

## Large Clones on Cliff Faces: Expanding by Rhizomes through Crevices

FENG-HONG LIU<sup>1</sup>, FEI-HAI YU<sup>1,\*</sup>, WEN-SHENG LIU<sup>1</sup>, BERTIL O. KRÜSI<sup>2</sup>, XIAO-HU CAI<sup>3</sup>,  
JAKOB J. SCHNELLER<sup>4</sup> and MING DONG<sup>1,\*</sup>

<sup>1</sup>State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China, <sup>2</sup>University of Applied Sciences Zürich-Wädenswil (HSW), Grüental Postfach 335, 8820 Wädenswil, Switzerland, <sup>3</sup>Sichuan Academy of Forestry, 18 Xinghui West Road, Chengdu 610081, Sichuan, China and <sup>4</sup>Institute of Systematic Botany, University of Zürich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland

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- **Background and Aims** Large clones of rhizomatous plants are found in many habitats, but little is known about whether such clones also occur on cliff faces where environmental conditions are extremely harsh and heterogeneous.
- **Methods** Using molecular (intersimple sequence repeat, ISSR) markers, the genotypic composition of a cliff-face population of *Oxyria sinensis* in Sichuan, China, was investigated.
- **Key Results** The 98 *O. sinensis* ramets sampled belonged to 12 different genotypes (clones). The three most frequent clones were represented with 45, 22 and 12 ramets, respectively; the remaining nine were represented with only one to five ramets. The three largest clones spanned at least 2.7 m in the vertical direction and 4.6–6.9 m in the horizontal direction on the cliff face.
- **Conclusions** On the cliff face, large clones of *O. sinensis* are formed by rhizomes growing along the crevices. Expansion by rhizomes may help *O. sinensis* to exploit the patchy resources and support establishment and growth of new ramets. Moreover, rooted ramets connected by rhizomes may effectively reduce the susceptibility of *O. sinensis* to rock fall and erosion and thus greatly improve the chances for long-term survival. The multi-clone structure indicates that sexual reproduction is also important for the long-term persistence of *O. sinensis* populations on cliffs.

**Key words:** Cliff ecology, clonal diversity, clonal growth, genet distribution, *Oxyria sinensis*, rhizomatous plants.

### INTRODUCTION

Cliff faces, i.e. the vertical or nearly vertical parts of cliffs, represent one of the harshest habitats for plants (Larson *et al.*, 2000). Because of their vertical nature and physical structure, favourable micro-sites on cliff faces are extremely rare and patchy (Larson *et al.*, 2000). Despite this, cliff faces provide a suitable habitat for many rare and often endemic plant species (Wagner *et al.*, 1994; Colas *et al.*, 1997; Larson *et al.*, 2000; de Lange and Norton, 2004; Tang *et al.*, 2004). However, the manner in which these plants adapt to cliff faces is poorly understood (Larson *et al.*, 2000).

It has been found that some rhizomatous clonal plants are very successful and form large clones in some extremely harsh habitats (Jónsdóttir and Watson, 1997), including deserts (Alpert, 1990; Danin and Orshan, 1995; Danin, 1996; Dong and Alaten, 1999; Yu *et al.*, 2004; Liu *et al.*, 2007), arctic and alpine tundras (Jonsson *et al.*, 1996; Steinger *et al.*, 1996; Körner, 2002; He *et al.*, 2007) and salt marshes (Hester *et al.*, 1994; Shumway, 1995; Amsberry *et al.*, 2000; Pennings and Calaway, 2000). However, little is known about the situation on cliff faces. In such extreme habitats, rhizomatous clonal plants capable of forming large clones may be in a better position than other life forms to overcome unfavourable conditions

and to access and exploit the rare favourable micro-sites (Alpert, 1990; Shumway, 1995; Dong and Alaten, 1999; Amsberry *et al.*, 2000; Pennings and Calaway, 2000; Yu *et al.*, 2004).

On cliff faces one frequently finds crevices where mineral soil, litter and water may accumulate and form a suitable substrate for plant growth (Larson *et al.*, 2000). Often the crevices are interconnected and form a wide-spread network, providing ideal passageways for rhizomatous plants (Tang *et al.*, 2004). For example, Tang *et al.* (2004) observed that the rare and endemic herb *Taihangia rupestris* could vegetatively propagate by rhizomes growing along the crevices of cliff faces in the Taihang Mountains of China. The rhizomes of *Oxyria sinensis* have also been observed to grow inside or along the larger crevices of cliff faces in the Qionglai Mountains, Sichuan, China (F-H. Yu and B. Krüsi, pers. observ.). However, very little is known about the ability of clonal growth by rhizomes and the adaptive status of this growth strategy on cliff faces.

The present paper deals with a cliff-face population of *Oxyria sinensis*, a rhizomatous perennial herb endemic to Guizhou, Sichuan, Tibet and Yunnan in China (Wu, 2004). On a cliff face in Sichuan, leaves from 98 ramets of *O. sinensis* were sampled and their genotypes were identified using molecular (intersimple sequence repeat, ISSR) markers. The objective was to address whether large clones of *O. sinensis* occur on the cliff face.

\* For correspondence. E-mail feihaiyu@ibcas.ac.cn or dongming@ibcas.ac.cn

## MATERIAL AND METHODS

*Species*

*Oxyria sinensis* Hemsley (Polygonaceae) occurs on steep, dry mountain slopes and vertical cliff faces, in openings of deciduous mountain forests and in grasslands at altitudes from 1600 to 3800 m a.s.l. (Wu, 2004). This species can propagate vegetatively by producing ligneous rhizomes with rooted ramets at the nodes. *O. sinensis* produces panicle inflorescences with unisexual flowers. It usually blooms from April to October and fruits from May to November. Individual ramets are 30–50 cm high (Wu, 2004).

*Study site and sampling*

The study site was a roughly 35-m-tall roadside cliff (31°34'95"N, 103°20'46"E), located between the mountain ranges of Qionglai and Longmeng in Li county, Sichuan province, China. The cliff was located at 1586 m a.s.l., near a river, and at the bottom of a fairly deep and narrow valley covered by deciduous woods. An area of about 6 × 8 m (height × length) at the lower part of the

cliff face was sparsely covered by a population of *O. sinensis*. In June, 2006, 98 ramets were sampled in the lower part (3.5 m high and 8 m long) of the population (Fig. 1A), and the relative location of each sample was noted. In the neighbourhood of the study area, six individuals from six different populations, separated from each other by at least 50 m, were also collected. All samples were dried with silica gel and kept at room temperature until DNA extraction.

*DNA extraction and PCR-ISSR*

Genomic DNA in the leaf tissue was isolated using the Plant Genomic DNA Purification Kit (Tiangen, China). ISSR primers (SBS Genetech Co. Ltd, Beijing, China) were screened using the six samples of *O. sinensis* collected from the six spatially and clearly separated populations. Four primers (811 [(GA)<sub>8</sub>C], 834 [(AG)<sub>8</sub>YT], 836 [(AG)<sub>8</sub>YA], 857 [(AG)<sub>8</sub>CG]) that were polymorphic and yielded clear, reproducible banding patterns were used. DNA amplifications were performed in a PTC-200 thermocycler (MJ Research, Watertown, MA, USA), with 25 μL PCR reaction mixture containing 12.5 μL *Taq* PCR

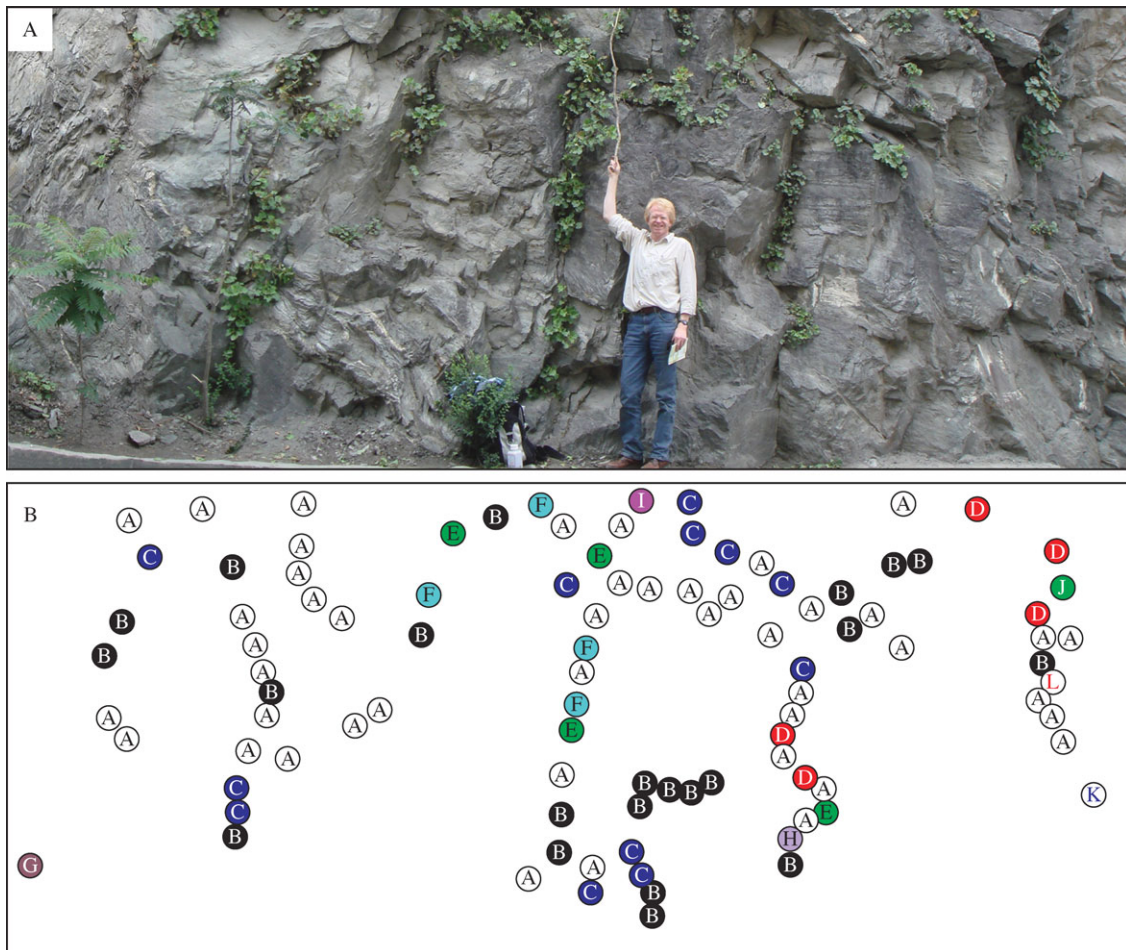


FIG. 1. (A) A photograph of the cliff-face population of *Oxyria sinensis* and (B) the distribution patterns of the *O. sinensis* clones. Circles represent the relative positions of the sampled ramets and A–L indicate the 12 different clones.

MasterMix (2× PCR buffer, 1.25 U *Taq* DNA polymerase, 0.5 mM of each dNTP and 3.0 μM MgCl<sub>2</sub>; Tiangen, China), 0.2 μM primer and 20–40 ng DNA template. The reaction was run for an initial 1.5 min at 94 °C, followed by 35 cycles of 40 s at 94 °C, 45 s at 50 °C (for primers 811, 834 and 836) or 52 °C (for primer 857), and 90 s at 72 °C, and then ended with 8 min at 72 °C. The amplification products were electrophoretically resolved on 1.5 % agarose gels in 0.5× TBE buffer and stained with ethidium bromide. Images were obtained using an Alpha Ease FC Imaging System (Alpha Innotech Corp., San Leandro, CA, USA).

## RESULTS AND DISCUSSION

Of the 98 *O. sinensis* ramets sampled, 12 different clones (genotypes) were identified (Fig. 1B). The three most frequent clones (*A*, *B* and *C*) were represented with 45, 22 and 12 ramets, respectively; the remaining nine (*D*–*L*) were represented by only one to five samples (Fig. 1B, Table 1). The three largest clones (*A*, *B* and *C*) spanned at least 2.7 m in the vertical direction and 4.6–6.9 m in the horizontal direction (Table 1), suggesting that *O. sinensis* is able to form quite large clones on the cliff face. Although rhizomatous plants form large clones in many different habitats (Parks and Werth, 1993; Jonsson *et al.*, 1996; Mitton and Grant, 1996; Wang *et al.*, 1999), this study provides the first evidence that this phenomenon also occurs in such extreme habitats as vertical cliff faces where favourable micro-sites are few and far apart.

The large clones of *O. sinensis* most likely result from clonal growth by rhizomes growing along the crevices of the cliff face. The seemingly discrete soils (patches) on the cliff face are connected by a network of crevices (Larson *et al.*, 2000), which provide passageways for the rhizomes of *O. sinensis*. This explanation is supported by the fact that rhizome connections between adjacent ramets of *O. sinensis* were clearly visible in some of the larger crevices (F-H. Yu and B. Krusi, pers. observ.).

On cliff faces where site conditions are generally very poor and highly heterogeneous, clonal growth by rhizomes

may have many advantages (Jónsdóttir and Watson, 1997; Larson *et al.*, 2000). For instance, it may allow *O. sinensis* to access and exploit the few favourable micro-sites on a cliff face by active or passive foraging (de Kroon *et al.*, 2005). By physiological integration, the *O. sinensis* ramets in patches with high resource availability may allow the clone to expand even if it must overcome larger lean patches. Moreover, because cliff habitats are subject to erosive processes (Larson *et al.*, 2000), large numbers of rooted ramets, interconnected by ligneous rhizomes, may effectively reduce the susceptibility of *O. sinensis* to rock fall and erosion, and thus greatly improve the chances for the long-term survival. However, more studies are needed to test experimentally the adaptive significance of forming large clones on cliff faces.

On the other hand, *O. sinensis* can form large clones only if: (1) the density of suitable micro-sites is sufficient; and (2) these micro-sites are accessible, e.g. via a system of interconnected crevices. As these conditions are only fulfilled by some cliff faces and frequently only in one section of a cliff face, extremely large clones of *O. sinensis* on cliff faces will most likely be rare. Where conditions for rhizomatous growth are not suitable, sexual reproduction must play a key role for the persistence of populations. The anemochorous seeds of *O. sinensis* may be very helpful in quickly colonizing new habitats over large distances. The multi-clone structure detected in the studied population suggests that *O. sinensis* also reproduces sexually. This occurs even though gravity and the scarcity of suitable germination sites do not facilitate the successful establishment of young plants on cliff faces. Thus, it seems that *O. sinensis* uses both sexual and asexual reproductions to colonize cliff faces and to sustain populations on such extreme habitats.

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TABLE 1. Frequency and distribution characteristics of the 12 *Oxyria sinensis* clones on the cliff face

Clone identity	Number of samples	Maximum vertical distance (m)	Maximum horizontal distance (m)	Area (m <sup>2</sup> )
A	45	6.87	2.74	18.82
B	22	6.66	2.85	18.95
C	12	4.62	2.74	12.66
D	5	1.93	1.89	3.65
E	4	1.66	1.45	2.40
F	4	2.36	1.99	4.67
G	1	—	—	—
H	1	—	—	—
I	1	—	—	—
J	1	—	—	—
K	1	—	—	—
L	1	—	—	—

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