Reproductive systems

Sex and the single lichen

Lichens are physiologically adapted for growth in dry, nutrient-deficient and temporarily thermally extreme habitats[1], but it is unclear how the reproductive strategies of lichen symbionts might have evolved to maximize colonization. We now show that sexually reproducing lichen-forming fungi can self-fertilize, and propose that this breeding system allows these symbiotic organisms to reproduce successfully in harsh environments.

Most lichenized fungi produce abundant sexual structures, and in many species sexual spores seem to provide the only means of dispersal. For example, 90% of lichens found in Great Britain and Ireland[2] produce ascomata (fruit bodies) containing sexually derived ascospores, whereas only 29% form symbiotic vegetative propagules. Sex in lichenized fungi has been assumed to equate with outcrossing[3], but failure to induce sexuality *in vitro* has prevented experimental investigation of their breeding systems.

We avoided this problem by using molecular markers to elucidate the sexual cycle. We compared the DNA fingerprints of single-spore progeny from a single ascoma: the presence or absence of genetic variation between sibling spores would show whether outcrossing (heterothallism) or self-fertilization (homothallism: allowing the fusion of two identical nuclei during meiosis) had occurred.

The crustose lichens *Graphis scripta* (order: Ostropales) and *Ochrolechia parella* (order: Pertusariales) fruit abundantly, but neither produces symbiotic vegetative propagules (Fig. 1a,b). We collected discrete, symmetrical lichen thalli at locations more than 10 m apart in south Wales, and induced excised individual ascomata to discharge spores. Extraction of DNA from cultured mycelia derived from single spores gave 218–263 randomly amplified polymorphic DNA (RAPD) markers[4] for each isolate. In both species, these markers revealed that spores from the same ascoma are genetically uniform (Fig. 1c), providing compelling evidence of homothallism.

This breeding system probably serves an ecological function similar to that of self-pollination in flowering plants[5–7]. It may confer a selective advantage by facilitating high spore output without the need for outcrossing; it would also promote the development of a lichen population from a single pioneer spore after dispersal into a new site. Genetic stability may be advantageous in abiotically extreme but relatively undisturbed habitats in which there is a low intensity of biotic interactions because it perpetuates successful genotypes adapted to the prevailing environmental regimes. Homothallism also retains certain benefits of sexual over asexual reproduction, including opportunistic outcrossing, as obligate selfing is rare in homothallic fungi[8].

Although we found both lichen fungi to be homothallic, the ascospore offspring derived from different conspecific thalli were genetically distinct (Fig. 1d). The average RAPD genetic divergence[9] observed in five isolates of *G. scripta* from one woodland was 15.2% (according to a neighbour-joining analysis of Jaccard's coefficient of band matching). Furthermore, ascospore progeny from different ascomata on 'single' thalli of *O. parella* showed RAPD polymorphisms, indicating that these lichen thalli may have been composed of at least two sexually active fungal genotypes.

Comparable variation within individual thalli was not detected in *G. scripta* (Fig. 1d). This difference is probably related to habitat: *G. scripta* is a pioneer, with thalli that probably arise from single spores, whereas the higher rates of spore deposition in the more densely occupied habitats of *O. parella* may cause frequent mergers between developing thalli. These 'mechanical hybridizations' are probably very common[10].

If homothallism is a general characteristic of lichen-forming fungi, including those in the order Lecanorales (which contains the most lichen-forming fungal species), it may explain the ecological paradox of the persistence of fruiting lichens in severe habitats where a stable, highly adapted genetic line would be most advantageous[11]. It has been shown that sexual reproduction is predominant in lichen communities growing at the frontiers of terrestrial life in polar regions[12,13]. Lichens may thus provide a model for the evolution of breeding systems in extreme environments.

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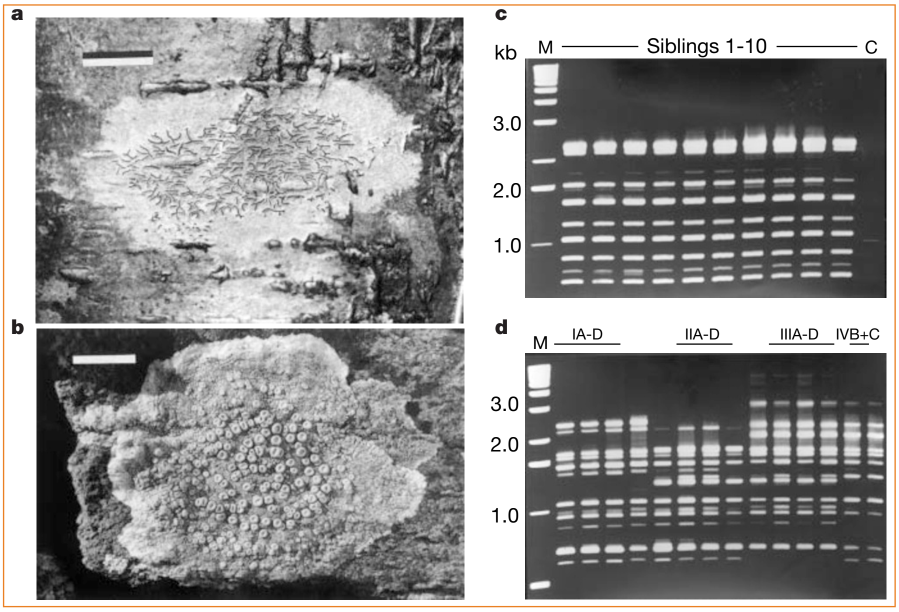

Figure 1 Sexual reproduction and genetic variation in lichen-forming fungi. **a, b,** The crustose lichens *Graphis scripta* and *Ochrolechia parella* growing on tree bark and maritime rocks, respectively (scale bars, 10 mm). Both species develop abundant ascomata (elongate in *G. scripta* and disc-shaped in *O. parella*), in which sexual ascospores are produced. **c,** Randomly amplified polymorphic DNA (RAPD) markers generated with primer OPAJ-03 showing uniformity in a sample of ten single-spore isolates from one ascoma of *G. scripta*. A set of ten progeny was collected from each of three separate thalli of both species and assessed for variability in RAPD profiles using a minimum of 30 primers (Operon Technologies). Consistent uniformity among the RAPD markers indicated that for both species all spores from the same ascoma were genetically identical. **d,** RAPD markers generated with primer OPAX-12 showing polymorphisms between isolates from four different thalli (I–IV) of *G. scripta* but uniformity among spores from 2–4 different ascomata (replicates A–D) on the same thallus. Eight thalli of *G. scripta* were compared, five of which were collected from the same woodland. For *O. parella*, 3–4 ascomata on each of three thalli were compared and polymorphisms found between both thalli and ascomata on the same thallus. M, size markers in kilobases; C, water control.

1. Kershaw, K. A. *Physiological Ecology of Lichens* (Cambridge Univ. Press, Cambridge, 1985).
2. Purvis, O. W. *et al.* (eds) *The Lichen Flora of Great Britain and Ireland* (Natural History Museum Publications, London, 1992).
3. Hestmark, G. *Oecologia* **92,** 305–312 (1992).
4. Murtagh. G. J., Dyer, P. S., McClure, P. C. & Crittenden, P. D. *Lichenologist* **31,** 257–267 (1999).
5. Jarne, P. & Charlesworth, D. *Annu. Rev. Ecol. Syst.* **24,** 441–466 (1993).
6. Jaine, S. K. *Annu. Rev. Ecol. Syst.* **7,** 469–496 (1976).
7. Richards, A. J. *Plant Breeding Systems* 2nd edn (Chapman & Hall, London, 1997).
8. Burnett, J. H. *Mycogenetics: An Introduction to the General Genetics of Fungi* (Wiley, London, 1975).
9. Weising, K., Nybom, H., Wolff, K. & Meyer, W. *DNA Fingerprinting in Plants and Fungi* (CRC, Boca Raton, 1995).
10. Jahns, H. M. & Ott, S. *Bibl. Lichenol.* **67,** 49–67 (1997).
11. Maynard Smith, J. *The Evolution of Sex* (Cambridge Univ. Press, Cambridge, 1978).
12. Fahselt, D., Maycock, P. F. & Wong, P. Y. *Lichenologist* **21,** 343–353 (1989).
13. Sancho, L. G. & Valladares, F. *Polar Biol.* **13,** 227–233 (1993).