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Viability of lichen photobionts after passing through the digestive tract of a land snail

A variety of terrestrial gastropods feed on lichens (Gerson & Seaward 1977; Seaward 1988; Fröberg *et al.* 1993; A. Baur *et al.* 1994; B. Baur *et al.* 1995). Lichen bionts in the snail's faeces that could withstand detrimental effects of digestive enzymes might develop into a new lichen thallus. This could be accomplished either by the combination of a mycobiont and a photobiont that derive from the same thallus (reconstitution), or by a combination of bionts from different thalli (relichenization).

Snail herbivory has frequently been observed in *Xanthoria parietina* (L.) Th. Fr. (A. Baur *et al.* 1994; B. Baur *et al.* 1995). We examined the viability of photobiont cells of *X. parietina* after they had passed through the digestive tract of the land snail *Helicigona lapicida* L. and compared it with the viability of photobiont cells in intact and squashed parts of the same lichen individual. The variable fluorescence of photosystem II can be used as a measure of the photosynthetic activity, which also indicates the viability of photobiont cells (Schreiber *et al.* 1986; Sonesson *et al.* 1995).

Lichens were collected on two limestone walls near Bårby källa, Mörbylånga par., and near Lilla Vickleby, Vickleby par., on the Baltic island of Öland, Sweden, on 3 March 2001. Specimens of *Helicigona lapicida* were collected in an abandoned quarry at Lilla Vickleby. Each lichen individual was divided into three pieces of similar size. One piece of lichen was used as food for the test snail, and the other pieces served as controls (intact and squashed) for the fluorescence measurements (see below). Twelve individuals of *H. lapicida* ($n=6$ for both lichen localities) were kept singly in transparent plastic dishes, 8·9 cm in diameter, lined with moist paper towelling. A piece of lichen, 1·5–3 cm in diameter, was provided as food for each snail. Snails that did not begin to graze within 2 h were replaced by other active individuals. After 58 h, the faeces of each snail were collected and kept moist for another 12 h. Under these conditions the photobionts maintain their photosynthetic activity (cf. Solhaug & Gauslaa 1996). Subsequently, the faeces were squashed by squeezing the side of a razor blade against the sample in a droplet of melted snow collected on Öland. In this way most of the photobiont cells were released. In a pilot experiment, samples from the same thallus had differed in fluorescence intensity when squashed either in distilled water or tap water (L. Fröberg, unpublished data). Therefore, we tried to simulate natural conditions by using melted snow. For fluorescence measurements the paste of squashed faeces was transferred into a pit of *c.* 0·1 ml made in a plexiglass block. Both control samples were kept moist in melted snow for 12 h prior to the experiment. One of them was squashed in the same way as the faeces. After adaptation to darkness for 10 min, the fluorescence intensity of photobiont cells was measured during application for 15 s of 650 nm excitation light using a plant efficiency analyser (Hansatech King's Lynn, Norfolk, UK). Parietin, the dominant lichen substance in *X. parietina*, absorbs light between 400 and 475 nm (Solhaug & Gauslaa 1996), and thus did not interfere with our measurements. The photosynthetic activity of the cells is presented as the ratio of increase in variable

fluorescence during illumination (F_v , variable fluorescence) to the maximum fluorescence intensity during the light pulse (F_m). The fluorescence activities (F_v/F_m) of intact and squashed thalli of *X. parietina* and that of lichen remains in faeces of *H. lapicida* are given as mean values with standard deviations in parenthesis, with $n=6$ for each locality. The fluorescence of photobionts averaged 0.73 (0.04) in intact lichen tissue from Bårby källa, and 0.74 (0.03) from Lilla Vickleby. The fluorescence of photobionts in squashed tissue was 0.64 (0.06) for sample from Bårby källa, and 0.65 (0.04) for samples from Lilla Vickleby. The fluorescence of photobionts in snail faeces was 0.41 (0.13) for samples from Bårby källa, and 0.44 (0.19) for samples from Lilla Vickleby. Thus, squashed lichen samples showed significantly lower F_v/F_m values than intact thalli at both localities (paired t -test; Bårby källa: $t=3.52$, d.f.=5, $P=0.0169$; Lilla Vickleby: $t=4.20$, d.f.=5, $P=0.0085$). Photobionts in lichen remains in faeces of *H. lapicida* also showed significantly reduced F_v/F_m values compared with those of intact thalli at both localities (Bårby källa: $t=6.30$, d.f.=5, $P=0.0015$; Lilla Vickleby: $t=3.63$, d.f.=5, $P=0.015$). The F_v/F_m values of lichen remains in faeces were even lower than those of squashed thalli at both localities (Bårby källa: $t=4.58$, d.f.=5, $P=0.006$; Lilla Vickleby: $t=2.78$, d.f.=5, $P=0.0389$). Thus, in comparison to intact lichens, squashed thalli of *X. parietina* showed an F_v/F_m ratio reduced by 12% (at both localities), whereas for lichen remains in faeces of *H. lapicida* the reduction was 41% (Lilla Vickleby) and 44% (Bårby källa), respectively.

To investigate how photobiont cells are morphologically affected by the passage through the snail's digestive tract, we measured the size of photobiont cells and of chloroplasts (the average of length and width) and the interspace (distance between cell wall and chloroplast membrane) using a microscope (magnification x 400) and compared them with corresponding values intact lichens (Table 1). Photobiont size did not differ between intact lichen thalli and lichens that had passed through the digestive tract of a snail (paired t -test, at both localities $P>0.32$). However, photobiont cells in lichen remains in faeces had a more or less shrunken chloroplast, indicated by a reduced size (Bårby källa: $t=2.50$, d.f.=5, $P=0.0545$; Lilla Vickleby: $t=3.22$, d.f.=5, $P=0.0234$). The space between cell wall and chloroplast was larger in lichen remains in faeces than in intact lichen thalli (Bårby källa: $t=4.93$, d.f.=5, $P=0.0044$; Lilla Vickleby: $t=2.99$, d.f.=5, $P=0.0306$). The results show that photobiont cells of *X. parietina* can partly withstand enzyme activity in the digestive tract of a gastropod.

The reduced photosynthetic activity of lichen remains in the faeces may result either from a few cells with full activity or from many cells with reduced activity. Most of the cells of the lichen remains in faeces were damaged and their chloroplasts were shrunken, but the samples also contained a few seemingly undamaged cells. It is, however, not possible to determine whether the undamaged cells have full photosynthetic activity, or if their activity is reduced. Furthermore, the reduced photosynthetic activity of photobionts in lichen remains in faeces could result either from mechanical damage through grazing (the altered situation for photobiont cells that have been released from the lichen thallus), or from the subsequent activity of digestive enzymes, or from a combination of both. To distinguish between these possibilities, we measured the fluorescence intensity of both squashed thalli and lichen remains in faeces. The squashed samples showed a reduced

Table 1.

The size of photobiont cells, chloroplasts and interspace (distance between cell wall and chloroplast membrane) in Xanthoria parietina from two localities

Locality	Treatment*	Cell size‡ (µm)	Chloroplast size‡ (µm)	Interspace‡ (µm)
Bårby källa	L	9.2±0.9	7.9±1.0	0.6±0.1
	Fe	9.3±0.6	6.7±0.7	1.2±0.3
Lilla Vickleby	L	10.4±0.7	9.1±0.7	0.6±0.1
	Fe	9.6±1.2	7.4±0.8	1.1±0.4

*Samples were taken from intact lichen thalli (L) and faeces (Fe) of *Helicigona lapicida* fed on *X. parietina*.

‡Values represent mean and S.D. of six samples (eight randomly selected cells measured in each sample).

activity, which was, however, less reduced than that of lichen remains in faeces. Thus, both mechanical damage and enzyme digestive activity seem to affect the photobiont cells negatively, but it is not possible to assess their relative impact. Further studies on the fluorescence of squashed and digested lichens are needed to understand better the mechanisms affecting the photosynthetic activity of released photobionts. The *Fv/Fm* values of intact lichen thalli found in the present study correspond to those of *X. parietina* given in Solhaug & Gauslaa (1996). If photobiont cells that have survived the passage of the digestive tract have the potential to reconstitute or relichenize, a viable mycobiont is also required in the faeces for the development of a new lichen. Mycobionts are probably more viable as spores than as hyphae from the thallus, because the latter may be adversely affected by digestive enzymes. In this context, the survival of the mycobiont spores after ingestion also needs to be examined, as well as the bionts' ability to reconstitute or relichenize within the faeces and to develop further.

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