Annals of Botany Page 1 of 10
doi:10.1093/aob/mcv059, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

Higher clonal integration in the facultative epiphytic fern *Selliguea griffithiana* growing in the forest canopy compared with the forest understorey

Hua-Zheng Lu[1,6], Wen-Yao Liu[1,*], Fei-Hai Yu[2], Liang Song[1], Xing-Liang Xu[3], Chuan-Sheng Wu[1,6], Yu-Long Zheng[1], Yang-Ping Li[1,6], He-De Gong[4], Ke Chen[1,6], Su Li[1], Xi Chen[1,6], Jin-Hua Qi[1] and Shu-Gang Lu[5,*]

[1]Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla 666303, China, [2]School of Nature Conservation, Beijing Forestry University, Beijing 100083, China, [3]Key Laboratory of Ecosystem Network Observation and Modeling, Chinese Academy of Sciences, Institute of Geographic Sciences and Natural Resources Research, Beijing 100101, China, [4]Faculty of Ecotourism, Southwest Forestry University, Kunming 650224, China, [5]Institute of Ecology and Geobotany, Yunnan University, Kunming 650091, China and [6]University of Chinese Academy of Sciences, Beijing 100049, China
** For correspondence. E-mail liuwy@xtbg.ac.cn or shuganglu@163.com*

Received: 24 January 2015 Returned for revision: 3 March 2015 Accepted: 30 March 2015

• **Background and Aims** The advantage of clonal integration (resource sharing between connected ramets of clonal plants) varies and a higher degree of integration is expected in more stressful and/or more heterogeneous habitats. Clonal facultative epiphytes occur in both forest canopies (epiphytic habitats) and forest understories (terrestrial habitats). Because environmental conditions, especially water and nutrients, are more stressful and heterogeneous in the canopy than in the understorey, this study hypothesizes that clonal integration is more important for facultative epiphytes in epiphytic habitats than in terrestrial habitats.

• **Methods** In a field experiment, an examination was made of the effects of rhizome connection (connected vs. disconnected, i.e. with vs. without clonal integration) on survival and growth of single ramets, both young and old, of the facultative epiphytic rhizomatous fern *Selliguea griffithiana* (Polypodiaceae) in both epiphytic and terrestrial habitats. In another field experiment, the effects of rhizome connection on performance of ramets were tested in small (10×10 cm^2) and large (20×20 cm^2) plots in both epiphytic and terrestrial habitats.

• **Key Results** Rhizome disconnection significantly decreased survival and growth of *S. griffithiana* in both experiments. The effects of rhizome disconnection on survival of single ramets and on ramet number and growth in plots were greater in epiphytic habitats than in terrestrial habitats.

• **Conclusions** Clonal integration contributes greatly to performance of facultative epiphytic ferns, and the effects were more important in forest canopies than in forest understories. The results therefore support the hypothesis that natural selection favours genotypes with a higher degree of integration in more stressful and heterogeneous environments.

Key words: Clonal integration, facultative epiphyte, forest canopy, forest understorey, habitat adaptation, ephiphytic fern, *Selliguea griffithiana*, Polypodiaceae, resource heterogeneity, resource sharing, clonal plant.

INTRODUCTION

Forest canopies possess an indispensable proportion of biodiversity, and are attracting increasing concerns from ecologists (Ellwood and Foster, 2004; May, 2010; Nadkarni *et al.*, 2011; Lowman and Schowalter, 2012). One important component of canopy biodiversity is the presence of epiphytes (obligate or facultative; Lowman and Schowalter, 2012; Zotz, 2013*b*), but how epiphytes adapt to forest canopies remains one of the key questions in canopy ecological research (Helbsing *et al.*, 2000; Zotz and Hietz, 2001; Benzing, 2004; Reyes-Garcia *et al.*, 2012). One notable pattern is that almost all non-vascular epiphytes (e.g. bryophytes; Jackson *et al.*, 1985; de Kroon and van Groenendael, 1997) and many vascular epiphytes (e.g. orchids, bromeliads, lycophytes and ferns; Dubuisson *et al.*, 2003; Duivenvoorden *et al.*, 2005; Wolf *et al.*, 2009; Mondragón and Ticktin, 2011; Tetsana *et al.*, 2014) are capable of clonal growth. However, how clonal traits such as physiological integration affect the adaptation of epiphytes to forest canopies is virtually unknown.

Facultative epiphytes grow in both forest canopies and forest understories (Benzing, 2004; Zotz, 2013*a*). Many facultative epiphytic ferns grow in the interstices of bark and in the junctions of tree trunks and branches of trees in forest canopies (epiphytic habitat) and also in the soil of forest understories (terrestrial habitats). Compared with terrestrial habitats, such epiphytic habitats have a smaller storage capacity for water and nutrients and lower physical stability with regard to airflow, and breakage of branches (Laube and Zotz, 2003; Cardelús and Mack, 2010; Lowman and Schowalter, 2012; Mondragón *et al.*, 2015). Also, the availability of water and nutrients is more variable and less predictable in epiphytic habitats than in terrestrial habitats (Benzing, 2004; Winkler and Zotz, 2010; Li *et al.*, 2014; Mondragón *et al.*, 2015). Furthermore, epiphytic habitats are characterized by more extreme fluctuations in moisture and temperature, higher light levels, higher wind speed, and more severe and variable vapour pressure deficits compared with terrestrial habitats (Théry, 2001; Zotz and Hietz, 2001; Lowman and Schowalter, 2012). Thus, environmental conditions are usually harsher and more heterogeneous in such epiphytic habitats (i.e. the interstices of bark and the junctions of tree trunks and branches) than in terrestrial habitats (Théry, 2001; Laube and Zotz, 2003, 2006; Lowman and Schowalter, 2012).

Physiological integration (translocations of photosynthates, water or nutrients between ramets interconnected by stolons, rhizomes or roots) is a key clonal trait that helps clonal plants to survive, grow and spread in stressful and/or heterogeneous environments (Jónsdóttir and Watson, 1997; Hutchings and Wijesinghe, 2008; Janeček *et al.*, 2008; Yu *et al.*, 2008; Xu *et al.*, 2012; Song *et al.*, 2013). In harsher or more heterogeneous environments, clonal plant species or genotypes with a higher degree of physiological integration are expected to be advantageous (Caraco and Kelly, 1991; Jónsdóttir and Watson, 1997; Alpert *et al.*, 2003; D'Hertefeldt *et al.*, 2014). Indeed, genotypes of the stoloniferous herb *Duchesnea indica* growing at higher (more stressful) altitude showed a higher degree (effect) of integration than those growing at lower (less stressful) altitude (Chen *et al.*, 2006), and genotypes of the stoloniferous herb *Fragaria chiloensis* from more heterogeneous dune habitats had a higher degree of integration than those from less heterogeneous grassland habitats (Alpert, 1999). In both epiphytic and terrestrial habitats of some clonal facultative epiphytes that form large clones spanning a large distance, ramets growing in resource-poor microsites are very likely to be connected to and thus get support from ramets growing in resource-rich microsites (Cornelissen *et al.*, 2014; Roiloa *et al.*, 2014), thereby increasing their survival and growth through clonal integration. As resources such as water and nutrients may be more limited and heterogeneously distributed in epiphytic than in terrestrial habitats, we expect that effects of clonal integration on survival and growth of facultative epiphytes are greater in epiphytic habitats (forest canopies) than in terrestrial habitats (forest understories).

The effects of clonal integration usually depend on the age of ramets (Hartnett and Bazzaz, 1983; Bullock *et al.*, 1994). As ramets establish and produce more leaves and roots, they become more independent of their parent ramets. Thus, with increasing age of offspring ramets, the strength of the source–sink relationship between parent and offspring ramets gradually decreases so that the degree of integration decreases (Hartnett and Bazzaz, 1983; Bullock *et al.*, 1994).

To test the effects of clonal integration on survival and growth of facultative epiphytes, we conducted two field experiments with the evergreen, facultatively epiphytic, rhizomatous fern *Selliguea griffithiana* in both epiphytic and terrestrial habitats in a montane moist evergreen broadleaved forest in south-west China. In these forests, precipitation occurs mostly in the wet season (May–October), with drought in the dry season (November–April) when our experiments were conducted. The forest canopies are more stressful and heterogeneous with regard to water and available nutrients compared with the forest understories (Qi *et al.*, 2012; You *et al.*, 2013*a*, *b*, *c*; Li *et al.*, 2014). Specifically, we addressed the following questions: (1) Does clonal integration increase survival and growth of *S. griffithiana* in the dry season in the montane moist evergreen broadleaved forest? (2) Is such an effect greater in epiphytic habitats than in terrestrial habitats? (3) Do younger ramets benefit more from clonal integration than older ramets, and does such an effect depend on habitat?

MATERIALS AND METHODS

Selliguea griffithiana (Hooker) Fraser-Jenkins (Polypodiaceae), synonymous with *Phymatopteris griffithiana* (Hooker) Pichi Sermolli or *Polypodium griffithianum* Hooker, is widely distributed in south-east, east and south Asia (Li *et al.*, 2012). In the tropical and subtropical forests of southern and south-west China, *S. griffithiana* is evergreen with overwintering simple fronds. It is a facultatively epiphytic fern and grows in both epiphytic and terrestrial habitats (Zhang, 2012). In epiphytic habitats *S. griffithiana* mainly inhabits interstices of bark and the junctions of tree trunks and branches (ramet height, 17·82 ± 0·50 cm; frond length, 12·08 ± 0·42 cm; mean ± s.e., $n = 60$), and in terrestrial habitats it grows in the soil or rocks of the forest understory (ramet height, 19·87 ± 0·68 cm; frond length, 12·48 ± 0·47 cm; mean ± s.e., $n = 60$). This fern can propagate via rhizomes (long-creeping rhizomes) and form vertical, perennial simple fronds (ramets) with adventitious roots (Flora of China, http://foc.eflora.cn/content.aspx?TaxonId=250098769). The mean distance between adjacent ramets along a rhizome is 4·21 ± 0·13 cm (mean ± s.e., $n = 120$). Both rhizomes and above-ground parts of ramets can last several years, and the rhizomes can remain connected even if above-ground parts of ramets die (our personal observations). The size of an interconnected clone varies greatly and depends on the age of the clone and the disturbance status of the forest. Violent disturbance by rodents, strong wind and storms can fragment the clones into different sizes, and fragmentation may also occur due to ageing. A clone of *S. griffithiana* may consist of several to hundred interconnected ramets spanning a few centimetres to several metres along trunks and branches of trees (our personal observations).

Study site

Montane moist evergreen broadleaved forests, characterized by high epiphyte abundance and high humidity (Cardelús *et al.*, 2006; Larrea and Werner, 2010; Li *et al.*, 2014), are an important global vegetation type and occur mainly in subtropical mountains at high altitude in Yunnan Province, south-west China (Song *et al.*, 2012). The subtropical montane moist evergreen broadleaved forest in the Xujiaba region (24 °32′N, 101 °01′E; 2000–2600 m above sea level) in Yunnan province, China, is a core area of the Ailao Mountain National Natural Reserve, covering 5100 ha on the northern crest of the Ailao Mountains (Li *et al.*, 2014). The forest is influenced by the south-west monsoon and is exposed to frequent and intense wind and mist events throughout the year (Wu *et al.*, 2014). Mean annual precipitation in the forest is 1902 mm, with 1630 mm (86 %) falling during the wet season (May–October) and 272 mm (14 %) during the dry season (November–April; Supplementary Data Fig. S1; Wu *et al.*, 2014). The potential evaporation is 383·7 mm in the wet season and 441·5 mm in the dry season (You *et al.*, 2013*a*, *b*, *c*). Therefore, seasonal drought occurs in the dry season (You *et al.*, 2013*a*, *b*, *c*). Mean annual relative humidity is 85 % and annual mean air temperature is 11·3 °C (Li *et al.*, 2014). The soil under the forest is typically brown earth. The texture is loam with a pH of 3.6. A 3- to 7-cm litter layer covers almost all the soil surface (Chen *et al.*, 2010). Our experiments were conducted in the dry season.

In this study site, the forest canopy has strong effects on the understory micrometeorology (You *et al.*, 2013*a*, *b*, *c*). Temperature in the forest understory was 2·4 °C lower than outside, and maximal temperature was lower and the minimal temperature was higher in the forest understory than outside the forest. Understorey radiation components are greatly reduced by the forest canopy (You *et al.*, 2013*a*, *b*, *c*). The microclimatic observation systems (Hobo U30, Onset Computer Corp., Bourne, MA, USA) showed that from November to April (the dry season) leaf wetness in the forest canopy was lower than in the forest floor ($t = 4{\cdot}1$, $P = 0{\cdot}010$, d.f. = 5), whereas photosynthetically active radiation (PAR) was the opposite ($t = 2{\cdot}6$, $P = 0{\cdot}047$, d.f. = 5). Inner branches of trees in the canopy are also light-limited, and photosynthesis mainly depends on unpredictable sun-flecks (Zhang *et al.*, 2009). Furthermore, for the epiphytes, water and nutrients are relatively richer in the junctions between trunks and branches of trees than in the trunks or branches, and even in the small scale of the junctions they are richer in the centre than outside the centre (Ingram and Nadkarni, 1993; Chen *et al.*, 2010).

In the montane moist evergreen broadleaved forests on Ailao Mountain, there are more than 600 epiphytic species, including 114 epiphytic ferns and lycophytes (Li *et al.*, 2014). Epiphytic ferns account for 52–53 % of vascular epiphytic biodiversity there (Xu and Liu, 2005; Hsu and Wolf, 2009).

Experimental design

Single-ramet experiment. On 28–30 November 2012, we selected 60 relatively young and 60 relatively old ramets of *S. griffithiana* from both an epiphytic habitat (in the interstices of bark and in the junctions of trunks or branches of host trees) and a terrestrial habitat (on the ground of the forest understorey). The young ramet was the first ramet nearest the tip of a rhizome, and the relatively old ramet was the second or the third ramet from the tip. Ramets from the same host trees (i.e. phorophytes) were at least 50 cm apart, and those from different phorophytes were at least 2 m apart. Epiphytic ramets (i.e. ramets on phorophytes or from epiphytic habitats) were on 20 phorophytes, and terrestrial ramets (i.e. ramets on the ground) were in three $20 \times 30\text{-m}^2$ sites. The 20 phorophytes were also located within the three sites. The genetic background of the ramets was unknown and we were unsure whether ramets on adjacent phorophytes or plots belonged to different genets. Half of the ramets in each location from each habitat were randomly assigned to rhizome disconnection (severed ramets) or connection (intact ramets) treatments. For the disconnection treatment, the two rhizome internodes at the two ends of the ramet were carefully exposed and cut off halfway from the ramet (Supplementary Data Fig. S2B). For the connection treatment, the rhizome internodes of the ramet were also carefully exposed, but no cut was made. After the treatments, the soil, if any, was backfilled. On 28–30 April 2013, the survival status of all ramets was noted and the surviving ramets were harvested. We measured frond length, width and thickness, and stipe length and diameter as morphological measures, and above-ground (lamina plus stipe) and below-ground (rhizome plus root) dry mass as growth measures. Before harvest, we also measured maximum quantum yield of PS II (F_v/F_m) as a physiological measure using a portable fluorometer (FSM-2; Hansatech, King's Lynn, UK).

Plot experiment. We selected 20 small plots (10×10 cm^2) and 20 large plots (20×20 cm^2). The initial number of ramets ranged from three to five in the small plots and from seven to ten in the large ones. It did not differ significantly between the connection and the disconnection treatments in either the small plots ($3{\cdot}65 \pm 0{\cdot}18$ vs. $3{\cdot}65 \pm 0{\cdot}20$; mean ± s.e., $n = 20$; $F < 0{\cdot}001$, $P = 1{\cdot}000$) or the large plots ($7{\cdot}30 \pm 0{\cdot}35$ vs. $7{\cdot}15 \pm 0{\cdot}33$; mean ± s.e., $n = 20$; $F = 0{\cdot}34$, $P = 0{\cdot}562$). Although ramet density in the small plots was twice that in the large plots, these were the values we commonly found in the field (Supplementary Data Fig. S2A). Some of the ramets within a plot were interconnected and some were not (personal observation during harvest). Plots were at least 2 m apart. In the terrestrial habitat the plots were located within three $20 \times 30\text{-m}^2$ sites, and in the epiphytic habitat, the plots were located on 20 phorophytes distributed also within the three sites. For the disconnection treatment in the epiphytic habitat, the rhizomes, roots and bryophytes along the edges of each plot were cut with a sharp blade so that ramets inside the plot were disconnected from those outside the plot. For the disconnection treatment in the terrestrial habitat, the soil along the edges of the plot was cut down with a sharp blade to a depth of 20–30 cm (Du *et al.*, 2010), which was sufficient to sever all rhizomes of *S. griffithiana*. Half (ten) of both the small and the large plots were used for the rhizome disconnection treatment and the other half (ten) were for the rhizome connection treatment (Fig. S2C). For the rhizome connection treatment, the microsite conditions along the edges of the plots were also disturbed in the same way as the rhizome disconnection treatment, except that the rhizomes were not severed (Fig. S2C). The experiment started on 30 November 2012 and ended on 30 April 2013. At the end of the experiment, we counted the number of surviving ramets in each plot, and measured maximum quantum yield of PS II (F_v/F_m) using a portable fluorometer (FSM-2) on two randomly selected ramets in each small plot and three randomly selected ramets in each large plot. We also measured frond length, width and thickness, and stipe length and diameter of each surviving ramet in each plot. We then harvested above-ground (lamina and stipe) and below-ground part (rhizomes and roots) of all surviving ramets in each plot.

Statistical analyses

We used a generalized linear model to test effects of rhizome connection (with vs. without clonal integration), habitat (epiphytic vs. terrestrial) and age (old vs. young) on the survival probability of single ramets. For this analysis, survival probability was modelled using a binomial error distribution and logit link function (McCullagh and Nelder, 1989). We used three-way ANOVA to test effects of rhizome connection, habitat, age and their interactions on growth (total, and above-ground and below-ground mass), morphology (frond length, width and thickness, stipe length and diameter) and physiology (F_v/F_m) of the ramets in the single-ramet experiment. For these analyses, measures of growth, morphology and physiology were modelled using a normal distribution.

For the plot experiment, we expressed the final growth data (number of ramets and biomass) on the basis of per initial ramet because the initial number of ramets differed greatly between the small and the large plots ($3{\cdot}65 \pm 0{\cdot}13$ vs. $7{\cdot}23 \pm 0{\cdot}24$; mean ± s.e., $n = 40$; $F = 193{\cdot}7$, $P < 0{\cdot}001$) and also between the two habitats (epiphytic vs. terrestrial: $6{\cdot}38 \pm 0{\cdot}36$ vs. $4{\cdot}50 \pm 0{\cdot}25$; mean ± s.e., $n = 40$; $F = 17{\cdot}0$, $P < 0{\cdot}001$). We used three-way ANOVA to test the effects of rhizome connection, habitat, plot size and their interactions on growth (number of ramets, total mass, above-ground and below-ground mass) per initial ramet, morphology (frond length, width and thickness, stipe length and diameter) and physiology (F_v/F_m) of the ramets in the plot experiment. All variables in the plot experiment were modelled using a normal distribution.

For variables that were analysed using ANOVA, data were checked for normality using a Kolmogorov–Smirnov test and homogeneity of variance using Levene's test. Data were transformed to the square-root or natural-logarithm to meet the assumptions of ANOVA if needed. Statistical analyses were carried out with SPSS 19.0 and R software (R Development Core Team, 2012).

RESULTS

Single-ramet experiment

Overall, rhizome disconnection (likelihood ratio $\chi^2 = 71{\cdot}20$, $P < 0{\cdot}001$) and habitat ($\chi^2 = 7{\cdot}28$, $P = 0{\cdot}007$) significantly affected survival probability of the single ramets of *S. griffithiana*, but ramet age ($\chi^2 = 0{\cdot}84$, $P = 0{\cdot}358$) did not (Fig. 1A). When the rhizome was connected, all ramets but one survived in both epiphytic and terrestrial habitats (Fig. 1A). However, when the rhizome was disconnected, survivorship decreased to 70 % in the terrestrial habitat and 45 % in the epiphytic habitat (Fig. 1A).

Rhizome disconnection significantly decreased total, above-ground and below-ground mass of the single ramet of *S. griffithiana*, and such an effect did not depend on habitat or ramet age (Table 1, Fig. 1B–D). Total and below-ground mass were significantly higher in the terrestrial habitat than in the epiphytic habitat (Table 1, Fig. 1B, D), and total and above-ground mass were significantly greater when the ramet was relatively old than when it was relatively young (Table 1, Fig. 1B, C).

Rhizome disconnection significantly decreased F_v/F_m of the single ramet of *S. griffithiana*; such an effect did not depend on habitat, but was greater when the ramet was relatively old than when it was young (Table 1: S × A effect $P < 0{\cdot}05$, Fig. 2A). Rhizome disconnection did not significantly affect frond length or frond width (Table 1, Fig. 2B, C, E), but significantly decreased frond thickness and stipe diameter (Table 1, Fig. 2D, F). The negative effect of disconnection on frond thickness was greater in the epiphytic habitat than in the terrestrial habitat (Table 1: S × H effect $P < 0{\cdot}001$, Fig. 2D), and when the ramet was relatively old than when it was young (Table 1: S × A effect $P < 0{\cdot}05$, Table 1, Fig. 2D).

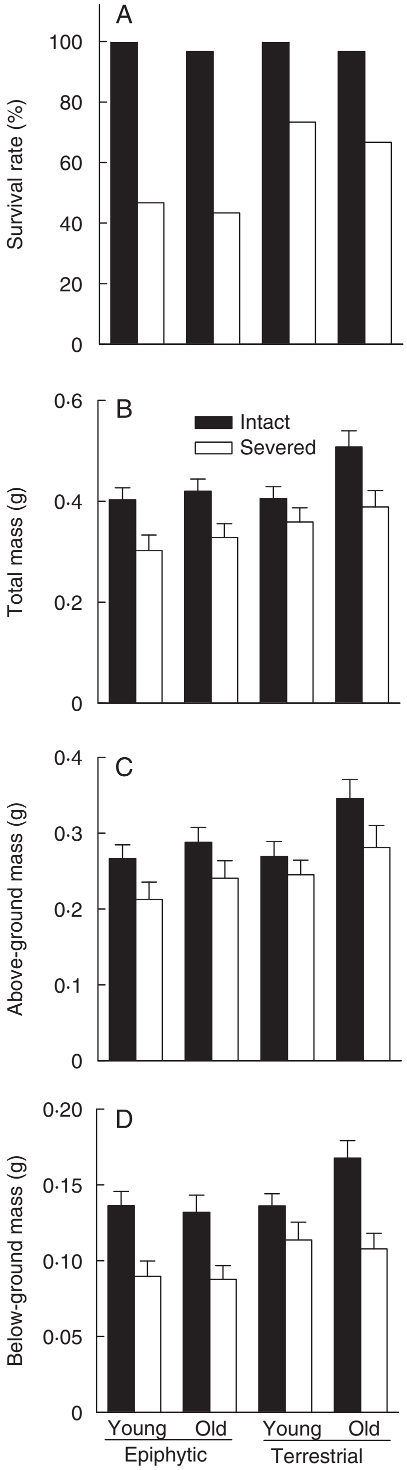

FIG. 1. Effects of habitat (epiphytic vs. terrestrial), severance (connected vs. disconnected) and age (young vs. old) on survival (A) and biomass (B–D) of *Selliguea griffithiana* in the single-ramet experiment. Means ± s.e. are given.

Plot experiment

On the basis of per initial ramet, rhizome disconnection significantly decreased number of ramets, and total, above-ground and below-ground mass of *S. griffithiana* in the plot experiment (Table 2: severance effect $P < 0{\cdot}01$). The negative effect of rhizome disconnection on the number of ramets was significantly larger in the epiphytic habitat than in the terrestrial habitat

TABLE 1. *Results of the three-way ANOVA (*F*-values) for the effects of severance (rhizome disconnection), habitat and age on ramet biomass,* F_v/F_m*, and frond and stipe morphology traits of* Selliguea griffithiana *in the single-ramet experiment*

Variable	Severance (S)	Habitat (H)	Age (A)	S × H	S × A	H × A	S × H × A
Total mass	17·63***	5·61*	3·99*	0·12	0·48	1·15	0·97
Above-ground mass†	8·00**	3·10$^{\#}$	5·18*	0·10	0·13	0·57	0·65
Below-ground mass	25·68***	4·84*	0·14	0·21	0·73	0·66	1·10
F_v/F_m‡	72·09***	12·20**	31·20***	1·27	13·93*	1·84	0·03
Frond length†	0·07	6·33*	4·02*	0·07	0·29	0·02	0·73
Frond width	1·84	1·33	0·34	0·29	0·02	0·55	0·13
Frond thickness	34·52***	0·53	0·17	42·16***	5·77*	1·85	0·16
Stipe length	3·34$^{\#}$	26·39***	2·85$^{\#}$	0·05	0·03	0·30	0·14
Stipe diameter	5·97*	7·16**	0·56	1·35	2·49	2·54	0·00

*** $P < 0·001$, **$P < 0·01$, *$P < 0·05$ and $^{\#}P < 0·1$.

† Square-root transformation.

‡ Log transformation.

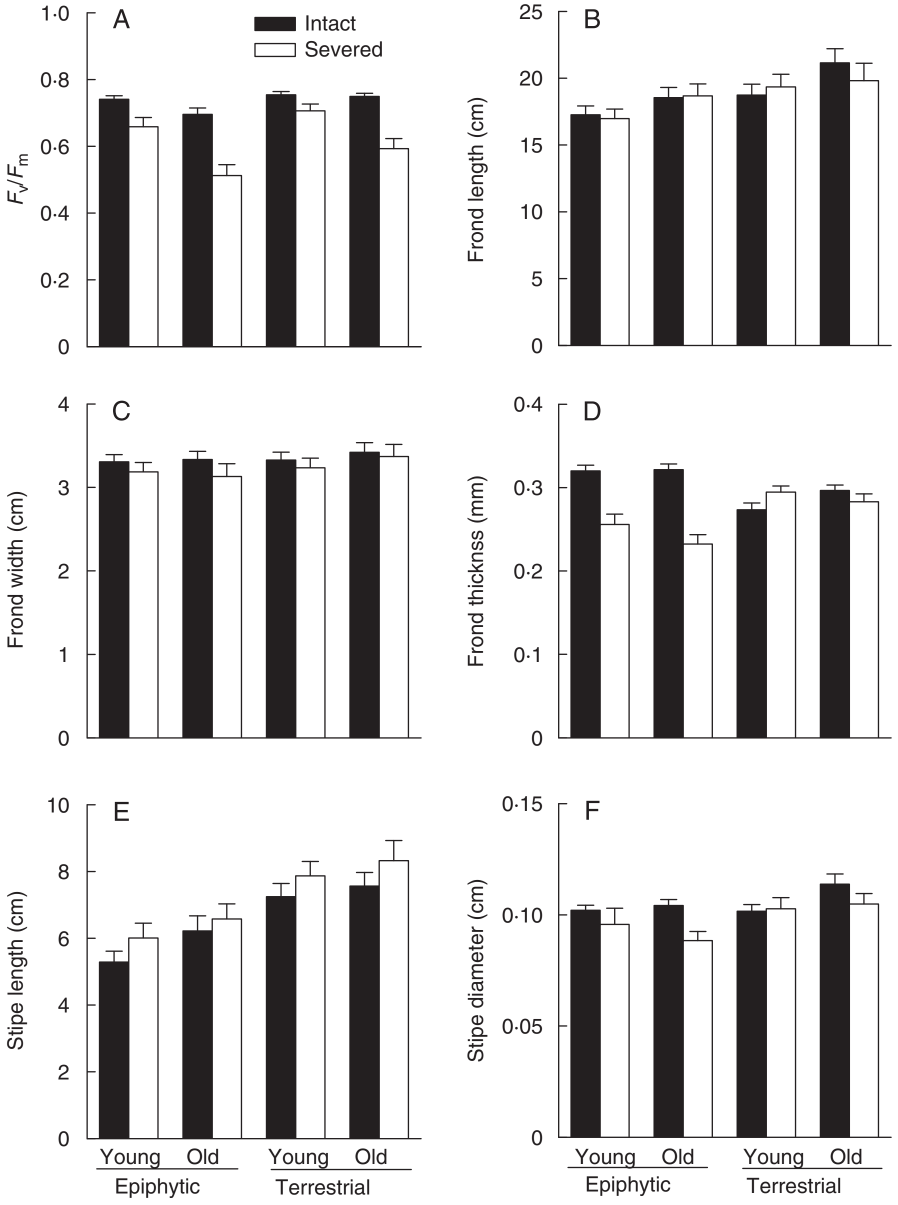

FIG. 2. Effects of habitat (epiphytic vs. terrestrial), severance (connected vs. disconnected) and age (young vs. old) on F_v/F_m (A), and morphology of frond (B–D) and stipe (E, F) of *Selliguea griffithiana* in the single-ramet experiment. Means ± s.e. are given.

TABLE 2. *Results of the three-way ANOVA* (F-*values*) *for effects of severance (rhizome disconnection), habitat and plot size on ramet number, biomass,* F_v/F_m, *and frond and stipe morphology traits of* Selliguea griffithiana *in the plot experiment*

Variable	Severance (S)	Habitat (H)	Plot size (P)	S × H	S × P	H × P	S × H × P
No. of ramets‡	8·57**	18·33***	4·55*	6·41*	7·06**	1·39	0·66
Total mass†	10·48**	0·04	0·15	3·13$^{\#}$	1·60	0·16	2·19
Above-ground mass	7·50**	2·20	2·82$^{\#}$	1·13	1·08	0·08	1·21
Below-ground mass	7·63**	0·36	0·24	2·90$^{\#}$	1·03	0·55	2·27
F_v/F_m	0·15	7·27**	1·54	0·05	1·76	0·36	0·87
Frond length‡	0·22	10·34**	1·41	0·97	0·34	1·59	1·49
Frond width	1·37	3·34$^{\#}$	0·87	0·35	1·05	0·91	0·56
Frond thickness	1·83	14·51***	5·99*	6·36*	5·38*	0·08	5·50*
Stipe length‡	0·15	57·56***	2·87$^{\#}$	0·06	0·46	0·06	0·54
Stipe diameter	1·20	39·21***	6·00*	1·49	1·39	0·02	0·94

*** $P < 0·001$, ** $P < 0·01$, * $P < 0·05$ and $^{\#}P < 0·1$.
† Square-root transformation.
‡ Log transformation.

(Fig. 3A, Table 2: S × H effect $P < 0·05$) and that on total and below-ground mass tended to be larger (Fig. 3B, D, Table 2: S × H effect $P < 0·1$). Also, the negative effect on the number of ramets was significantly greater when the plot size was small than when it was large (Fig. 3A, Table 2: S × P effect $P < 0·01$). There was no significant effect of severance × habitat × plot size on ramet number or biomass (Table 2).

Rhizome disconnection had little effect on F_v/F_m, frond length and width, or stipe length and diameter in the plot experiment (Table 2, Fig. 4A–C, E, F). Frond thickness was significantly larger in the small plot in the epiphytic habitat, but did not differ in the large plot in the epiphytic habitat or in the small or large plot in the terrestrial habitat (Table 2: S × H, S × P and S × H × P effect $P < 0·05$, Fig. 4D)

DISCUSSION

Both the single-ramet and the plot experiment showed that rhizome connection contributed greatly to survival and growth of the facultative epiphytic fern *S. griffithiana* in both forest canopies and forest understories. The effects were mainly due to resource sharing (clonal integration), although it is unclear what resources were shared, which could be addressed using isotope labelling in future studies (Jackson *et al.*, 1985). In forests where epiphytes are abundant, such as the subtropical montane moist evergreen broadleaved forest of our study area, environmental conditions are harsh (You *et al.*, 2013*a*, *b*, *c*) and facultative epiphytes growing in canopies may suffer frequently from water and nutrient shortage and those in understories from low light (Chazdon and Pearcy, 1991; Poorter *et al.*, 2005; Lowman and Schowalter, 2012; Qi *et al.*, 2012; Li *et al.*, 2014). Even in the rainy season in tropical rain forests, short-term drought stress occurs regularly in the canopy (Windsor, 1990; Zotz and Thomas, 1999; Watkins *et al.*, 2007). Furthermore, water, nutrients and light are distributed heterogeneously at small scales both in space and in time (Cardelús and Mack, 2010; Bartels and Chen, 2010; Freschi *et al.*, 2010; Lowman and Schowalter, 2012; Reyes-Garcia *et al.*, 2012). In such stressful and heterogeneous environments, keeping rhizome connection and thus the functioning of clonal integration may confer facultatively clonal epiphytes with great advantages by alleviating the negative effects of water and nutrient shortage and/or low light and making better use of the heterogeneously distributed resources (de Kroon *et al.*, 1991; Alpert, 1999; Alpert *et al.*, 2003; Hutchings and Wijesinghe, 2008; Janeček *et al.*, 2008; Song *et al.*, 2013).

Results of both experiments also suggested that the effects of clonal integration on survival and/or growth of *S. griffithiana* were stronger in forest canopies than in forest understories, supporting the hypothesis that stronger integration is favoured in more stressful and/or more heterogeneous environments (Caraco and Kelly, 1991; Jónsdóttir and Watson, 1997; Alpert, 1999; Alpert *et al.*, 2003; Chen *et al.*, 2006; Nilsson and D'Hertefeldt, 2008; D'Hertefeldt *et al.*, 2014). Facultative epiphytes growing in forest canopies usually suffer from water and nutrient stress more frequently than those in forest understories (Lowman and Schowalter, 2012; Qi *et al.*, 2012; Li *et al.*, 2014). Furthermore, other abiotic conditions such as temperature were more spatially and temporally variable in forest canopies than in forest understories (Lowman and Schowalter, 2012; You *et al.*, 2013*b*, *c*). Both theoretical (Caraco and Kelly, 1991; Alpert, 1999) and empirical (Alpert, 1999; Alpert *et al.*, 2003; Chen *et al.*, 2006; D'Hertefeldt *et al.*, 2014) studies have shown that genotypes with a high degree of clonal integration were favoured when growing in more stressful and/or more heterogeneous environments. Forest canopies are harsher and more heterogeneous in resource supply (e.g. water and nutrients) than forest understories (Laube and Zotz, 2003; You *et al.*, 2013*b*, *c*; Li *et al.*, 2014), and thus may favour genotypes of epiphytes with a higher degree of integration (Alpert *et al.*, 2003). We therefore hypothesize that there may exist genetic differentiation between the *S. griffithiana* ramets in forest canopies and those in forest understories. This hypothesis could be tested by a reciprocal transplant experiment between the two habitats using two sets of identical ramets collected from forest canopies and forest understories, respectively.

Unlike the rhizomes of terrestrial plants on forest floors, rhizomes of epiphytes in forest canopies may have an additional function of anchoring epiphytes to phorophytes because many adventitious roots can be formed along the rhizomes (Schneider, 2000, 2013; Tsutsumi and Kato, 2006). So far, however, there has been little study to test the effect of rhizome connection on the anchoring capability of epiphytes

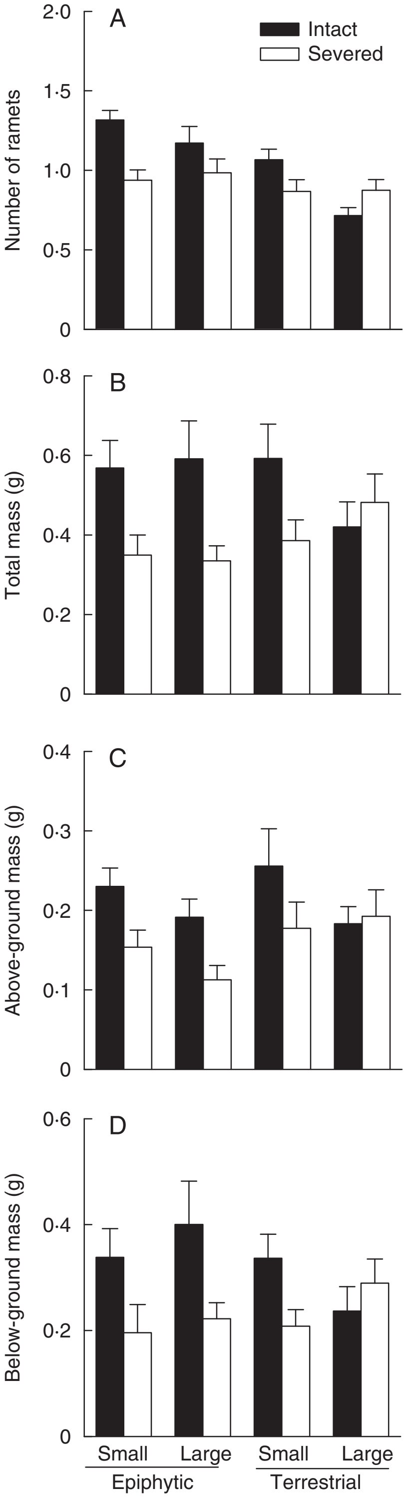

FIG. 3. Effects of habitat (epiphytic vs. terrestrial), severance (connected vs. disconnected) and plot size (small vs. large) on ramet number (A) and biomass (B–D) per initial ramet of *Selliguea griffithiana* in the plot experiment. Means ± s.e. are given.

to phorophytes. We therefore hypothesize that connected ramets of epiphytes would be more easily anchored to phorophytes than disconnected ramets, and that selection thus may favour epiphytes with long-lasting, connected rhizomes than those with short-lasting rhizomes. We did observe that rhizomes of *S. griffithiana* and adventitious roots along rhizomes remained connected and alive even if some ramets along the rhizomes had died. However, our experimental set-up did not allow us to separate the effects of clonal integration from those of anchoring. It would be promising to design experiments to test the roles of rhizome connection in anchoring of epiphytes to phorophytes.

The effect of rhizome connection on survival and growth of *S. griffithiana* did not vary with the age of the ramet, although the effect on F_v/F_m was larger in the relatively old than in the young ramets. Previous studies showed that the effect of clonal integration on survival or growth was more positive for offspring ramets than for parent ramets (Nielsen and Pedersen, 2000; Cullen *et al*., 2005) or for young ramets than for old ramets (Alpert *et al*., 2002). There are two possible explanations for this discrepancy. First, in our study the old ramets were only relatively old, and their size was very similar to that of the young ramets. Therefore, they may have the same resource collecting and stress-resistance abilities, and depend on clonal integration to a similar degree (see Du *et al*., 2010). In future studies, ramets differing more greatly in age should be used to test the potential dependence of physiological integration on ramet age. Second, for this facultatively epiphytic fern, the extent of clonal integration was likely to be very high in both canopies and understories. Therefore, more ramets contribute to the resources imported by the single ramet, no matter whether it was young or old.

Although below-ground mass per initial ramet was much higher in the plot experiment (epiphytic & terrestrial: 0·289 ± 0·031 vs. 0·268 ± 0·006 g, respectively; mean ± s.e., $n = 40$; Fig. 3D) than in the single-ramet experiment (epiphytic & terrestrial: 0·134 ± 0·007 vs. 0·151 ± 0·007 g, respectively; mean ± s.e., $n = 60$; Fig. 1D), the two experiments produced similar results regarding the effect of clonal integration and its dependence on habitats (Tables 1 and 2; Figs 1 and 3). Thus, our study provides robust evidence that clonal integration plays important roles in the adaption of the clonal facultatively epiphytic fern in subtropical forests, especially in the dry season when drought occurs (You *et al*., 2013*a*, *b*, *c*). On the other hand, it has been suggested that clonal integration may confer costs to clonal plants (Jackson *et al*., 1985). For instance, it may decrease performance of donor ramets that export resources (Wang *et al*., 2009) and allow systemic diseases to spread within clonal networks (Jackson *et al*., 1985; D’Hertefeldt and van der Putten, 1998). Furthermore, maintaining clonal connection needs additional energy to deal with the respiration of inter-ramet connectors (Jackson *et al*., 1985). However, the design of our experiments did not allow us to test the potential costs of clonal integration in the epiphytic fern because we did not monitor survival and growth of donor ramets. Further studies could be designed to examine the costs of integration in both forest canopies and understories.

CONCLUSIONS

The effects of rhizome connection on survival and growth of the facultatively epiphytic fern were more important in forest canopies than in forest understories. The effects of rhizome

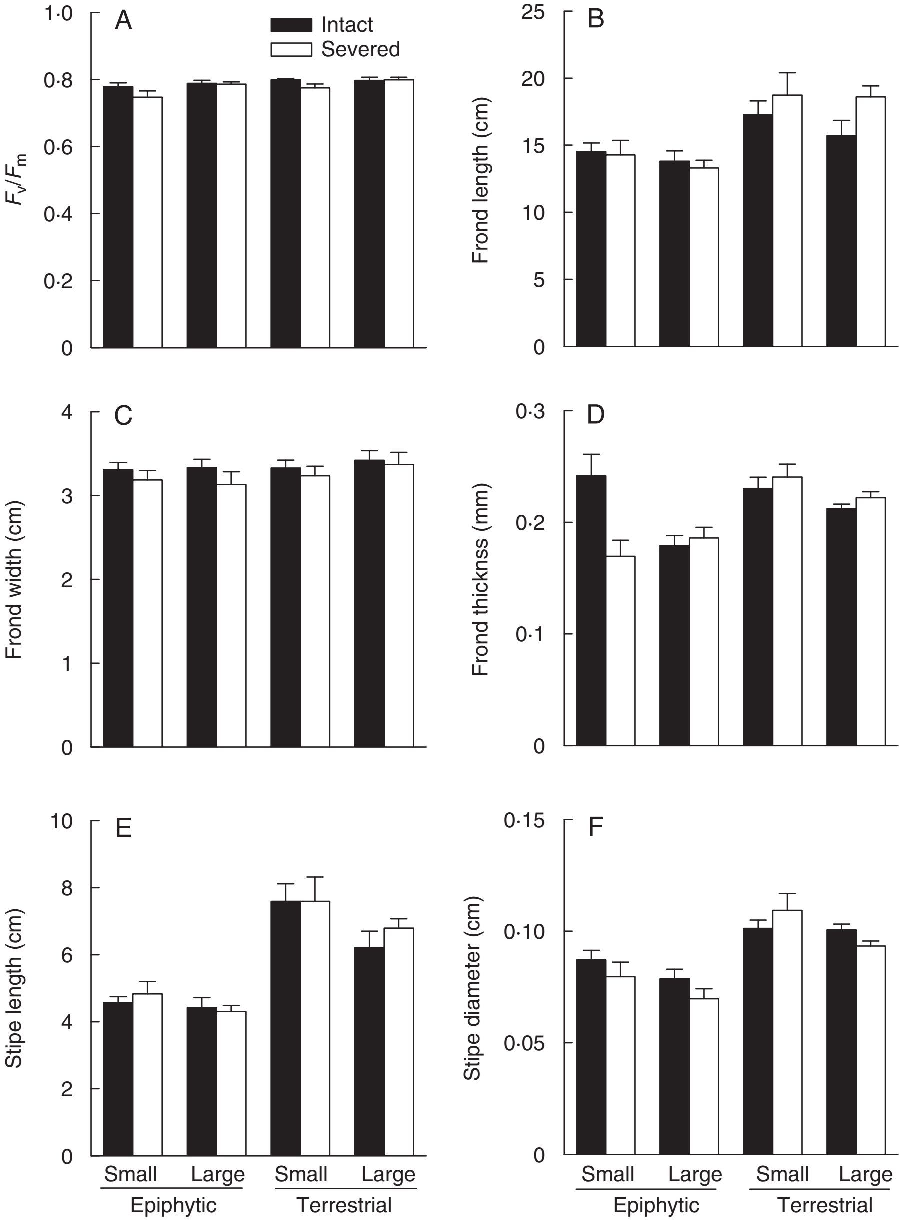

FIG. 4. Effects of habitat (epiphytic vs. terrestrial), severance (connected vs. disconnected) and plot size (small vs. large) on F_v/F_m (A), and morphology of frond (B–D) and stipe (E, F) of *Selliguea griffithiana* in the plot experiment. Means ± s.e. are given.

connections were mainly due to resource sharing (clonal integration), but increasing anchoring capacity may be an additional mechanism. Further studies should be designed to separate the effects of clonal integration from those of anchoring, and a reciprocal transplant experiment and a multi-species comparison are also needed to address the general question of the vital roles of rhizome connection in forest canopies and forest understories.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Figure S1: rainfall and air temperature in the dry and wet seasons. Figure S2: locations of epiphytes and experimental design.

ACKNOWLEDGMENTS

We thank Drs Jacques Frédéric and Bo Wang as well as two anonymous reviewers for their constructive comments, and Dr Yue-Hua Hu for suggestions regarding data analysis. We also thank Ailaoshan Station for Subtropical Forest Ecosystem Studies for providing experimental sites and background data. This study was supported by the National Natural Science Foundation of China (Nos. 31300459 and U1133605) and the Yunnan Natural Science Foundation (No. 2013FB076).

LITERATURE CITED

Alpert P. 1999. Clonal integration in *Fragaria chiloensis* differs between populations: ramets from grassland are selfish. *Oecologia* **120**: 69–76.

Alpert P, Holzapfel C, Benson JM. 2002. Hormonal modification of resource sharing in the clonal plant *Fragaria chiloensis*. *Functional Ecology* **16**: 191–197.

Alpert P, Holzapfel C, Slominski C. 2003. Differences in performance between genotypes of *Fragaria chiloensis* with different degrees of resource sharing. *Journal of Ecology* **91**: 27–35.

Bartels SF, Chen HY. 2010. Is understory plant species diversity driven by resource quantity or resource heterogeneity? *Ecology* **91**: 1931–1938.

Benzing DH. 2004. Vascular epiphytes. In: MD Lowman, HB Rinker. eds. *Forest canopies*, 2nd edn. Burlington: Elsevier Academic Press, 175–211, 544.

Bullock J, Mortimer A, Begon M. 1994. Physiological integration among tillers of *Holcus lanatus*: age-dependence and responses to clipping and competition. *New Phytologist* **128**: 737–747.

Caraco T, Kelly CK. 1991. On the adaptive value of physiological integraton in clonal plants. *Ecology* **72**: 81–93.

Cardelús CL, Mack MC. 2010. The nutrient status of epiphytes and their host trees along an elevational gradient in Costa Rica. *Plant Ecology* **207**: 25–37.

Cardelús CL, Colwell RK, Watkins JE. 2006. Vascular epiphyte distribution patterns: explaining the mid-elevation richness peak. *Journal of Ecology* **94**: 144–156.

Chazdon RL, Pearcy RW. 1991. The importance of sunflecks for forest understory plants. *BioScience* **41**: 760–766.

Chen J-S, Lei N-F, Yu D, Dong M. 2006. Differential effects of clonal integration on performance in the stoloniferous herb *Duchesnea indica*, as growing at two sites with different altitude. *Plant Ecology* **183**: 147–156.

Chen L, Liu W-Y, Wang G-S. 2010. Estimation of epiphytic biomass and nutrient pools in the subtropical montane cloud forest in the Ailao Mountains, south-western China. *Ecological Research* **25**: 315–325.

Cornelissen JHC, Song Y-B, Yu F-H, Dong M. 2014. Plant traits and ecosystem effects of clonality: a new research agenda. *Annals of Botany* **114**: 369–376.

Cullen BR, Chapman DF, Quigley PE. 2005. Carbon resource sharing and rhizome expansion of *Phalaris aquatica* plants in grazed pastures. *Functional Plant Biology* **32**: 79–85.

D'Hertefeldt T, van der Putten WH. 1998. Physiological integration of the clonal plant *Carex arenaria* and its response to soil-borne pathogens. *Oikos* **81**: 229–237.

D'Hertefeldt T, Eneström JM, Pettersson LB. 2014. Geographic and habitat origin influence biomass production and storage translocation in the clonal plant *Aegopodium podagraria*. *PLoS One* **9**: e85407.

de Kroon H, van Groenendael J. 1997. *The ecology and evolution of clonal plants*. Leiden: Backhuys Publishers.

de Kroon H, Whigham DF, Watson MA. 1991. Developmental ecology of mayapple: effects of rhizome severing, fertilization and timing of shoot senescence. *Functional Ecology* **5**: 360–368.

Du J, Wang N, Alpert P, Yu M-J, Yu F-H, Dong M. 2010. Clonal integration increases performance of ramets of the fern *Diplopterygium glaucum* in an evergreen forest in southeastern China. *Flora* **205**: 399–403.

Dubuisson JY, Hennequin S, Rakotondrainibe F, Schneider H. 2003. Ecological diversity and adaptive tendencies in the tropical fern *Trichomanes* L. (Hymenophyllaceae) with special reference to epiphytic and climbing habits. *Botanical Journal of the Linnean Society* **142**: 41–63.

Duivenvoorden JF, Vasco A, Callejas R. 2005. A first quantitative census of vascular epiphytes in rain forests of Colombian Amazonia. *Biodiversity and Conservation* **14**: 739–758.

Ellwood MD, Foster WA. 2004. Doubling the estimate of invertebrate biomass in a rainforest canopy. *Nature* **429**: 549–551.

Freschi L, Takahashi CA, Cambui CA *et al.* 2010. Specific leaf areas of the tank bromeliad *Guzmania monostachia* perform distinct functions in response to water shortage. *Journal of Plant Physiology* **167**: 526–533.

Hartnett D, Bazzaz F. 1983. Physiological integration among intraclonal ramets in *Solidago canadensis*. *Ecology* **64**: 779–788.

Helbsing S, Riederer M, Zotz G. 2000. Cuticles of vascular epiphytes: efficient barriers for water loss after stomatal closure? *Annals of Botany* **86**: 765–769.

Hsu RCC, Wolf JHD. 2009. Diversity and phytogeography of vascular epiphytes in a tropical–subtropical transition island, Taiwan. *Flora* **204**: 612–627.

Hutchings MJ, Wijesinghe DK. 2008. Performance of a clonal species in patchy environments: effects of environmental context on yield at local and whole-plant scales. *Evolutionary Ecology* **22**: 313–324.

Ingram SW, Nadkarni NM. 1993. Composition and distribution of epiphytic organic-matter in a neotropical cloud forest, Costa-Rica. *Biotropica* **25**: 370–383.

Jackson JBC, Buss LW, Cook RE. 1985. *Population biology and evolution of clonal organisms*. New Haven, CT: Yale University Press.

Janeček Š, Kantorová J, Bartoš M, Klimešová J. 2008. Integration in the clonal plant *Eriophorum angustifolium*: an experiment with a three-member-clonal system in a patchy environment. *Evolutionary Ecology* **22**: 325–336.

Jónsdóttir IS, Watson MA. 1997. Extensive physiological integration: an adaptive trait in resource-poor environments? In: H de Kroon, J van Groenendael, eds. *The ecology and evolution of clonal plants*. Leiden: Backhuys Publishers, 109–136.

Larrea ML, Werner FA. 2010. Response of vascular epiphyte diversity to different land-use intensities in a neotropical montane wet forest. *Forest Ecology and Management* **260**: 1950–1955.

Laube S, Zotz G. 2003. Which abiotic factors limit vegetative growth in a vascular epiphyte? *Functional Ecology* **17**: 598–604.

Laube S, Zotz G. 2006. Neither host-specific nor random: vascular epiphytes on three tree species in a Panamanian lowland forest. *Annals of Botany* **97**: 1103–1114.

Li C-X, Lu S-G, Ma J-Y *et al.* 2012. From the Himalayan region or the Malay Archipelago: molecular dating to trace the origin of a fern genus *Phymatopteris* (Polypodiaceae). *Chinese Science Bulletin* **57**: 4569–4577.

Li S, Liu W-Y, Li D-W *et al.* 2014. Slower rates of litter decomposition of dominant epiphytes in the canopy than on the forest floor in a subtropical montane forest, southwest China. *Soil Biology and Biochemistry* **70**: 211–220.

Lowman MD, Schowalter TD. 2012. Plant science in forest canopies – the first 30 years of advances and challenges (1980–2010). *New Phytologist* **194**: 12–27.

May RM. 2010. Tropical arthropod species, more or less? *Science* **329**: 41–42.

McCullagh P, Nelder JA. 1989. *Generalized linear models*, 2nd edn. London: Chapman and Hall.

Mondragón D, Ticktin T. 2011. Demographic effects of harvesting epiphytic bromeliads and an alternative approach to collection. *Conservation Biology* **25**: 797–807.

Mondragón D, Valverde T, Hernandez-Apolinar M. 2015. Population ecology of epiphytic angiosperms: a review. *Tropical Ecology* **56**: 1–39.

Nadkarni NM, Parker GG, Lowman MD. 2011. Forest canopy studies as an emerging field of science. *Annals of Forest Science* **68**: 217–224.

Nielsen SL, Pedersen MF. 2000. Growth, photosynthesis and nutrient content of seedlings and mature plants of *Cymodocea nodosa* – the importance of clonal integration. *Aquatic Botany* **68**: 265–271.

Nilsson J, D'Hertefeldt T. 2008. Origin matters for level of resource sharing in the clonal herb *Aegopodium podagraria*. *Evolutionary Ecology* **22**: 437–448.

Poorter L, Bongers F, Sterck FJ, Wöll H. 2005. Beyond the regeneration phase: differentiation of height-light trajectories among tropical tree species. *Journal of Ecology* **93**: 256–267.

Qi J-H, Zhang Y-J, Zhang Y-P *et al.* 2012. Water-holding capacity of an evergreen broadleaf forest in Ailao Mountain and its functions in mitigating the effects of Southwest China drought. *Acta Ecologica Sinica* **32**: 1692–1702.

R Development Core Team. 2012. *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: http://www.R-project.org.

Reyes-Garcia C, Mejia-Chang M, Griffiths H. 2012. High but not dry: diverse epiphytic bromeliad adaptations to exposure within a seasonally dry tropical forest community. *New Phytologist* **193**: 745–754.

Roiloa SR, Antelo B, Retuerto R. 2014. Physiological integration modifies δ^{15}N in the clonal plant *Fragaria vesca*, suggesting preferential transport of nitrogen to water-stressed offspring. *Annals of Botany* **114**: 399–411.

Schneider H. 2000. Morphology and anatomy of roots in the filmy fern tribe Trichomaneae H. Schneider (Hymenophyllaceae, Filicatae) and the evolution of rootless taxa. *Botanical Journal of the Linnean Society* **132**: 29–46.

Schneider H. 2013. Evolutionary morphology of ferns (Monilophytes). In: BA Ambrose, M Purugganan, eds. *Annual plant reviews: the evolution of plant form*. Oxford: Wiley-Blackwell, 1115–1140.

Song L, Liu W-Y, Nadkarni NM. 2012. Response of non-vascular epiphytes to simulated climate change in a montane moist evergreen broad-leaved forest in southwest China. *Biological Conservation* **152**: 127–135.

Song Y-B, Yu F-H, Keser LH *et al.* 2013. United we stand, divided we fall: a meta-analysis of experiments on clonal integration and its relationship to invasiveness. *Oecologia* **171**: 317–327.

Tetsana N, Pedersen HÆ, Sridith K. 2014. Character intercorrelation and the potential role of phenotypic plasticity in orchids: a case study of the epiphyte *Liparis resupinata*. *Plant Systematics and Evolution* **300**: 517–526.

Théry M. 2001. Forest light and its influence on habitat selection. In: K Eduard, AJD Linsenmair, B Fiala, MR Speight, eds. *Tropical forest canopies: ecology and management*. Dordrecht: Springer, 251–261.

Tsutsumi C, Kato M. 2006. Evolution of epiphytes in Davalliaceae and related ferns. *Botanical Journal of the Linnean Society* **151**: 495–510.

Wang N, Yu F-H, Li P-X *et al.* 2009. Clonal integration supports the expansion from terrestrial to aquatic environments of the amphibious stoloniferous herb *Alternanthera philoxeroides*. *Plant Biology* **11**: 483–489.

Watkins JE, Rundel PW, Cardelús CL. 2007. The influence of life form on carbon and nitrogen relationships in tropical rainforest ferns. *Oecologia* **153**: 225–232.

Windsor DM. 1990. *Climate and moisture variability in a tropical forest: long-term records from Barro Colorado Island, Panama*. Washington, DC: Smithsonian Institution Press.

Winkler U, Zotz G. 2010. 'And then there were three': highly efficient uptake of potassium by foliar trichomes of epiphytic bromeliads. *Annals of Botany* **106**: 421–427.

Wolf JHD, Gradstein SR, Nadkarni NM. 2009. A protocol for sampling vascular epiphyte richness and abundance. *Journal of Tropical Ecology* **25**: 107–121.

Wu C-S, Zhang Y-P, Xu X-L *et al.* 2014. Influence of interactions between litter decomposition and rhizosphere activity on soil respiration and on the temperature sensitivity in a subtropical montane forest in SW China. *Plant and Soil* **381**: 215–224.

Xu H-Q, Liu W-Y. 2005. Species diversity and distribution of epiphytes in the montane moist evergreen broad-leaved forest in Ailao Mountain, Yunnan. *Biodiversity Science* **13**: 137–147.

Xu L, Yu F-H, van Drunen E, Schieving F, Dong M, Anten NPR. 2012. Trampling, defoliation and physiological integration affect growth, morphological and mechanical properties of a root-suckering clonal tree. *Annals of Botany* **109**: 1001–1008.

You G-Y, Zhang Y-P, Liu Y-H *et al.* 2013a. Investigation of temperature and aridity at different elevations of Mt. Ailao, SW China. *International Journal of Biometeorology* **57**: 487–492.

You G-Y, Zhang Y-P, Liu Y-H *et al.* 2013b. On the attribution of changing pan evaporation in a nature reserve in SW China. *Hydrological Processes* **27**: 2676–2682.

You G-Y, Zhang Y-P, Schaefer D *et al.* 2013c. Observed air/soil temperature trends in open land and understory of a subtropical mountain forest, SW China. *International Journal of Climatology* **33**: 1308–1316.

Yu F-H, Wang N, He W-M, Chu Y, Dong M. 2008. Adaptation of rhizome connections in drylands: increasing tolerance of clones to wind erosion. *Annals of Botany* **102**: 571–577.

Zhang Q, Chen J-W, Li B-G, Cao K-F. 2009. Epiphytes and hemiepiphytes have slower photosynthetic response to lightflecks than terrestrial plants: evidence from ferns and figs. *Journal of Tropical Ecology* **25**: 465–472.

Zhang X-C. 2012. *Lycophytes and ferns of China*. Beijing: Peking University Press.

Zotz G. 2013a. 'Hemiepiphyte': a confusing term and its history. *Annals of Botany* **111**: 1015–1020.

Zotz G. 2013b. The systematic distribution of vascular epiphytes – a critical update. *Botanical Journal of the Linnean Society* **171**: 453–481.

Zotz G, Hietz P. 2001. The physiological ecology of vascular epiphytes: current knowledge, open questions. *Journal of Experimental Botany* **52**: 2067–2078.

Zotz G, Thomas V. 1999. How much water is in the tank? Model calculations for two epiphytic bromeliads. *Annals of Botany* **83**: 183–192.