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Shade-induced changes in the branching pattern of a stoloniferous herb: functional response or allometric effect?

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Abstract Shade-induced changes in the branching pattern of clonal plants can lead to conspicuous modifications of their growth form and architecture. It has been hypothesized that reduced branching in shade may be an adaptive trait, enabling clonal plants to escape from unfavourable patches in a heterogeneous environment by allocating resources preferentially to the growth of the main axis (i.e. linear expansion), rather than to local proliferation by branching. However, such an adaptionist interpretation may be unjustified if (1) branching frequency is a function of the ontogenetic stage of plants, and if (2) shading slows down the ontogenetic development of plants, thereby delaying branch formation. In this case, architectural differences between sun- and shade-grown individuals, harvested at the same chronological age, may not represent a functional response to changes in light conditions, but may be a by-product of effects of shade on the rate of plant development. To distinguish between these two alternatives, individuals of the stoloniferous herb *Potentilla reptans* were subjected to three experimental light conditions: a control treatment providing full daylight, and two shade treatments: neutral shade (13% of ambient PPFD; no changes in light spectral composition) and simulated canopy shade (13% PPFD and a reduced red:far-red ratio). Plant development was followed throughout the experiment by daily monitoring primary stolon growth as well as branch and leaf initiation. Biomass and clonal offspring production were measured when plants were harvested. At the end of the experiment shaded plants had produced significantly fewer branches than clones grown in full daylight. In all three treatments, however, initiation of secondary stolons occurred at the same developmental stage of individual ramets. Shading significantly slowed down the ontogenetic development of plants and this resulted in the observed differences in branching patterns between sun- and shade-grown individuals, when compared at the same chronological age. These results hence provide evidence that shade-induced changes in the branching pattern of clonal plants can be due to purely allometric effects. Implications for interpreting architectural changes in terms of functional shade-avoidance responses are discussed.

Key words Allometry · Architectural plasticity · Clonal growth · Plant development · Plastochron

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Introduction

Sun- and shade-grown individuals of many plant species may show visually conspicuous differences in architecture (e.g. Warming 1909), caused by environmentally induced changes in branching patterns, internode and petiole lengths and patterns of biomass allocation (Corré 1983 a, b; Slade and Hutchings 1987; Evans 1992; Schmitt and Wulff 1993). Such plasticity has often been interpreted as a functional response to changes in the environment, enabling plants to maximise their performance in spatially and temporally variable habitats. A number of concepts and hypotheses have been formulated to explain the proximal as well as the ultimate causes and consequences of plastic changes in plant architecture in response to shading (Grime 1979; Harper 1985; Grime et al. 1986; Hutchings 1988; Casal and Smith 1989; Ballaré 1994; Hutchings and de Kroon 1994).

For stoloniferous herbs, the adoption of a more linear growth form by reduced branching under shaded conditions has been interpreted as part of an avoidance strategy enabling horizontally spreading clones to escape from unfavourable resource conditions by giving priority to the extension of the main axis rather than to resource investments into local proliferation. In other

words, a plastic change in the branching pattern of stolons may represent a functional response minimizing the "residence time" of clones in shaded micro sites of spatially heterogeneous environments (Hutchings 1988; Hutchings and Slade 1988; de Kroon and Schieving 1990; Hutchings and de Kroon 1994). In mechanistic terms, adopting a more linear growth form would require greater lateral bud suppression under shaded conditions, causing individual ramets of expanding clones to form branches at a later stage in their ontogenetic development.

Such true plasticity in branching patterns, however, can easily be confounded with ontogenetic, allometric effects due to differences in the developmental stage of sun- and shade-grown individuals harvested at the same chronological age. Branch initiation does not usually occur at a constant rate during the first stages of the ontogenetic development of ramets (i.e. it is subject to "ontogenetic drift", *sensu* Evans 1972), which means that differences in ontogenetic development of clones can cause apparent differences in branching intensity. If environmental conditions (e.g. resource availability, toxic substances) decrease the rate of ontogenetic development, as described for various plant species (Silk 1980; Ackerly et al. 1992; Coleman and McConnaughay 1995; Jamadagni et al. 1995), differences in branching intensity between sun- and shade-grown individuals could be a result of ontogenetic drift (Coleman et al. 1994). In other words, ramets might branch at the same developmental stage in high- and low-light environments, and the differences in clonal architecture observable after a certain period of (chronological) time would simply be a consequence of (developmental) stage differences between plants. In this case shade-induced changes in branching frequency would represent an unavoidable side-effect of environmentally induced changes in the rate of ontogenetic development rather than a functional response to variable resource supply (Coleman et al. 1994).

Alterations in plant architecture due to shading represent complex interactive effects between aspects of growth (biomass production), morphogenesis (morphological plasticity), and ontogenetic development. These processes are triggered by environmental factors related to the light conditions in shaded habitats, such as decreased levels of radiation (photosynthetic photon flux density, PPFD, as a source of energy) and specific changes in the light spectral quality (a reduction of the red:far-red ratio; Casal and Smith 1989; Ballaré 1994; Aphalo and Ballaré 1995), which influence plant growth and development in different ways. Changes in light spectral quality are known to have pronounced effects on plant morphogenesis, such as internode elongation (e.g. Schmitt and Wulff 1993) and branching (e.g. Deregibus et al. 1985; Casal et al. 1987; Robin et al. 1994), while light quantity mainly affects plant traits related to growth and biomass production (Corré 1983 a, b; Hutchings and Slade 1988; Schmitt and Wulff 1993).

The experiment presented here aims to distinguish between plasticity in the timing of branch initiation and the effects of ontogenetic drift on branching patterns. Both of these processes, which are not mutually exclusive, may lead to linear growth forms in stoloniferous plants growing in shaded environments. The study specifically addresses the following questions:

1. Do shade-grown individuals of a stoloniferous species branch at a later developmental stage than light-grown plants or can their linear growth form be explained by shade-induced changes in the rate of whole-plant development?
2. Do simulated canopy shade and artificial shade in which the spectral quality of transmitted light remains unaltered have the same (or different) effects on branching patterns?

These questions will be answered by presenting detailed results on the developmental timing of clone expansion, and of branch and leaf initiation on primary ramets in the stoloniferous species *Potentilla reptans*. This species has previously been shown to adopt a linear growth form in shaded environments (Huber 1995). Plants were grown in full daylight and under two experimental shading conditions, in which the effect of simulated canopy shade were compared to those of spectrally neutral shading.

Materials and methods

The species

The stoloniferous, rosette-forming herb *Potentilla reptans* L. (Rosaceae) occurs in open habitats. It is especially common in regularly disturbed environments such as river shores, roadside verges and pastures. Established rosettes produce horizontally growing stems (i.e. primary stolons) which may root at their nodes and thereby give rise to potentially independent daughter rosettes (cf. Stuefer et al. 1994; Huber 1995, 1996). The terms rosettes and ramets will be used interchangeably in this paper.

Each primary meristem on the sympodial stolons produces one module consisting of an internode, a node with two secondary meristems and an embryonic flower. One of the secondary meristems at each node continues the growth of the main axis (i.e. primary stolon), while the other forms a ramet (Wolf 1908; Barrenscheen 1991; Huber 1995). Rosettes consist of an indeterminate number of leaves produced by a strictly vegetative meristem. Stolons may branch by activating buds in the axils of rosette leaves. Previous experiments have shown that shade-grown individuals of *P. reptans* produce considerably fewer side branches (i.e. secondary stolons) than equally-aged individuals grown in full daylight (Huber 1995; J.F. Stuefer, unpublished work).

The experiment

In spring 1990, rosettes of *P. reptans* were collected from a natural population in the vicinity of Utrecht (The Netherlands) and allowed to proliferate for three successive years in the experimental garden of Utrecht University. In May 1993, 50 similar-sized rosettes of unknown genetic identity were randomly taken from this garden population and used as experimental material. Ramets were excavated and size-standardized by removing all but the youngest un-

folded leaf from the rosettes, and by cutting roots to a length of 3 cm. Ramets were then planted into plastic pots (13 cm in depth and diameter) filled with river sand. Plants were watered daily with tap water, and fertilized weekly with a nutrient solution which had previously been shown to provide non-limiting nutrition for *P. reptans* (H. Huber and J.F. Stuefer, unpublished work; amounts equivalent to 10 kg N, 4 kg P and 10 kg K ha^{-1} $week^{-1}$). This watering and fertilization regime was continued until the end of the experiment.

After 4 weeks of pre-cultivation (in mid-June 1993), 30 plants were selected for uniform size and randomly assigned to one of the three experimental treatments. All primary stolons except one were removed from the rosettes at this point. The experiment was thus started with plants consisting of a rooted ramet (the "mother rosette") and one primary stolon with one fully elongated internode. Additional primary stolons formed on the mother rosette during the experiment were regularly removed once per week to standardize for plant size and architectural complexity among replicates. A pilot study had shown that removing additional primary stolons on mother rosettes of *P. reptans* has no effect on the developmental growth rate of a remaining stolon (H. Huber and J.F. Stuefer, unpublished work). All ramets produced on the primary stolons were rooted in plastic trays (15 cm × 15 cm × 100 cm) and supplied with water and nutrients as described above. The experiment was carried out in an open greenhouse covered by transparent plastic (light transmission: 90% PPFD; no effects on the red:far-red ratio of transmitted light).

Three experimental treatments were applied to ten replicate plants each (Table 1). Control plants (**C**) were exposed to high light conditions. They were grown in cages covered by a colourless plastic film to make micro-climatic conditions comparable between treatments. Light intensities (PPFD) inside control cages were approximately 85% of light intensities outside the greenhouse. This value of PPFD will subsequently be referred to as full daylight (100% in Table 1). In two shading treatments, whole plants (i.e. mother rosettes and their stolons) were grown in cages covered by one of two shading materials. "Neutral" shading (**N**) was imposed by means of two layers of black shade cloth which reduced light intensities to 13% of full daylight, without affecting spectral light quality (Table 1). Simulated canopy shade (**S**) was imposed by the use of one layer of black shade cloth and one layer of a plastic film (Lee Colortran International, Andover, Hants, UK; film no. 144), which in combination transmitted the same amount of PPFD (13%) as in the neutral-shade treatment, and reduced the red:far-red ratio inside cages to values around 0.2 (Table 1).

Shade cages were constructed in a way which allowed air circulation within cages and facilitated exchange of air with the surrounding environment in the greenhouse. Microclimatic measurements revealed no differences in wind speed and air humidity between treatments. Air temperatures within cages were automatically recorded (multi-channel Delta-Logger, Delta-T Devices, Burwell, Cambridge, UK) once an hour throughout the experiment. In mid-July 1993, all plants were harvested, washed free of sand, dried to constant mass at 72°C, and weighed.

Table 1 Light quantity (% photosynthetic photon flux density, PPFD relative to the light availability in the control treatment, measured with a LICOR-LI-185a light meter), and light spectral quality (red:far-red ratio; measured with a LICOR-LI-1800 spectro-radiometer), and the mean daily air temperature (in °C ± 1 SE) in the three experimental treatments

	% PPFD	Red:far-red ratio	Mean temperature
Control treatment (C)	100	1.14	19.7 ± 0.1
Neutral shade (N)	13	1.17	18.8 ± 0.1
Simulated canopy shade (S)	13	0.22	19.4 ± 0.1

Growth conditions

Daily means for air temperatures within cages varied between 15°C and 26°C during the experiment. On average, temperatures in the control and in the simulated canopy shade treatment were higher than in the neutral shading treatment (Table 1). Differences in mean daily air temperature between the two shade treatments were around 0.5°C on cloudy days, and reached maxima of about 2.5°C on clear days. The relationship between mean air temperature within cages and the daily increase in stolon length was significant for the control treatment ($r^2 = 0.26$, $P = 0.009$) but not for the shading treatments (neutral shade: $r^2 = 0.09$, $P > 0.05$; simulated canopy shade: $r^2 = 0.01$, $P > 0.05$). In this regression analysis the mean temperature of the 24 h preceding developmental measurements was used as a predicting variable for stolon growth realized in the same period of time.

Plant development

The developmental stage of plants was quantified by means of plastochron indices (Erickson and Michelini 1957; formulas are given below). The plastochron index is based on the production rate of modular units (e.g. leaves, stem internodes) on extending plant axes. Numerous studies have shown that the plastochron index is a precise and reliable measure of the developmental stage of plants (Lamoreaux et al. 1978; Maksymowych 1990). It should be noted that the plastochron index is not based on biomass increments over time, and can hence be determined in a non-destructive way.

The duration of one plastochron is defined as the time interval between two recurring events in the (modular) development of plants (Askenasy 1878), such as between the formation of two successive internodes on a stolon, or the initiation of two successive leaves on a ramet. The developmental stage of a plant can be quantified by the plastochron index (**PI**) which is the number of plastochrons (i.e. the number of recurring developmental events such as the production of stem internodes) realized by the main axis (shoot) of the plant.

Birch and Hutchings (1992 a, b) have shown that the PI is a useful and precise measure for describing patterns of plant development in clonal species. The original method of calculating PIs assumes constant developmental rates over time (cf. Erickson and Michelini 1957). Since this assumption is likely to be violated if plants are grown outside strictly controlled environments such as climate chambers, temperature-controlled greenhouses), an alternative method proposed by Hill and Lord (1990) was used to calculate PIs in this experiment. Whole-plant development was quantified by the the developmental stage of the primary stolon, expressed in units of plastochron (Birch and Hutchings 1992 a, b). Note that one plastochron corresponds to the production of one module on the primary stolon of the experimental plants. An internode on extending primary stolons was considered as formed when it had reached a reference length of 40 mm.

Local plastochron indices (LPI; Birch and Hutchings 1992a) were calculated for leaf and stolon formation. The LPI is defined as the developmental stage of a plant part relative to the developmental stage of the whole plant (Birch and Hutchings 1992b). The LPI is based on the PI of the main shoot. In our case the LPI of a plant part such as of a primary rosette at first branching is equal to the number of plastochrons which have elapsed on the main stolon between the formation of the rosette and the initiation of its first branch. The LPI thus permits comparison of the developmental stage at which differently-aged ramets undergo a certain developmental event, such as leaf or branch initiation. In accordance with Birch and Hutchings (1992a) we use the PI of the main axis as a measure of the developmental stage of the whole plant, and the LPI as a measure of the relative developmental stage of plant parts (e.g. ramets). The LPI is expressed in numbers of plastochrons.

Developmental measurements were made every day starting one week after light treatments had been applied, and continued until the day of harvest. Measurements were made every morning between 8.30 and 10.00 a.m. Parameters recorded daily included the length of all elongating internodes, the initiation of the 1st and the 2nd leaf and the initiation of branches (i.e. secondary stolons) on all primary ramets of each replicate. Leaves and branches were considered as initiated when they had exceeded a reference length of 10 mm.

The plastochron index (PI) was calculated according to the following two formulas (see Hill and Lord 1990).

$$\mathrm{PI} = i + \frac{t_2 - t_{\mathrm{R(i)}}}{t_{\mathrm{R(i+1)}} - t_{\mathrm{R(i)}}} \qquad \text{(Eq. 1)}$$

$$t_{\mathrm{R(i)}} = t_2 - (t_2 - t_1)\left(\frac{\ln[L(n_\mathrm{i}, t_2)] - \ln(R)}{\ln[L(n_\mathrm{i}, t_2)] - \ln[L(n_\mathrm{i}, t_1)]}\right) \qquad \text{(Eq. 2)}$$

where PI is the Plastochron index of the plant at t_2, $t_{\mathrm{R(i)}}$ is the time (in days) when the organ at node i equals the reference length, i is the node number on the primary stolon, whose organ (internode, leaf) is just longer than the reference length R, t_1 is the time (day) of observation of the organ at node i before it has passed the reference length, t_2 is the time (day) of observation of the organ at node i after it has passed the reference length, n_i is the organ (internode, leaf) at the i^{th} node, which is the youngest organ exceeding the reference length (R) at time t_2, L (n_i, t_1) is the length of the organ at node i at time t_1, L (n_i, t_2) is the length of the organ at node i at time t_2, and R is the reference length chosen in the exponential phase of organ growth.

Note that this approach to calculating PIs requires that an organ (e.g. elongating internode on the primary stolon) is measured twice within its exponential growth phase. For further details on the calculation of the plastochron index see Hill and Lord (1990). The equation (Eq. 1) given above differs slightly from the corresponding formula in Hill and Lord (1990), as their "mean plastochron at node i" has been replaced by the duration of the last fully realized plastochron ($t_{\mathrm{R(i+1)}} - t_{\mathrm{R(i)}}$). As a consequence the PI of plants can not be estimated for the stage when the last internode passes the reference length R. The LPI of an organ at node i can be calcultaed by subtracting the node number i from the PI of the whole plant.

Statistical analysis

Treatment effects were tested by means of one-way analysis of variance (ANOVA) followed by two orthogonal planned comparisons (Sokal and Rohlf 1981). Comparisons were carried out by using the CONTRAST statement in the SAS procedure GLM (SAS 1988). The first contrast compared the high-light treatment with the two shading treatments; the second contrast was a comparison between the simulated canopy and the neutral shade treatment. Data on branch and leaf initiation were analysed by means of a nested ANOVA (plants nested within treatments). Repeated measurement ANOVA (Potvin et al. 1990) was used to test for treatment effects on the daily increase of PI values and on the length of primary stolons. The day of measurement was used as repeated variable in these analyses. Differences in the developmental timing (i.e. LPI values) of branch and leaf initiation on rosettes of the primary stolons were tested with a nested ANOVA (plants nested within treatments). The statistical program package SAS (SAS 1988) was used for all calculations.

Results

The ontogenetic development of plants, measured as the daily increase in plastochron index (PI) of the primary stolon, showed an approximately linear relationship with chronological time in all treatments (Fig. 1). Under high light conditions the mean duration of one plastochron (i.e. time interval between the formation of two successive ramets on the primary stolon) was 3.29 days. Shading slowed down whole-plant development (Fig. 1, Table 2). In the neutral shade treatment the mean duration of one plastochron was slightly less than 4 days. Under simulated canopy shade it took on average 3.44 days to produce a new ramet on the extending primary stolon (Table 2).

In all three treatments daughter rosettes produced their first leaf about one plastochron after the ramet had been formed (Table 3). In shade-grown individuals the initiation of the first leaf on daughter rosettes tended to take place at an earlier developmental stage than in full daylight. If the age of ramets was expressed in days the initiation of the second leaf appeared to be significantly delayed by shading, while the age at which ramets produced their first leaf was not significantly different between the control and the shading treatments, but differed between the two shade types (Table 3).

Plants in all treatments formed secondary stolons during the experiment. At the time of harvest shade-grown individuals of *P. reptans* had produced fewer branches than plants grown under high light conditions. The average number of secondary stolons per primary rosette (Table 2, "branching index") was considerably lower for plants from both shading treatments as compared to plants grown in full daylight.

The chronological age of ramets initiating their first branch was significantly different between treatments (Table 3). Ramets exposed to neutral and to simulated canopy shade produced their first branch at an average

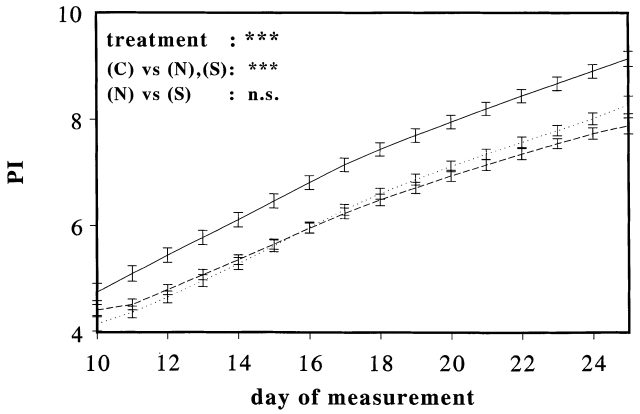

Fig. 1 Increase in plastochron index (PI) over time. The *lines* indicate treatment means (± 1 SE) (treatment codes: — control, - - - neutral shade, ... simulated canopy shade). Significance indicators relate to overall treatment effects (tested with a repeated measurement analysis of variance, RM-ANOVA), and to the results of the two orthogonal contrasts comparing the control (i.e. high light) treatment (C) with the two shade treatments [(S), (N); contrast 1], and the neutral (N) with the simulated canopy shade treatment [(S); contrast 2]. The day of measurement was used as repeated factor in the RM-ANOVA. This factor and its interactions with overall treatment effects as well as with both of the contrasts were all significant at $P < 0.001$. Significance levels as in Table 2

Table 2 Mean values (± 1 SE) of parameters describing the growth and development of *Potentilla reptans*, plants grown under three different light conditions. Data were analyzed by one-way ANOVA followed by two orthogonal contrasts (see Methods). *Asterisks* next to the variable names indicate significance levels for treatment effects. The two columns at the right-hand side of the table give the results of the two planned comparisons

	Treatment			Contrasts	
	Control treatment (C)	Neutral shade (N)	Simul. canopy shade (S)	(C) vs. (N), (S)	(N) vs. (S)
Total plant biomass [g]***	9.0 ± 0.26	1.9 ± 0.10	2.8 ± 0.13	***	**
Number of primary ramets [n] ***	10.6 ± 0.16	9.1 ± 0.18	9.6 ± 0.16	***	*
Number of secondary ramets [n] ***	24.9 ± 1.81	2.7 ± 0.56	4.3 ± 0.78	***	n.s.
Total stolon length [cm] **	121.2 ± 2.01	109.9 ± 2.67	120.3 ± 2.89	n.s.	**
Stolon extension rate[a] [mm/day] ***	35.9 ± 1.6	32.8 ± 1.3	37.2 ± 1.7	n.s.	**
Duration of 1 plastochron [days] ***	3.29 ± 0.04	3.90 ± 0.07	3.44 ± 0.03	***	***
Internode length [cm] ***	11.4 ± 0.16	12.1 ± 0.25	12.5 ± 0.16	***	n.s.
Petiole length [cm] ***	8.7 ± 0.93	30.6 ± 1.87	29.0 ± 0.81	***	n.s.
Branching index[b] ***	1.56 ± 0.08	0.38 ± 0.06	0.42 ± 0.05	***	n.s.

(n.s. $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$
[a] Calculated by means of repeated measurements ANOVA. The repeated factor (i.e. day of measurement) was significant at $P < 0.001$
[b] The branching index was calculated as the total number of secondary stolons divided by the number of nodes on the primary stolon

Table 3 Timing of branch and leaf initiation on primary ramets of *Potentilla reptans*, grown under three different light conditions. Values represent means (± 1 SE) for (**a**) the developmental stage (i.e. local plastochron index, LPI), and (**b**) the chronological age of ramets (i.e. age in days) when they formed their first two branches and their first and second rosette leaf, respectively. Means are based on measurements on all ramets on the primary stolon. Treatment effects were tested by nested ANOVA (plants nested within treatments), followed by two orthogonal contrasts (see Methods). The nested factor was non-significant in all cases. Indication of statistical significance as in Table 2

	Treatments			Contrasts	
	Control treatment (C)	Neutral shade (N)	Simul. canopy shade (S)	(C) vs (N), (S)	(N) vs (S)
(a) LPI					
First leaf ***	1.06 ± 0.02	0.99 ± 0.02	0.92 ± 0.02	**	*
Second leaf **	1.90 ± 0.03	2.07 ± 0.05	1.96 ± 0.04	**	*
First branch (n.s.)	3.13 ± 0.15	3.76 ± 0.28	3.36 ± 0.13	n.s.	n.s.
Second branch (n.s.)	4.70 ± 0.32	[a]	4.75 ± 0.20	n.s.	[a]
(b) days					
First leaf *	3.62 ± 0.09	4.00 ± 0.11	3.52 ± 0.12	n.s.	**
Second leaf ***	6.36 ± 0.22	8.01 ± 0.35	7.14 ± 1.20	***	*
First branch ***	8.73 ± 0.22	15.14 ± 0.67	13.23 ± 0.56	***	**
Second branch ***	12.93 ± 0.74	20.46 ± 1.80	17.84 ± 0.64	***	n.s.

[a] Most primary ramets of plants exposed to neutral shade produced the second stolon too late to estimate their developmental stage with the method of Hill and Lord (1990)

age of about 15 and 13 days respectively, while ramets grown in full daylight branched at an age of slightly less than 9 days (Table 3). This apparent delay in secondary stolon formation by shading, however, disappeared when branching was related to the developmental stage of ramets (i.e. their LPI value) rather than to their chronological age (Table 3). Primary ramets of shaded (both treatments) and unshaded plants did not differ in their developmental stage when they produced the first branch (Table 3).

The timing of secondary stolon formation showed a clear relationship with ontogenetic development. In unshaded plants the developmental time-lag between the formation of successive branches on the same rosette decreased significantly over time (Fig. 2). Table 3 suggests a similar pattern for shade-grown plants with respect to the first two side branches on primary ramets. However, individual ramets of shaded plants did not produce as many branches as those of unshaded plants during the experimental period, which made it impossible to calculate the time intervals between the initiation of four successive branches on shaded ramets.

Both types of shading significantly decreased total biomass and ramet production (Table 2). Differences in the number of daughter rosettes were mainly due to effects of shade on branching frequency, which resulted in

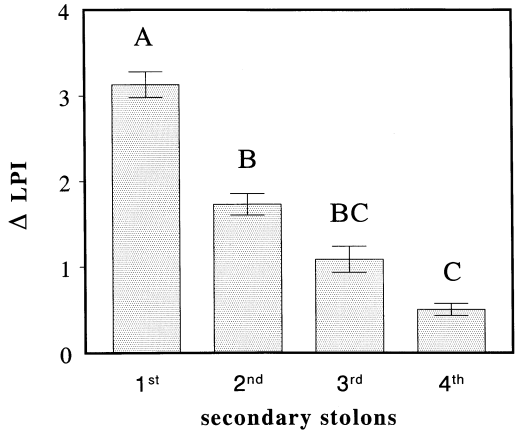

Fig. 2 Developmental time-lag (measured as differences in developmental stage of ramets, ΔLPI) between the formation of two successive branches on individual primary ramets of *Potentilla reptans* grown under high-light conditions. *Bars* represent mean values (± 1 SE). Different *letters* indicate statistically significant differences at $P < 0.05$

a lower number of secondary ramets in shaded plants. Individuals grown under simulated canopy shade accumulated significantly more biomass than plants exposed to neutral shade (Table 2). This effect was probably due to enhanced biomass allocation to leaves in plants subjected to simulated canopy shade (data not shown). There was no significant difference in the extension rate (i.e. daily increase in length) of the primary stolon between plants from the control and the shading treatments. Individuals grown under simulated canopy shade showed a slightly higher stolon extension rate than plants grown under neutral shade (Table 2).

There was a significant effect of shading on the average length of internodes (Table 2). In the two shading treatments internodes were about 10% longer than in the control treatment. Petioles were shortest under high light conditions and more than three times longer in the two shading treatments. Plants subjected to neutral and to simulated canopy shade did not differ significantly in petiole length (Table 2).

Discussion

After 4 weeks of growth, sun- and shade-grown individuals of *P. reptans* showed conspicuous differences in branching patterns, as well as in the number of ramets and total biomass produced. Clones exposed to either of the two shade treatments had produced significantly fewer secondary and higher order branches than clones grown in full daylight and thus exhibited a much more linear growth form than unshaded plants at the end of the experiment. Shading significantly slowed down the rate of ontogenetic plant development, as quantified by the increase in plastochron index over time, implying that shaded and unshaded plants reached the same ontogenetic stage at different points in (chronological) time. The initiation of secondary stolons occurred at a constant developmental stage of ramets in all three treatments. This indicates that the apparent shade effects on branching patterns were due to allometric differences between plants compared on the basis of the same chronological age. They can thus not be interpreted as adaptive change in plant architecture in response to shading.

Branching patterns

At harvest, shaded plants had produced significantly fewer branches and secondary ramets than plants grown under full daylight. These findings are in agreement with the results of numerous experimental studies which also reported significant shade effects on branching intensity in stoloniferous species (Hutchings and Slade 1988; Sutherland and Stillman 1988; Evans 1992; Dong 1994; Hutchings and de Kroon 1994), when compared on a common time scale. The strong effects of shade on branching intensity were also responsible for the low total number of ramets produced by shaded as compared with unshaded plants, which largely explained differences in total clone biomass between shaded and unshaded plants (Table 2).

The results of this study show that in *P. reptans* branching does not occur at a constant rate during the ontogenetic development of ramets. After an initial lag-phase, which lasted for about three to four plastochrons, ramets started to initiate side branches from the axillary buds of rosette leaves. The rate at which lateral stolons were produced during the initial branching phase was not constant in time but showed a clear tendency to increase: the first four branches on a rosette were initiated after successively shorter intervals (Fig. 2). The systematic relationship between patterns of stolon initiation and the ontogenetic stage of ramets means that, in *P. reptans*, branching rates are clearly subject to ontogenetic drift (*sensu* Evans 1972; Coleman and McConnaughay 1995) during the phase of ramet development studied in this experiment.

Allometric effects

The rate at which clones developed through time (i.e. the rate of ramet production on the main axis) differed significantly between treatments. Shading generally slowed down the ontogenetic development of plants, which was reflected in a smaller increase in numbers of ramets on the primary stolon per unit of chronological time. This consequently resulted in a slight difference in total numbers of primary ramets between shaded and unshaded plants. Such shade effects on the rate of whole-plant development imply that individuals raised in different light conditions will not reach the same developmental stage in a given period of time, i.e. plants harvested at the same time will inevitably differ in on-

togenetic stage (Evans 1972; Coleman et al. 1994). Small effects on the rate of primary stolon extension may result in major differences in clonal architecture and total numbers of ramets over time, because changes in the (linear) rate of stolon extension are amplified by (exponential) branching during the first stages of clone development.

The *chronological* time to first branching of a primary ramet was significantly longer under shaded than under high light conditions, leading to a considerably lower number of side branches being produced by shade-grown individuals during the course of the experiment. If expressed on a common time scale, shading thus significantly retarded branch initiation in *P. reptans*. However, the *developmental* timing of branch formation was not affected by either of the two shade treatments. This result strongly suggests that the visually conspicuous differences in branching patterns between sun- and shade-grown individuals were not due to an enhanced suppression of lateral buds in shaded conditions, but were a consequence of the ontogenetic dissimilarity of plants at the time of harvesting (cf. Coleman et al. 1994; Coleman and McConnaughay 1995). Thus, had shaded plants been given enough time to reach the same developmental stage as plants grown in full daylight, branching patterns would probably not have differed between treatments.

The developmental timing of branch initiation proved to be independent of environmental conditions. Neither reduced levels of light quantity (neutral shade treatment) nor additional changes in the red:far-red ratio (simulated canopy shade treatment) had significant effects on the ontogenetic stage at which ramets initiated their first branches. This is a clear indication that, in *P. reptans*, branching patterns do not respond plastically to shading via phytochrome-mediated effects on lateral bud outgrowth, as found in other species (Deregibus et al. 1985; Casal et al. 1987; Robin et al. 1994). It also indicates that in this species apical dominance is not enhanced by light limitation. Temperature differences between treatments may have affected the rate of plant development (as shown for other species; cf. Ackerly et al. 1992), in addition to light conditions. However, as primary ramets in all three treatments started to branch at the same developmental stage, potential temperature effects on whole-plant development would not obscure the conclusion that the developmental timing of branch initiation was independent of environmental conditions.

Functional adjustments of plant architecture?

Clear plasticity in the activation and suppression of lateral branches has been reported for root systems of grasses (Drew et al. 1973; Drew 1975; Robinson 1994). In these systems the main axis continues growth at a rate which is more or less independent of local resource availability. It is the initiation and the development of secondary and higher order branches which responds in a plastic way to spatial variation in nutrient supply, thereby enhancing resource uptake from nutrient-rich soil patches. It has been suggested that this response may be part of a "foraging" strategy, in which the primary axis assumes the task of *exploring* new territory by constant expansion growth, while laterals are responsible for *exploiting* favourable micro sites in the sampled soil volume (Gersani and Sachs 1992; Hutchings and de Kroon 1994).

In analogy to such foraging responses of roots, shade-induced changes in branching patterns of stoloniferous species have frequently been interpreted as a functional response to light limitation, which may maximize resource capture in a heterogeneous environment by minimizing the time and energy expenditure of clones for producing and supporting ramets in shaded micro-sites (Sutherland and Stillman 1988; Hutchings 1988; de Kroon and Schieving 1990). The results of this study suggest that this shade-avoidance hypothesis has to be rejected for expanding clones of *P. reptans*, because differences in branching patterns observable at the end of the experiment were not due to plastic changes in the developmental timing of branch initiation but to environmentally-induced changes in the rate of plant development (i.e. a result of ontogenetic drift). Similiar responses have been observed in non-clonal species (Ackerly et al. 1992, Silk 1980). Potential confusion between ontogenetic effects and functional responses may also obstruct ecological interpretations of allocation patterns, as recently shown by Coleman and McConnaughay (1995) and Gedroc et al. (1996).

The results of this study indicate that *P. reptans* may not exhibit functional adjustments of plant architecture to shading in terms of a plastic suppression of side branches. As recently proposed by de Kroon and Hutchings (1995) the degree of shade-induced internode elongation found in this experiment (about 10%, see Table 2) is also unlikely to promote selective ramet placement into favourable patches of a heterogeneous environment. However, the species does show strong shade-avoidance responses in terms of petiole elongation, as found in this (Table 2) and in other studies (Huber 1995, 1996). It is suggested that clonal species such as *P. reptans* may avoid shading in a vertical direction (i.e. by petiole elongation) rather than in the horizontal direction (e.g. by reduced branching and/or internode elongation; Huber and Wiggerman 1997). This may be a more profitable shade-avoidance response under field conditions, because in many herbaceous canopies patterns of light availability seem to be much more predictable in a vertical than in a horizontal direction (Huber 1996; Stuefer 1996; J.F.Stuefer and M.E. Pérez-Corona, unpublished work).

Conclusion

Phenotypic variation in branching patterns of clonal plants can be caused by two fundamentally different

processes, namely by (1) plasticity in activation or suppression of lateral buds, and (2) by environmentally induced changes in the rate of ontogenetic plant development. Both are inducible responses to changes in environmental conditions, matching the classical definition of phenotypic plasticity (Bradshaw 1965). However, "not all plasticity needs to be adaptive [as] any developmental system that is not buffered against changes in the environment will be phenotypically plastic" (Stearns 1982). We conclude from the results of this study that the linear growth form of shaded *P. reptans* clones can not be considered as a functional component of an adaptive shade-avoidance response. We propose that the observed shade effects on ontogenetic plant development represent unbuffered variability imposed by resource limitation, and that differences in branching patterns observable at a specific point in time were the result of allometric effects. This means that they are unlikely to represent a functional plant response to the environment and that they may rather be considered as an artefact of comparing plants at a different ontogenetic stage.

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