures that project outwards from the outer cortex of the thallus. Soredia and isidia can be dispersed by wind, rain or small animals (Büdel & Scheidegger, 1996; Dobson 2003; Purvis, 2000). In the short term, vegetative propagules have the advantage that both the mycobiont and the photobiont are dispersed simultaneously and so can rapidly establish a new thallus (Ott, 1987a, b). However, the most commonly produced lichen propagules are sexually derived ascospores that are exclusively fungal in origin. For example, an analysis of the 1350 lichen species listed by Purvis *et al.*, (1992) in *The Lichen Flora of Great Britain and Ireland* reveals that 90% produce ascomata containing sexually derived ascospores, whereas only 29% form symbiotic vegetative propagules. Whilst ascospores might be effective in long distance dispersal of the fungus, as lichen propagules they have the inherent problem that they are not normally packaged with a photobiont and so chance encounter with a suitable photobiont is necessary in order to reestablish the symbiosis (see later). Only a very few lichens, such as *Staurothele* species, carry algal cells in their fruiting bodies and eject these together with fungal spores (Purvis, 2000). Thus, vegetative and sexual reproductive strategies appear to serve two complementary functions: a means of rapid spread of locally-adapted genotypes in a given local environment via asexual propagules, and long-distance dispersal of potentially novel genotypes via sexually derived ascospores.

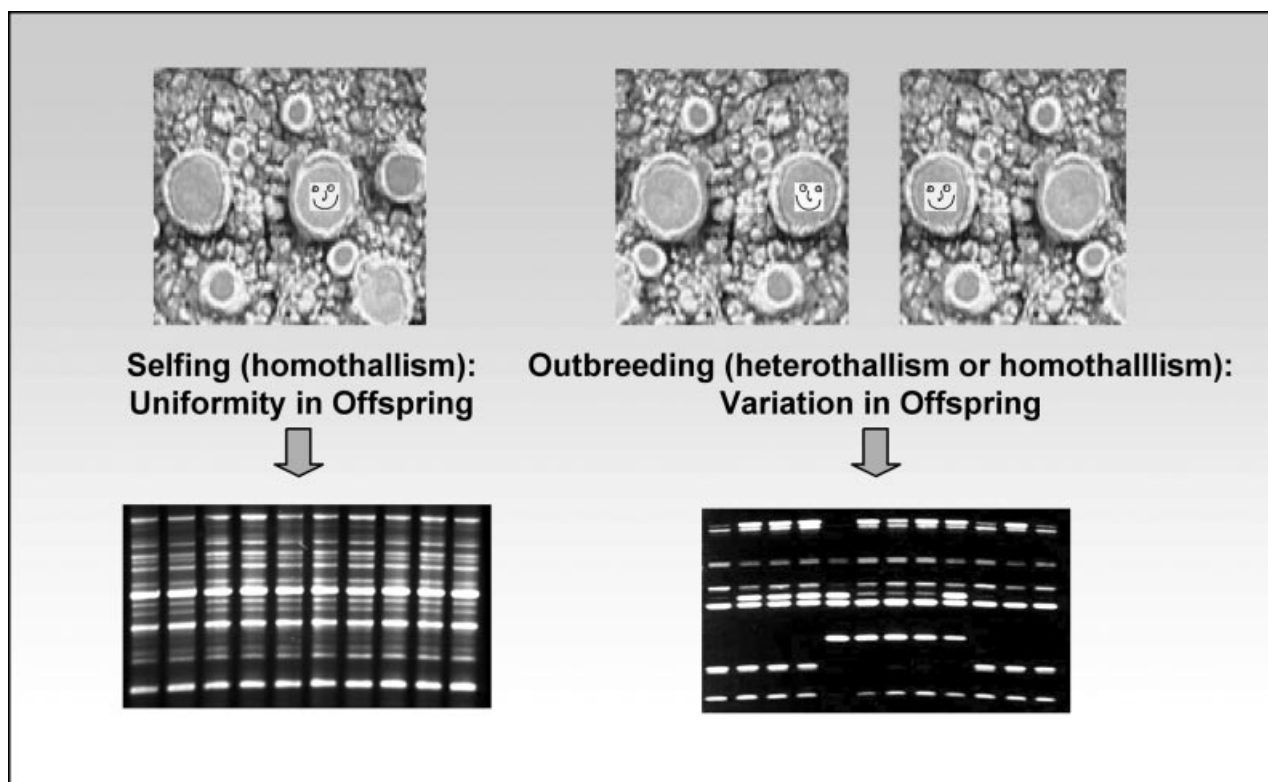
Anatomical details of fruit body development (mainly apothecia and perithecia) have been studied in lichens (Jahns *et al.*, 1995) but have offered little information about underlying genetic mechanisms. The nature of sex in lichen-forming fungi therefore remains poorly understood. For example, do lichens have any special adaptations for 'extreme sex'?

### Breeding systems in lichen-forming fungi

As in ascomycete fungi in general, sex in lichen fungi can occur via one of two principal breeding systems. These are **heterothallism** in which individuals are self-sterile and there is a requirement for a compatible partner of different mating type for sexual reproduction to occur, and **homothallism** in which individuals are self-fertile, although they may retain the ability to mate with similar genetic strains (Dyer *et al.*, 1992). Inherent experimental difficulties, such as very slow growth



**Fig 1** Rock outcrops of the Haupt Nunataks, Wilkes Land, Antarctica, on which sexually reproducing lichens occur. **Fig 2** Rocky fell-field near Casey Station (Wilkes Land), Antarctica, with dense cover of the black fruticose lichen *Usnea sphacelata*. **Fig 3** Fruticose thalli of *Usnea aurantiaco-atra* in the Falkland Islands, bearing disc-shaped apothecia (arrowed). Scale bar = 3cm. **Fig 4** Crustose thalli of *Buellia frigida* (grey-black) and *Caloplaca* spp. (cf. *C. saxicola*, orange) on Antarctic rocks bearing apothecia (arrowed). Scale bar = 2 cm. **Fig 5** The fruticose lichen *Teloschistes capensis* (orange) on a rocky ridge top (c. 150m altitude) in the coastal Namib Desert, Namibia.



**Fig 6** Rationale for use of DNA fingerprinting techniques to determine sexual breeding systems in lichen-forming fungi.

rates, and the failure to induce sex *in vitro* have meant that little is known about the nature of sex in lichen-forming fungi. However, recent studies taking advantage of advances in axenic culture of mycobionts and DNA analyses, are now providing insights into lichen sex for the first time.

Initial studies on gene flow in lichens were performed using chemical markers. Lichens produce a diverse array of secondary metabolites, some of which have been used to distinguish 'chemotypes' – chemical forms of a species that may be correlated with distinct geographical and ecological distributions (Culberson & Culberson, 1967; Elix, 1996). In a pioneering study, Culberson *et al.* (1988) attempted to elucidate the sexual cycle in *Cladonia chlorophaea* using chemical markers. Earlier investigations on North American populations of *C. chlorophaea* had distinguished 14 distinct chemotypes, which were interpreted as sibling species (Culberson, 1986). Culberson *et al.* (1988) analyzed secondary products in progeny of individuals of *C. chlorophaea* taken from populations of mixed chemotypes. This produced evidence of occasional interbreeding between some chemotypes that grew together in the same lichen mat while other chemotypes appeared to be reproductively isolated in spite of physical proximity. Similar methods were used to investigate the breeding system in *Ramalina siliquosa*, a species complex in which six chemotype sibling

species were known; again evidence of occasional outcrossing was found (Culberson *et al.*, 1993). However, these results could be explained by both heterothallism, in which outcrossing is obligatory, and homothallism in which selfing predominates but outcrossing occurs occasionally.

More recently in our group, molecular genetic markers were used by Murtagh *et al.* (2000) to unequivocally determine the mode of reproduction in two species of crustose lichen-forming fungi: *Graphis scripta* (found on tree bark) and *Ochrolechia parella* (found on rocky sea shores). Random amplified polymorphic DNA (RAPD) fingerprint markers were generated for sets of single-spore sibling progeny isolated from the same apothecium. The resulting DNA fingerprint patterns within sets of progeny were found to be uniform, demonstrating homothallic reproductive systems in both species (Fig 6). It was also noted that ascospore progeny derived from different thalli were genetically distinct from each other, establishing that the lichen populations of *G. scripta* and *O. parella* were not clonal (Murtagh *et al.*, 2000; Dyer *et al.*, 2001).

A similar approach was used by Honegger *et al.* (2004), who investigated breeding systems in the lichen-forming fungal genus *Xanthoria*. A homothallic system was again found in the common cosmopolitan *X. parietina*, but a heterothallic system in five other species. Meanwhile, evidence for outcrossing and

recombination has also been reported in the literature for the lichenized fungi *Letharia gracilis*, *L. lupine*, *L. vulpina* (Kroken and Taylor, 2001; Högberg *et al.*, 2002) and *Lobaria pulmonaria* (Zoller *et al.*, 1999), using data derived from sequencing of specific DNA regions.

To investigate this topic further, we are examining lichen species from a wider range of environments, including both temperate and Antarctic habitats, in an attempt to discover which, if any, breeding system predominates. Results are so far available for three species of *Cladonia* (Fig 7): *C. floerkeana*, a pioneer species on nutrient poor soils such as those in heathlands; *C. portentosa*, a 'reindeer' lichen dominant at later stages of succession in heathland; and *C. galindezii*, a lichen with limited distribution on peaty soils primarily in the Antarctic (Purvis *et al.*, 1992; Øvstedal, 1988). These species were chosen for their contrasting ecologies and reproductive strategies, and because they belong to *Lecanorales*: the major lichen-forming fungal order (Seymour *et al.*, 2005). Using similar methods to those of Murtagh *et al.* (2000), DNA fingerprint markers (both RAPD and AFLP) were produced for sets of single-spore sibling progeny. However, this time spores from the same apothecium were consistently not genetically uniform, indicating a heterothallic breeding system in all three species (Fig 6). These findings were supported by results from a novel approach, using the presence of 'mating-type' (MAT) genes to identify the breeding system in operation. Sexual compatibility in filamentous heterothallic ascomycete fungi is determined by mating-type (MAT) genes, which act as the 'master regulators' of sexual development with isolates of compatible MAT-1 and MAT-2 genotypes required for sex to occur (Dyer *et al.*, 1992; Coppin *et al.*, 1997). A PCR approach was used to show that MAT-2 mating-type genes were found in only half of the sibling-spore progeny of all three species, indicating segregation of mating-type genes consistent with heterothallism.

Further work at the University of Nottingham is exploring the breeding systems of other Antarctic and temperate lichen species, including those in the genera *Buellia* and *Caloplaca* (Fig 4), and *Carbonia vorticosa*, a species that occurs at latitude 86°S at the southerly limits for life on earth (Øvstedal & Lewis-Smith, 2001).

### Significance of lichen sexuality

Given that sexual reproduction is a common feature of lichens in extreme habitats, it is intriguing to speculate why sex persists in such environments. At least three explanations can be put forward:

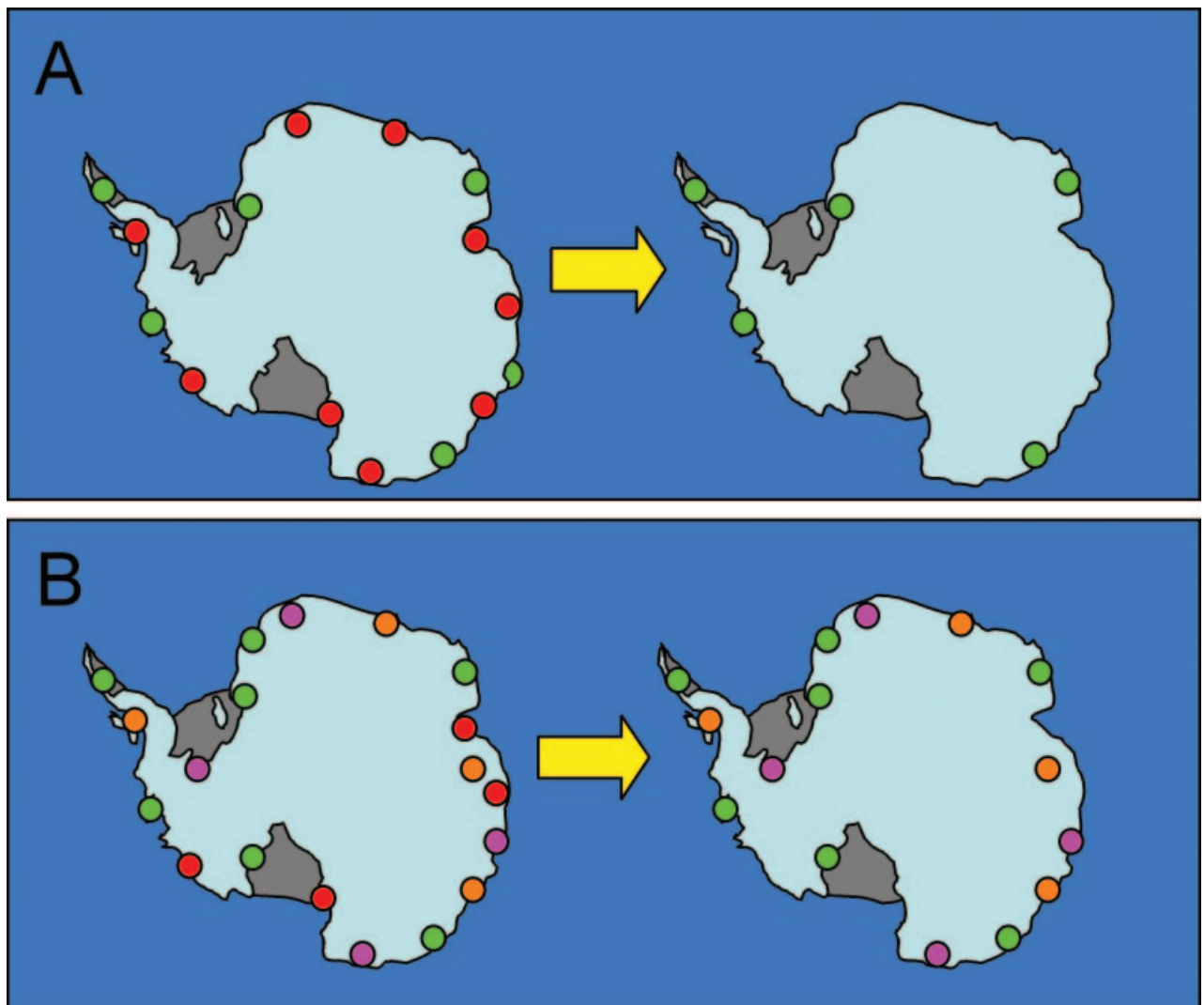
**Sex enables spore production for dispersal.** The

number of spores produced in asci of lichen-forming fungi is species dependent and ranges between one and a number in excess of 100, but is most frequently eight. Sexual spores are generally smaller than vegetative structures such as isidia and soredia so are likely to be dispersed over longer distances. They can also be produced in larger numbers with less metabolic investment per propagule. Dispersal might also be achieved by asexual sporulation, since conidia are produced by many lichenized ascomycetes. However, sexual spores may be preferred because they are more resistant to adverse environmental conditions, and can be stored safely within ascospores whilst awaiting conditions favourable for spore dispersal and germination (Dyer *et al.*, 1992). Indeed, the advantages of prodigious spore production might explain why some lichen-forming fungi exhibit homothallic breeding systems because such self-fertile species produce spores without the necessity to encounter a compatible partner. For example, contact with an isolate of the opposite mating type might be an infrequent event on an isolated nunatak in the Antarctic, or in new habitats such as recently exposed stonework or the bark of a young tree. Also, a single spore of a homothallic mycobiont dispersed into a new habitat could, following lichenization, produce ascospores without need for a mate and thereby establish a new resident population (Murtagh *et al.*, 2000). But then what of heterothallic species? Intriguingly, we have noted that in many Antarctic lichen species the contents of asci are regularly discharged as a unit of eight adhering spores. A similar observation was made by Honegger *et al.* (2004) regarding ascospore discharge in various *Xanthoria* species. Perhaps 'spore-clumping' is an adaptive strategy in extreme environments to ensure that individuals of both mating types are dispersed together, thereby facilitating sex and consequent spore production.

**Sex generates novel genetic diversity within populations.** To ensure the long-term survival and evolution of a species, there is normally a requirement for genetic diversity to be present in a population to facilitate response to environmental change by the selection of genotypes suited to, or able to tolerate, new abiotic or biotic pressures. Sexual reproduction involving heterothallism, and to a lesser extent homothallism, provides a mechanism for generating such genetic diversity within lichen populations. Little variation, associated with extensive clonal development and/or inbreeding, might place lichen populations at risk if a particularly widespread genotype was unable to withstand altered climatic conditions or compete with new strains of co-occurring organisms (Fig 9). The



**Fig 7** Podetia of *Cladonia coccifera* on peaty soil bearing apothecia (red). Scale bar = 1 cm. **Fig 8** The crustose lichen *Ophioparma ventosa* bearing abundant apothecia (reddish-brown). Scale bar = 0.3 cm.



**Fig 9** Importance of genetic variation in survival and evolution of species. Climate change in the Antarctic may lead to widespread loss of resident lichen populations if little variation is present and a dominant genotype (shown in red) is sensitive to change (A); whereas the presence of variation increases the likelihood that some genotypes (shown in green, orange, purple) will tolerate change and so there will be less impact (B).

impact of predicted climate change is a particular concern for the Antarctic environment, and we currently have studies underway to try to determine the extent of genetic variation present in resident lichen populations. Conversely, outbreeding runs the risk of breaking up favourable sets of genes. This might explain why some lichenized fungi have evolved homothallic breeding systems because this would preserve highly adapted (and successful) genotypes. This might be particularly relevant to extreme environments, which although highly stressful are often relatively stable and subject to low biotic pressure (Murtagh *et al.*, 2000). Therefore for particular species of lichen-forming fungi, the presence of a heterothallic or homothallic breeding system is likely to be the outcome of an evolutionary balance between the need to promote increased variation through outbreeding and the advantages of self-fertility in harsh environments.

**Sex enables escape from parasites.** Despite their slow growth rates, lichens have their very own specialised parasites (Honegger, 2001). Included in the 'lichenicolous fungi' are species that have a devastating effect on lichens. Sexual reproduction and dispersal of ascospores, exclusively of the mycobiont, offers a means to escape the offending parasite.

### Chemical warfare, survival and sex

'Lichen sex' also has other special characteristics. Unlike most non-lichenized ascomycetes, which produce ephemeral fruiting bodies only when environmental conditions are favourable, lichen sexual reproductive ascomata are long lived, most persisting for several years during which time they can release large numbers of ascospores. Indeed, some thalli can be viewed as a positive orgy of sex (Fig 8). This constant exposure means that mechanisms must exist to prevent thalli and ascomata being consumed by herbivores or out-competed by other microbes and lichens (Purvis, 2000). Lichens are known to produce a spectrum of chemical compounds, some of which are antagonistic or inhibitory substances used as self-defence in 'chemical warfare'. Intriguingly, some of these substances may have pharmacological activity and be of benefit to mankind (Crittenden & Porter, 1991). Lichens as a group have yet to be intensively screened for bioactive compounds as they are considered too slow growing for large-scale production, but new opportunities to clone and express lichen metabolites in more amenable systems may allow future exploitation and reveal a wealth of compounds (Miao *et al.*, 2001). In addition, the colours of many thalli and ascomata

are attributable to secondary products; some of these, and others that are not coloured, are thought to be essential for protection from UV-radiation.

### Sex, relationship breakdowns and new partnerships

One other feature of lichen sex is that it results in a breakdown of the symbiotic relationship, because ascospores are in almost all instances discharged free of the photobiont. Upon germination, the new fungal mycelium therefore needs to meet a compatible photobiont to form a new lichen (Ott, 1987 a, b; Büdel & Scheidegger, 1996; Honegger, 2001). This might explain why lichens are so sexually prolific, since large numbers of ascospores are probably required to ensure that even a few spores land on a suitable substratum, germinate successfully and are then able to encounter an appropriate algal partner.

The process of 're-lichenization' has been studied, and what might be viewed as quite scandalous interactions have come to light. For example, Ott (1987b) examined early stages of development in *Xanthoria parietina* and found that germinating spores were able to grow over a small area and form a temporary symbiotic relationship with a non species-specific photobiont(s). This photobiont could then be replaced by a species-specific partner at a later stage by 'theft' from nearby soredia or even other lichen thalli. i.e. the lichen fungus took up with one partner before enticing a better option from another individual! Indeed, it is believed that the process of fungal spores landing on an existing lichen (containing a suitable alga), and then being incorporated into, or 'taking over' from, the host mycobiont may be widespread, although the reality of this process (termed 'cleptobiosis') is disputed by some authors (Honegger, 2001; Dobson, 2003). This appears to be especially important for lichen fungi that form symbioses with algae in the genus *Trebouxia*; these are found infrequently in the free-living state in nature (Ott, 1987a, b; Sanders & Lücking, 2002). In other lichen symbioses re-lichenization may simply rely on chance encounter with a free-living form of a suitable photobiont partner (for further details see Hammer, 1993; Hestmark, 1992; Ott, 1987a, b),

### Conclusions

Lichens have a remarkable ability to survive in the harshest environments and yet despite this appear to have a diverse and continuous sex life. Perhaps the fact that lichen-forming fungi rely on an intimate and stable

relationship with symbiotic partners living together predisposes them to sex? Ongoing research now aims to understand why some lichen-forming fungi exhibit a homothallic, whilst others a heterothallic, sexual breeding system. Lichens may yet prove to be a paradigm for understanding 'sex in the extreme'.

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