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Regeneration of *Pseudocyphellaria aurata* transplanted on a tree in Japan

To conserve endangered and/or rare lichens, transplantation methods are considered useful tools to establish new populations (Scheidegger *et al*. 1995, 1998; Hilmo 1999; Zoller *et al*. 2000; Lidén *et al*. 2004; Hilmo *et al*. 2011; Gustafsson *et al*. 2013). However, transplantation experiments have been applied to only a limited number of species. Further lichenological knowledge should be accumulated, especially for endangered and rare species.

Pseudocyphellaria aurata (Ach.) Vain. (*Lobariaceae*, Ascomycota) is a cosmopolitan species and widespread in tropical regions of the world. It is also found in drier, warmer, coastal areas in cool temperate regions (Galloway & Arvidsson 1990). The populations in Japan, however, are rather rare and ranked in some local red lists (e.g. The Committee of the Red Data Book Chiba 2009; Saitama Prefecture 2011). We performed transplantation studies on *P. aurata* using soredia and thallus fragments. This paper describes the growth process into juvenile thalli in each case.

Original material for two transplantation studies was collected from the trunk of *Liriodendron tulipifera* in Takihara, Kimitsu-city, Chiba Prefecture, Japan (35°13′27″N, 140°06′45″E), at 100 m elev. on 31 March 2009 (*Y. Kon* 09031, TNS). Transplantation experiments followed the modified methods of Scheidegger (1995) and Zoller *et al*. (2000).

In the first study, soredia of *P. aurata* were detached from one lobe using a sterile needle, and transplanted into a multi-layered gauze sheet. The sheet was 4×4 cm in size and consisted of four layers of surgical gauze (Hakujuji Co. Ltd, Japan). Approximately 30 soredia were put between the first and second layer of the sheet, which was sprayed with distilled water in advance. Five sets of the sheet were prepared and attached by stainless steel nails onto the east side of a *L. tulipifera* trunk (110 cm in diameter) arranged in a vertical line at a height of *c*. 1·5 m from the ground. The transplantation took place between 31 March 2009 and 18 July 2011 (*c*. 28 months), at the same location where the specimen of *P. aurata* was originally collected.

In the second study round thallus discs (6 mm in diameter, without thallus margin) were punched out from the lobes of a thallus. Five thallus discs were attached to the east side of a *L. tulipifera* trunk at a height of *c*. 1·5 m from the ground. They were fixed by a nylon mesh (*c*. 2×2 cm in size) which was stapled onto the bark. The transplantation period was between 31 March 2009 and 9 May 2010 (*c*. 14 months).

Growth was checked every month in the field. When morphological changes were observed, a cotton gauze sheet or a disc was taken off the tree trunk and details of the morphology were checked under a stereomicroscope in the laboratory.

The original soredia of *P. aurata* were globular in shape [96 ± 27 µm diam. (mean ± SD), $n = 50$] and pale yellow in colour on the thallus (Fig. 1A). Six months after the transplantation, brownish hyphae which were anchored onto the cotton fibres of the gauze were developed from most soredia (in 19 of 30 soredia on a sheet) (Fig. 1B). Thirteen months after the transplantation, the soredia had developed into cylindrical primordia which were up to *c*. 400 µm long and 125 µm wide ($182 \pm 73 \times 106 \pm 21$ µm, $n = 17$) (Fig. 1C). After twenty-eight months the isidium-shaped primordia grew up into spathuliform lobules which were up to 540 µm long and 360 µm wide ($228 \pm 100 \times 199 \pm 60$ µm, $n = 14$) (Fig. 1D). Pseudocyphellae and tomenta were not observed on the surface of the lobules in this developmental stage.

In the second study, no morphological alterations were observed during the first four months after transplantation. After seven months some protuberances had formed along the margin of the thallus fragment; these developed further into lobules after 14 months ($175 \pm 60 \times 146 \pm 32$ µm, $n = 18$) (Fig. 2). Pseudocyphellae and tomenta were not observed on the surface of the lobules in this developmental stage.

Regeneration of juvenile thalli from transplanted diaspores have been reported in

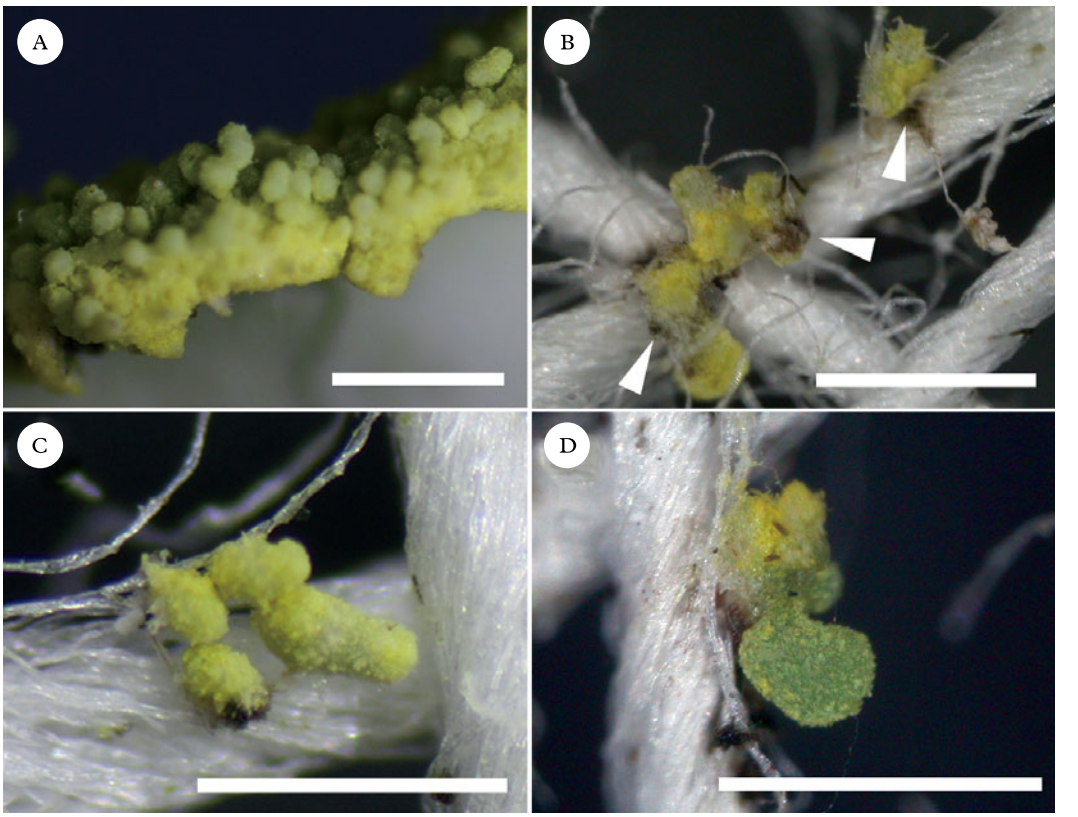

FIG. 1. Regeneration of lobules from soredia in *Pseudocyphellaria aurata*. A, soredia on the original thallus; B, soredia after 6 months of transplantation, arrows indicate brownish hyphae which might play a role in fixing the diaspores onto the cotton fibres; C, soredia 13 months after transplantation; D, a lobule formed on soredium 28 months after transplantation. Scale = 0·5 mm. In colour online.

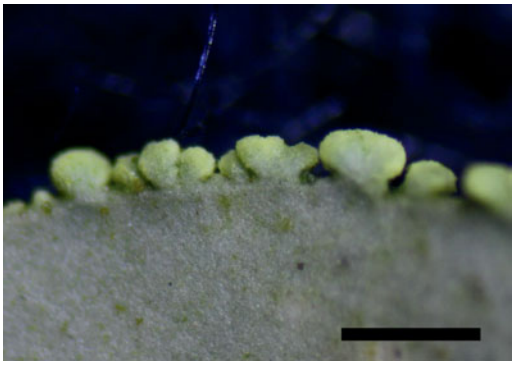

FIG. 2. Regenerated lobules from the cut-end of thallus fragments of *Pseudocyphellaria aurata*. Scale = 0·5 mm. In colour online.

various species (Table 1). The regeneration process from a soredium into a lobule in *Pseudocyphellaria aurata* is similar to that in *Lobaria pulmonaria* (Scheidegger 1995) but differs from those in *Parmotrema clavuliferum* and *Lobaria scrobiculata*, in which a cluster of soredia at first redifferentiate into an undifferentiated mass of tissue (callus) and then lobules form from the callus (Hilmo & Ott 2002; Kon & Ohmura 2010). In contrast, the regeneration process from the cut-ends of thallus lobes into lobules was similar to that in *Parmotrema tinctorum* (Kon & Kashiwadani 2005).

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Table 1. *Regeneration period from diaspores or thallus fragments into juvenile thalli in various lichens*

Species	Source	Regeneration period (months)	Reference
Hypogymnia physodes	Soredia	12	Zoller *et al*. (2000)
Leptogium saturninum	Isidia	16	Zoller *et al*. (2000)
Lobaria plumonaria	Soredia	15	Scheidegger (1995)
L. scrobiculata	Soredia	29	Hilmo & Ott (2002)
Menegazzia terebrata	Soredia	16	Zoller *et al*. (2000)
Parmelia sulcata	Soredia	12	Schuster *et al*. (1985)
Parmotrema clavuliferum	Soredia	12	Kon & Ohmura (2010)
P. tinctorum	Isidia	12	Kon & Kashiwadani (2005)
	Thallus fragments	6	Kon & Ohmura (2008) Ohmura *et al*. (2009)
Physcia tenella	Soredia	9	Schuster *et al*. (1985)
Pseudocyphellaria aurata	Soredia	28	This study
	Thallus fragments	14	This study
Ramalina yasudae	Soredia	18	Kon & Ohmura (2010)
Sticta fuliginosa	Isidia	8	Zoller *et al*. (2000)
Usnea antarctica	Soredia	36	Ott (2004)
Xanthoria parietina	Thallus fragments	5	Honegger (1996)

The regeneration period of 28 months from diaspores into lobules in *Pseudocyphellaria aurata* is much longer than in most other lichen species, for which periods between (8–)12 and 18(–36) months have been reported. The shortest known regeneration period from diaspores in a natural habitat was eight months in *Sticta fuliginosa* (Zoller *et al*. 2000) and the longest period was 36 months in *Usnea antarctica* (Ott 2004). The slow regeneration in *U. antarctica* might be caused by the cold and harsh Antarctica environment. Similarly, soredia of *Lobaria scrobiculata* in a boreal spruce forest took 29 months until the first tiny lobule (0·1 mm long) differentiated (Hilmo & Ott 2002). The regeneration period in lichens may be affected by various environmental factors such as temperature, humidity, light intensity, substratum preference, and air pollution. For *P. aurata*, which is a tropical species, the temperate climate in Japan might not be favourable. Gauze is also an artificial substratum with no nutrients. Further research is needed to clarify the growth rate of this species in a transplantation experiment with replication across trees and sites.

The regeneration period from a thallus fragment into a lobule in *P. aurata* is shorter than that from a soredium (14 months from a thallus fragment compared to 28 months from a soredium). This behaviour is also observed in *Parmotrema tinctorum* (six months from thallus fragments and 12 months from isidia) (Kon & Kashiwadani 2005; Kon & Ohmura 2008; Ohmura *et al*. 2009). Therefore, for cultivation purposes *in situ*, transplantation of thallus fragments may be more effective compared to methods starting from soredia and isidia. However, in conservation practice, this will not always be possible and in small populations of rare species the transplantation of vegetative diaspores may be more advisable.

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