

## Clonal splitters and integrators in harsh environments of the Trans-Himalaya

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**Abstract** Individuals of clonal plants consist of physically and physiologically connected ramets. In splitters, they are integrated for a time shorter than ramet generation time (i.e. the time it takes to produce the first offspring ramet), whereas in integrators connections between ramets persist for a longer time. It has been predicted that integrators should prevail in stressful environments, such as habitats poor in nutrients, whereas splitters are expected to dominate in benign habitats, such as fertile areas with a moderate climate. I tested these predictions in four dry mountain areas of the Trans-Himalaya, in high altitudes subjected to multiple stresses. In accordance with the expectations I found that clonal plants with integrated ramets reach higher mean and maximum altitudes than splitters. Integrators were over-represented in nutrient-poor habitats, such as dry semi-deserts, sandy steppes and in subnival habitats, whereas splitters preferentially colonised mesic habitats, saline sites and wetlands. While there was no difference in the representation of splitters and integrators in habitats with an unstable surface, such as scree, dunes and water bodies, fully integrated clonal plants preferred very stable environments, such as banks of streams covered by closed-canopy vegetation. Most relationships between clonal integration and environmental factors were explainable by the phylogenetic relationship between the species, only the significant preference of splitters for shaded environments persisted in phylogenetically corrected analysis. The results indicate that clonal integration belongs to a set of evolutionarily conservative plant traits, usually shared by related species. Consequently, the adaptive value of clonal integration in individual habitats remains questionable.

**Keywords** Clonal integration · Elevation · Indicator value · Ladakh · Nutrient availability · Phylogenetic correction · Salinity · Shading · Soil moisture · Substrate stability

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## Introduction

Most plants, at least in areas with a seasonal climate, are clonal (de Kroon and van Groenendael 1997; Klimeš et al. 1997). This implies that they consist of two or more connected individuals—ramets. Participating in the resulting network of physically connected units, the ramets are usually connected also physiologically. They may exchange resources, such as water and nutrients, thereby buffering heterogeneity of the environment to some extent (Hutchings and de Kroon 1994). In plants which are not disturbed, the extent of the connected systems generally depends on the rate of plant branching and on the rate of splitting, i.e. disintegration of the oldest, basal part of the system. If the rate of branching steadily exceeds the rate of splitting, large connected systems develop, and such a plant is called an integrator. If these rates are comparable, small connected systems emerge and such plants are called splitters (van Groenendael et al. 1996).

The contrast between the behaviour of splitters and integrators has received considerable attention, especially in modelling studies where long-term dynamics of clonal plants differing in growth characteristics can relatively easily be seen. An environment consisting of “good” (resource-rich) and “bad” (resource-poor) patches can be created and manipulated, and interactions between plant growth and the environment can be modelled, resulting in a number of definitive predictions. For example, Oborny et al. (2000) and Magori et al. (2003) suggested that in environments with resources concentrated in a low number of small patches, integration is favoured. In contrast, increased size of resource-rich patches supports splitters, even if the total amount of resources is the same. If the total amount of resources decreases, then splitters succeed whereas integrators perform better if the total amount of resources is higher (Oborny et al. 2000). In another review, Pitelka and Ashmun (1985) presented contrasting predictions. Based on literature survey and field observations, they expected splitting in stressful and resource-poor environments, because maintenance of connections between ramets is costly. In such environments, dispersal is favoured, both by seeds and vegetative propagules, which are sometimes highly specialised, such as turions, bulbils and detachable buds. Extensive clonal integration is expected in environments which are (1) patchy in space and time, (2) patchy in space but not in time, where “guerrilla” growth forms with long spacers and low density of ramets are expected and (3) stable both in space and time where “phalanx” growth forms with tightly packed modules are expected (Pitelka and Ashmun 1985).

The relationships between persistence of spacers connecting ramets and habitat preference of plants along moisture, nutrient, temperature and light gradients were studied in Central European flora by van Groenendael et al. (1996). In a species set consisting of ca. 2,300 species, they distinguished clonal plants with spacers persisting for 1–2 seasons and longer, and used Ellenberg indicator values as a measure of habitat preference. They found that clonal plants with short-lived spacers are over-represented in wet, nutrient-rich and shaded habitats, but no difference was found for temperature (altitude).

Jónsdóttir and Watson (1997) used persistence time of functional ramet connections and ramet longevity to delimit integration in clonal plants. Based on a literature study of about 50 species, they concluded that clonal plants exhibiting full integration are limited to nutrient-poor environments whereas splitters do not show such specialisation and occur both in resource-poor and resource-rich environments.

In numerous studies published in the course of the last decade, mostly based on experimental results, the preponderance of splitters in resource-rich environments and preponderance of integrators in resource-poor environments were corroborated. However, except for van Groenendael et al. (1996), a single or relatively few species have been

usually included, and a single environmental factor was considered. Thus, more comprehensive analyses, based on a relatively complete species lists, in which several environmental factors are considered simultaneously, are still rare.

In this paper I tested the differences in environmental preference of integrators and splitters in the high mountains of the Trans-Himalaya, using the concept developed by Jónsdóttir and Watson (1997), and considering several environmental gradients. The area chosen for the study has a harsh climate where plants are subjected to multiple stresses. Due to that, habitat types and plant communities are relatively sharply delimited there and markedly differ in species composition. Therefore, selection pressure by the environment is strong and is not masked by biotic interactions (Körner 1999). Also, due to the remoteness of the area, the local flora is hardly modified by human activities, except for grazing, and by plant invasions. I used data from four geographical areas, 750–3,100 km<sup>2</sup> in size, covering one of the most prominent altitudinal gradients in the world which spans from 3,550 to 6,060 m above sea level. Besides calculating the relationship between clonal integration and habitat preference for species treated as independent units (TIPS), I accounted for the effect of relatedness of the species. This removes the part of variation which is explainable by the phylogenetical relationships among the species and which is possibly not adaptive. I used phylogenetically corrected procedures calculating phylogenetically independent contrasts (PICS) between plant traits for pairs of related species.

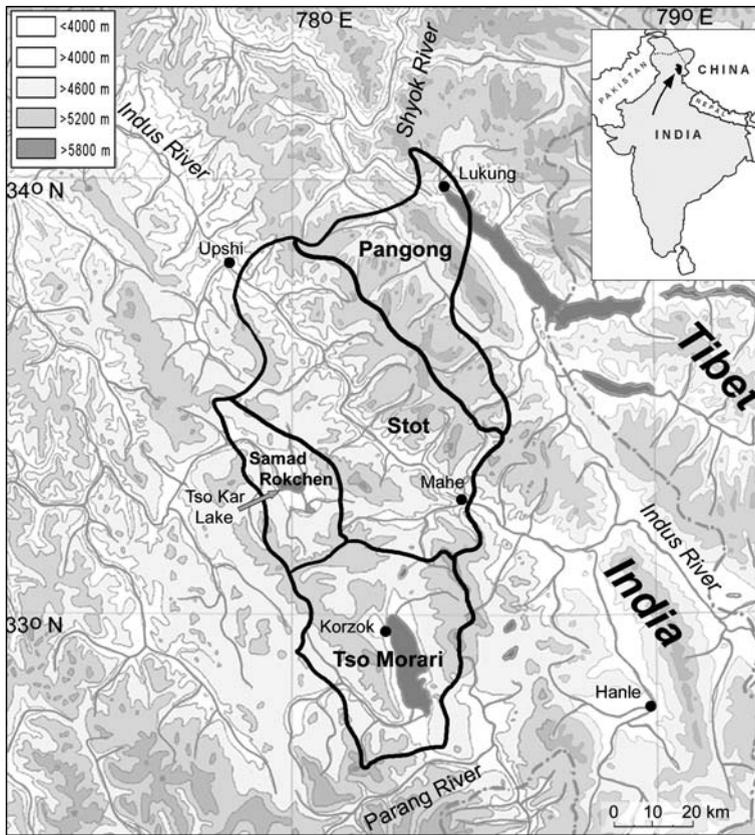
## Materials and methods

### Study region

The study region covers in total 6,912 km<sup>2</sup>, and is situated in Ladakh, Jammu and Kashmir State, India. The shape of the region is approximately elliptic, with the major axis running north-south (32°41.5′–33°59.7′ N, and 77°47.0′–78°33.4′ E). It covers the south-western-most extension of the high level Tibetan Plateau including three basins without external drainage, and its rim towards the high-mountain ranges of the Inner Himalayas. Elevation in the study region ranges from 3,550 m at the bottom of the Indus Valley to 6,622 m above sea level at Chhamser Kangri peak (Kapadia 1999). Vascular plants were found up to 6,060 m. Four non-overlapping areas, about 760–3,080 km<sup>2</sup> in size, were selected in the study region (Fig. 1, Table 1), to see how robust the relationships between clonal integration and environmental variables are.

No climatic station is located in the study region. However, data from nearest stations (Dickoré 1995; Hartmann 1997, 1999; Miehe et al. 2001) show that the study region is generally arid. It is rarely affected by monsoonal precipitation, which usually fails to cross the high crest of the main Himalayan range (Bhattacharyya 1989). At lower and middle elevations evaporation values are much higher than precipitation. Along the Indus Valley precipitation decreases from 115 mm at Leh (3,514 m, 34°09′ N, 77°34′ E, about 50 km NW of the study region) to 54 mm at Gar in SW Tibet (4,232 m, 32°07′ N 80°04′ E, c. 160 km SE of the study region).

There are a variety of substrates ranging from siliceous rocks of the Tso Morari Complex to calcareous or saline sediments. Low precipitation determines vegetation in the largest part of the study region. Accordingly, small areas at the relatively lowest elevations



**Fig. 1** The four study areas in E Ladakh, NW India, delimited by *black line*. Dots denote villages. None of the lakes (*dark grey*) has an outlet

**Table 1** Basic characteristics of the four study areas

	Pangong	Samad Rokchen	Stot	Tso Morari
Coordinates: N	33°24.8'–33°59.7'	33°6.0'–33°29.5'	33°3.8'–33°51.1'	32°41.5'–33°10.0'
Coordinates: E	78°5.6'–78°33.4'	77°47.0'–78°8.5'	77°51.5'–78°33.4'	77°59.8'–78°30.3'
Minimum elevation [m a.s.l.]	4,300	4,540	3,550	4,500
Maximum elevation [m a.s.l.]	5,920	5,900	5,990	6,060
Area [km <sup>2</sup> ]	1,292	758	3,082	1,780
Number of localities	104	206	491	481
Number of records	2,207	4,052	12,725	8,034

Minimum and maximum elevations concern localities where vascular plants were recorded

of the Indus Valley are occupied by semi-desert. Steppe vegetation prevails in the whole studied region and extends up to an altitude of ca. 5,300 m. It is dominated by several species of *Stipa* and *Artemisia*. Other frequent species include *Oxytropis microphilla* (Pall.)

DC., *Tanacetum fruticosum* Ledeb. and *Ptilotrichum canescens* (DC.) C. A. Meyer. Plant cover ranges from 10 to 40% in the steppe. Higher up, a narrow belt of alpine grasslands occurs, where *Kobresia pygmaea* (C. B. Clarke) C. B. Clarke in Hook. f. and *Carex pseudofoetida* Kük. usually dominate. Above an altitude of 5,300–5,700 m, sparse subnival vegetation is developed, characterised by *Astragalus confertus* Benth. ex Bunge, *Thylacospermum caespitosum* (Camb.) Schischk. and *Potentilla pamirica* Wolf. Vascular plants occur regularly up to 5,900 m, locally up to 6,060 m above sea level. Detailed surveys of vegetation of various regions of Ladakh focused on semi-deserts, steppes and alpine grasslands have been published by Hartmann (1983, 1984, 1987, 1990, 1995, 1997, 1999), vegetation of the Tso Kar basin was studied by Rawat and Adhikari (2005), landscape types by Kala and Mathur (2002) and altitudinal distribution of life forms of vascular plants by Klimeš (2003). Less attention has been paid to azonal vegetation, especially of wetlands, which occupy relatively large areas of flat and broad valley bottoms in the whole region and to saline habitats. Patches with some characteristic plants, such as *Pegaeophyton scapiflorum* (Hook. f. and Thomson) Marq. & Shaw and *Saxifraga nanella* Engl. & Irmscher, are found near the snow line. Along the Indus River and its tributaries are quite a few villages in the study region, whereas on the plains situated at about 4,550 m above sea level small permanent settlements are exceptional. Barley and less often oat is cultivated on a small scale up to 4,700 m. Synanthropic vegetation includes plant assemblages developed on eutrophicated ground by stables of domestic animals and near villages up to 5,400 m above sea level. Steppe and alpine turf vegetation is grazed by sheep, goats and yaks up to 5,600 m. The grazing effect of wild ungulates seems to be negligible due to the low densities of most species (Fox et al. 1991; Mallon 1991). During recent decades the region has been characterised by continuous overgrazing due to high numbers of domestic sheep, goats and yaks (Jina 1995; Holzner and Kriechbaum 1998).

#### Data sampling and analysis

##### *Floristics*

I collected field data on seven expeditions lasting four to 7 weeks each, from 1998 to 2003, and in 2005. Altogether I recorded the vascular flora at 1,282 localities, each ~1 ha in size. The localities were non-randomly distributed, which is mainly due to the part of the study area being devoid of vascular plants (very unstable slopes, glaciers, lakes and extremely high elevations). However, I attempted to cover all elevations and habitats in all parts of the delimited areas. As I aimed at recording as many vascular plant species as possible, most localities included various habitats and vegetation types. Depending on habitat diversity, accessibility, species richness and elevation, plants at individual localities were searched for about 30 min to 1 day. Number of records was 27,018 and included 540 species. The elevation of all localities was estimated with an altimeter (Thommen TX, Revue Thommen<sup>®</sup>, Waldenburg, Switzerland).

##### *Indicator values*

I evaluated the species composition of the vegetation using records from 369 plots, each 100 m<sup>2</sup>. I used a stratified placement of the plots, to cover the visually assessed variation in habitat and vegetation type. In each plot I assessed five environmental variables, considered (according to literature and personal field experience) to be the most important for habitat differentiation and easily assessable. I scored them using the following scales.

- (1) Stability of the soil/substrate surface (the inverse of disturbance frequency): 1—unstable (scree, dunes and solifluction soils), 2—partly stable (grasslands, steppes), 3—stable (rocky crevices, *Kobresia pygmaea* mats).
- (2) Light availability: 1—shaded (gorges, shaded rocky crevices, walls of stream banks), 2—partially shaded (dense vegetation cover), 3—full light (sparse vegetation cover).
- (3) Soil moisture: 1—dry (substrate usually without visible traces of water), 2—mesic, 3—wet (water level regularly but transiently above soil surface), 4—permanent surface water.
- (4) Nutrient availability: 1—low (semi-deserts, steppes), 2—medium, 3—high (stables, animal resting places).
- (5) Soil salinity: 1—no salt deposits on soil surface, 2—salt deposits rare, 3—salts forming a continuous crust.

I used two approaches to assign the indicator values to individual species. (1) Modal values of the environmental variables evaluated for plots in which individual species were recorded, were used as indicator values of particular species. For species missing from the 369 plots, I estimated the indicator values using my field notes, available literature concerning vegetation of the study area (e.g. Hartmann 1999) and comments given on herbarium specimens studied at K, BM, BM, LE, GOTT, DD, BSD and WII (about 10,000 specimens examined; abbreviations of herbaria follow Holmgren et al. 1990). (2) Indicator values of a species were calculated as arithmetical means of indicator values estimated for individual plots in which that species was recorded, weighed by the logarithm of its cover. Only species recorded in three or more plots were considered. As PICS results based on these two approaches were essentially the same, I present here only results from the later approach.

### *Clonality*

Clonal plants were defined as plants that multiply vegetatively on a regular basis, i.e. consisting of potentially independent ramets or spreading by vegetatively originated propagules. Non-clonal plants included (1) annual and perennial species with a main (tap) root of the primary root system and without adventitious roots and buds, (2) pleiocorm plants (Lampe 1999) with short below-ground branches bearing very few or no roots, which are unable to survive when detached from the rest of the plant, (3) root-splitters that multiply vegetatively only when very old (senile), and spread over a very limited distance, and (4) apomicts (agamospermy: seeds are produced without meiosis and fertilisation) lacking other modes of vegetative multiplication.

### *Clonal integration*

Clonal integration categories followed a simplified classification by Jónsdóttir and Watson (1997) with two resulting plant categories: integrators and splitters. For assignment of individual species to the integration categories I used the rules developed by Jónsdóttir and Watson (1997). They defined disintegrators (splitters) as plants in which persistence time of functional ramet connections ( $C$ ) is the same or shorter than the time it takes to develop the first offspring ramet (ramet generation time— $G$ ;  $C \leq G$ ), and integrators as plants with a longer persistence of the spacers ( $C > G$ ). Using ramet longevity ( $R$ ) as the third variable, they divided integrators into four sub-categories, combining restrictive ( $C < R$ ) versus full ( $C = R$ ) integration and small versus large clonal fragments. To obtain two species groups

comparable in size (number of species), I did not use categories differing in ramet generation time, and merged splitters with restrictive integrators, calling them splitters.

I evaluated the longevity of ramets, longevity of functional ramet connections and ramet generation time using (1) species descriptions and pictures available in literature, (2) studies of herbarium specimens (see above), and (3) repeated observations in the field, carried out from 1997 to 2006, which were focused on morphology of below-ground organs; when necessary, dissections of spacers between ramets were performed, to reveal whether ramet connections are functional. In general, clonal plants with woody spacers belonged to integrators whereas all caespitose graminoids forming large turfs and producing mono- or bicyclic shoots, plants with above-ground stolons and water plants were splitters. All other species were considered individually and their assignment the integration categories usually required a careful evaluation of all available data.

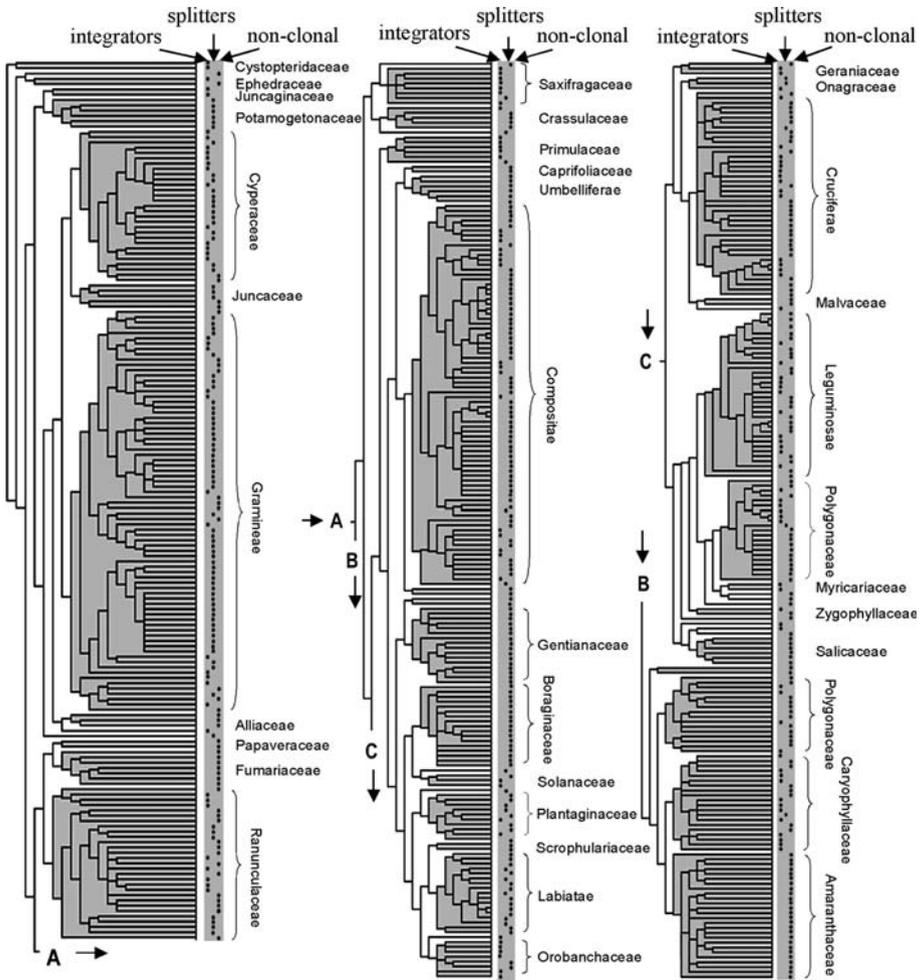
### Data analysis

I performed two types of analyses to assess the correlation between clonal integration and habitat preference. As the scales used for the indicator values were ordinal (rather than metric) in this study (similarly to Ellenberg 1991, and others), calculation of their arithmetic mean and variation was problematic. Therefore, I treated them as nominal variables. Using  $\chi^2$  tests, I tested the hypotheses that the distribution of the number of splitter species does not differ from the corresponding distribution obtained for integrators.

### The effect of phylogeny on the association of plant traits with integration

I used the supertree of German vascular plants developed by Durka (2002) with updates from Soltis et al. (2005) for higher hierarchical ranks (Fig. 2). In contrast to most supertrees available in literature, this supertree includes taxonomical ranks below families so that the number of polytomies is relatively low. For groups including genera missing from the German flora I used the following updates: *Amaranthaceae* (Kadereit et al. 2003; Müller and Borsh 2005; Kadereit et al. 2006), *Compositae* (Funk et al. 2005), *Leguminosae* (Wojciechowski et al. 2004) and *Cruciferae* (Al-Shehbaz et al. 2006; Bailey et al. 2006; Beilstein et al. 2006). In the case of several genera, such as *Saussurea* (Kita et al. 2004) and several genera of *Cruciferae* (Yue et al. 2006; Warwick et al. 2002, 2004a, b, 2006; O’Kane and Al-Shehbaz 2003), phylogenetic analyses based on molecular data were available even for Asian species. Lower (within-genus) polytomies remained sometimes unresolved, especially in genera containing numerous species of Asian distribution (e.g. *Elymus*, *Taraxacum*, *Ranunculus*, *Artemisia* and *Oxytropis*). As the flora of Ladakh is truly Holarctic, with a negligible proportion of Paleotropical elements (Dickoré 1995; Dickoré and Miehe 2002), the supertree by Durka (2002) developed for the German flora could be used in most cases down to the genus level. On the other hand, as the flora of the four study areas is relatively species-poor, numerous genera were represented by one or two species so that phylogenetical relationship of species within genera was often straightforward.

I tested the evolutionary independence of plant integration and habitat preference using PICS (Harvey and Pagel 1991). As clonal integration categories were defined as a dichotomous variable, I used the “brunch” procedure (Purvis 1991). This approach assumes that under the null model half of the contrast in indicator values calculated at nodes are positive and half of them negative ( $H_{01}$ ), and the mean value of the contrasts is zero ( $H_{02}$ ). Therefore, I used the two-tailed sign test ( $H_{01}$ ) and the one-tailed *t*-test ( $H_{02}$ ) for testing. As these two approaches gave the same results, I present here data based on the



**Fig. 2** Phylogenetic tree of the flora of E Ladakh. Families which include >1 species are shaded, names of families represented by single species were omitted. Clonal integrators, splitters and non-clonal plants are marked in three columns

second approach. In contrast to the TIPS analyses, I combined data from the four areas for PICS, to increase the power of the tests.

The calculations were performed using STATISTICA for Windows, Version 7.0, and the CAIC program (Purvis and Rambaut 1995).

**Results**

**Species numbers**

The number of species of vascular plants recorded in the four study areas ranged from 279 to 486, with a total of 540 (Table 2). Even if the study areas were situated close to each

**Table 2** Number and percentage of species recorded in the four study areas according to integration category

	Pangong	%	SR	%	Stot	%	TM	%	Total	%
Non-clonals	140	49.6	136	48.7	271	55.8	170	51.2	301	55.7
Integrators	80	28.4	84	30.1	125	25.7	97	29.2	136	25.2
Splitters	62	22.0	59	21.1	90	18.5	65	19.6	103	19.1
Totals	282	100	279	100	486	100	332	100	540	100

SR—Samad Rokchen, TM—Tso Morari

other, they were well differentiated by species composition, as only 197 species were recorded in all four areas. The highest species density was observed between 4,500 and 5,000 m above sea level, slowly declining towards both ends of the elevation gradient. The number of non-clonal plants was surprisingly high in all four study areas: about 50% of plants were non-clonal, which is considerably more than the proportion of integrators (25–30%) and splitters (19–22%).

### Elevation

The mean and maximum elevation of integrators was higher than that of splitters in all four areas, whereas no difference was found between splitters and non-clonal plants, except for the Stot area where non-clonal plants were concentrated at the lowest elevations (Table 3). However, this tendency disappeared when PICS were calculated (mean elevation:  $t = 1.28$ ,  $P = 0.2$ ; maximum elevation:  $t = 1.54$ ,  $P = 0.13$ ).

**Table 3** Mean (a) and maximum (b) elevations of non-clonal plants, integrators and splitters in the four study areas

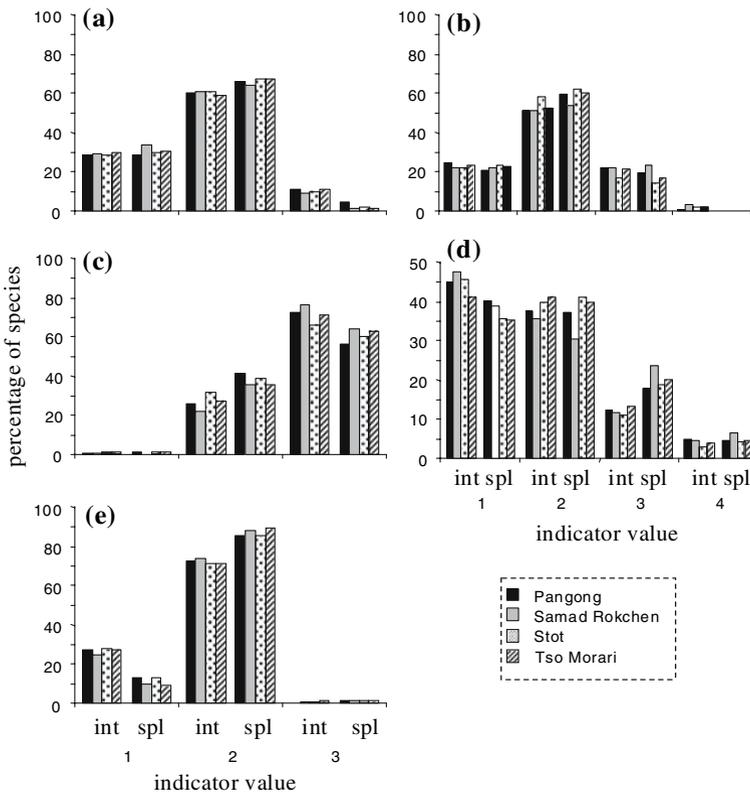
	Pangong		Samad Rokchen		Stot		Tso Morari	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>(a) Mean</b>								
Non-clonals	4,707 <sup>a</sup>	346	4,928 <sup>a</sup>	243	4,440 <sup>a</sup>	473	4,860 <sup>a</sup>	260
Integrators	4,936 <sup>b</sup>	363	5,055 <sup>b</sup>	269	4,792 <sup>c</sup>	485	5,012 <sup>b</sup>	321
Splitters	4,747 <sup>a</sup>	291	4,856 <sup>a</sup>	230	4,593 <sup>b</sup>	422	4,886 <sup>a</sup>	296
<i>F</i>	11.95		12		24.55		8.93	
<i>P</i>	<0.00001		<0.00001		<0.00001		<0.00002	
<b>(b) Maximum</b>								
Non-clonals	4,899 <sup>a</sup>	438	5,134 <sup>a</sup>	346	4,803 <sup>a</sup>	596	5,097 <sup>a</sup>	414
Integrators	5,181 <sup>b</sup>	416	5,321 <sup>b</sup>	363	5,209 <sup>c</sup>	564	5,329 <sup>b</sup>	461
Splitters	4,982 <sup>a</sup>	435	5,047 <sup>a</sup>	370	4,998 <sup>b</sup>	524	5,106 <sup>a</sup>	447
<i>F</i>	10.95		12		21.84		9.55	
<i>P</i>	<0.00001		<0.00001		<0.00001		<0.0001	

Identical superscripts within columns denote mean values not different at 0.05 (Tukey HSD)

Indicator values

The proportion of integrators living in areas with a low surface stability corresponded well to the values found in splitters (Fig. 3). In contrast, integrators were over-represented in very stable habitats and under-represented in habitats with a medium stability. This tendency was quite strong in all four study areas ( $\chi^2 = 7.3\text{--}61.7$ ,  $P < 0.05$ ). However, when phylogenetically corrected analysis was performed, the association between surface stability and clonal integration disappeared ( $t = 0.94$ ,  $P = 0.36$ ). Tolerance to shading significantly differed between splitters and integrators in one area only (Pangong;  $\chi^2 = 8.4$ ,  $P < 0.05$ ), where unexpectedly high numbers of integrators were found in open habitats. In other areas the same tendency was observed but the difference was smaller. The effect of shading persisted even after phylogenetical relationships were removed ( $t = -2.0$ ,  $P = 0.04$ ).

The results obtained for soil moisture were unexpected, as no difference was found between integrators and splitters. The PICS analysis confirmed this non-significant relationship ( $t = -0.79$ ,  $P = 0.4$ ).



**Fig. 3** Relative distribution of species indicator values for plants belonging to integrators (*int*) and splitters (*spl*) in the four studied areas. (a) Stability of the soil/substrate surface, (b) soil moisture, (c) light availability, (d) soil salinity, (e) nutrient availability

As expected, nutrient availability affected the distribution of integrators and splitters very strongly ( $\chi^2 = 16.1\text{--}40.1$ ,  $P < 0.005$ ). While all clonal plants were very rare in habitats with excessive nutrients, in nutrient-poor conditions of all study areas a prominent role was played by integrators. Consequently, splitters preferred environments with a medium availability of nutrients. However, the effect for nutrients disappeared when the analysis was corrected for phylogeny ( $t = 0.1$ ,  $P = 0.9$ ). The effect of salinity was not so strong and it was statistically significant in two study areas only (Samad Rokchen:  $\chi^2 = 7.8$ , Stot:  $\chi^2 = 7.9$ , both  $P < 0.05$ ), although the general trend was the same in all areas. Integrators were over-represented in non-saline habitats and under-represented in saline conditions. In hyper-saline habitats no difference was found between the representation of splitters and integrators (Fig. 3). Using PICS, non-significant results were obtained for the relationship between clonal integration and salinity ( $t = 0.39$ ,  $P = 0.7$ ).

## Discussion

In high mountains, environmental conditions are usually considered stressful, as several factors, especially climatic ones, are less conducive to plant growth than in lowlands (Körner 1999). I found that in all four study areas integrators reached significantly higher mean and maximum altitudes than non-clonal plants and clonal splitters. This differs from the results by van Groenendael et al. (1996), who did not find any difference in indicator values of temperature for clonal plants with short- and long-lived spacers. Even if the difference in methods used here and by van Groenendael et al. (1996) may be partly responsible for the obtained discrepancy, a more plausible explanation is found in the extent of the altitudinal gradient. While the altitudinal gradient considered by van Groenendael et al. (1996) extended from lowlands to the lower alpine belt, my altitudinal gradient spanned from the subalpine belt to the upper limit of vascular plant growth. Therefore, extreme altitudes hosting a specific flora of high alpine and subnival belts, where integrators play a significant role, were completely missing from the data set used by van Groenendael et al. (1996). The prominent role of integrators in high-altitudes relates not only to nutrient economy in poor and patchy environments, but also to storage of carbon in spacers and its efficient utilisation during the short vegetation season (Callaghan et al. 1997). The number of studies in which integration was compared along an altitudinal gradient is relatively low, however, in agreement with the predictions by Jónsdóttir and Watson (1997), Chen et al. (2004, 2006) reported that *Fragaria vesca* L. and *Duchesnea indica* (Andrews) Focke benefited from integration only at higher altitudes where the environment is more stressful than at lower altitudes.

The pattern found for nutrient availability and substrate stability was strongly significant and consistent for the four study areas. The preponderance of clonal integrators in nutrient-poor conditions found in this study was already predicted by Jónsdóttir and Watson (1997). The same pattern has been reported also for populations belonging to one species (Jacquemyn et al. 2005), however, in other studies the level of integration was independent of nutrient availability (D'Hertefeldt and Falkengren-Grerup 2002). These discrepancies could be caused by the fact that clonal integrators form a heterogeneous group of plants. In the four areas included in this study most clonal integrators in nutrient-poor conditions shared the following characteristics: individual ramets being connected with the persisting tap root for a long time, spacers being placed close to the soil surface, being somewhat woody and therefore relatively costly both for development and maintenance, having short spacer length and slow rates of lateral spreading, amount of roots being developed on

offsprings and spacers strongly limited (e.g. *Potentilla bifurca* L., *Saussurea bracteata* Decne. in Jacquem. and *Thylacospermum caespitosum*). In contrast, a few integrators of nutrient-poor habitats developed markedly long spacers placed deep in the soil [*Leymus secalinus* (Georgi) Tzvelev, *Trikeria hookeri* (Stapf) Bor and *Carex moorcroftii* Falc. ex Boott]. However, all these species are of an exceptionally strong stature which enables them to develop rhizomes in spite of the hard soil texture. The third main group of integrators colonising nutrient-poor environments includes plants with short epigeoecic below-ground stems (i.e. originating in the above-ground and pulled below the soil surface by contracting roots; e.g. *Leontopodium pusillum* (Beauv.) Hand.-Mazz., *Geranium himalayense* Klotzsch ex Hoffmeist. & al. and *Triglochin palustre*).

Indicator values for substrate stability have not been tabulated so far. However, substrate stability, as the inverse of disturbance, has a pronounced effect on plants, especially in high mountains (Körner 1999). In the subnival belt repeated freezing and thawing is responsible for low stability of the soils (polygonal soils and solifluction streams; see Callaghan et al. 1997). The perpetual movement of the soil heavily disturbs plants and may damage connections between their ramets (Klimeš 2003). In contrast, on stream banks, in dense alpine grasslands and in rocky crevices, the soil surface is fixed, so that spacers connecting ramets can persist over many years. Whereas, I found that integrators are over-represented in habitats with a stable soil surface, at the opposite end of the gradient, where soil surface is unstable, no difference was found. Wetlands, as representatives of habitats with an unstable substrate, are usually colonised by typical splitters [e.g. *Potamogeton* spp., *Juncus thomsonii*, *Catabrosa aquatica* (L.) P. Beauv.] whereas screes, which also belong to unstable habitats, are dominated by integrators. Long-term maintenance of spacers is costly in wetlands because of rotting, which supports splitting. Besides, in contrast to wetlands, only the upper layer is usually unstable in screes, whereas lower layers are fixed. Most plants colonising screes are long-lived and develop a strong tap root with a root crown hidden deep in the scree. Sprouts bearing leaves, flowers and fruits are often weak. They develop only few roots on below-ground stems which can be easily broken and detached from the root crown. However, if they are damaged, new sprouts originating from the root crown replace them. Therefore, clonal growth of these plants, investing into persistence and regeneration, is limited [e.g. *Physochlaina praealta* (D. Don) Hook. f., *Cicer microphyllum* Benth. in Royle, *Dracocephalum heterophyllum* Benth.]. Plants of the subnival belt, where disturbance is frequent and severe, invest into persistence, but as soil is moving there relatively slowly and regenerative growth is limited by a short vegetation season, it is profitable to invest into the quality of the spacers, and prevent their damage. Therefore, some plants colonising polygonal and solifluction soils, such as *Nepeta longibracteata* Benth., *Alyssum klimesii* Al-Shehbaz and *Desideria pumila* (Kurz) Al-Shehbaz, develop strong and partly woody spacers. It has been suggested that in heavily and very frequently disturbed habitats clonal plants are replaced by seeders, i.e. plants investing their resources preferably into seed production (Bellingham and Sparrow 2000). Even if frequency of disturbance in the subnival belt is very high (the soil freezes and thaws two times a day during the whole vegetation season), disturbance intensity is moderate and investment into a high quality of spacers connecting the ramets and into morphological and structural adaptations of roots (e.g. Jonasson and Callaghan 1992) may enable persistence of clonal plants even in this habitat. In accordance to the prediction by Klimešová and Klimeš (2003, 2007), the only plant colonising the flushes permanently disturbed in the vegetation season, is a root-sprouter (*P. scapiflorum*).

The failure to find any difference in the representation of clonal integrators and splitters along the soil moisture gradient is surprising, as a lack of moisture certainly belongs to the

important stressors in the study area. However, when looking at individual species, it becomes clear that virtually all splitters among plants of dry habitats belong to turf grasses and sedges (e.g. *Stipa orientalis* Trin. in Ledeb., *Hordeum brevisubulatum* (Trin.) Link, *Elymus jacquemontii* (Hook. f.) Tzvelev). These plants develop compact clones, usually consisting of hundreds of individuals (tillers), broken up into physiologically independent units. Plants developing this growth form are utilising the consolidator strategy (Briske and Derner 1998), with an efficient economy of carbon and nitrogen utilisation. In this way, they approach the fully integrated plants, which also benefit from an efficient economy of basic nutrients in heterogeneous and generally poor environments. Besides, the tough leaf bases and compact growth form caused by intra-vaginal tillering protect the plant bud bank from desiccation and freezing. The preponderance of hemicryptophytic graminoids with intra-vaginal tillering in steppe and alpine communities was documented also in other regions (e.g., Klimeš et al. 1997; Halassy et al. 2005).

Shading hardly belongs to the critical environmental factors in the studied areas, as real woodlands are missing and the above-ground biomass of herbaceous vegetation is generally low, being efficiently suppressed by the adverse climate and grazing. While integrators showed a weak tendency of over-representation in open habitats, some representatives of plants colonising shaded sites below ledges, such as *Saxifraga cernua* L., behaved as extreme splitters, producing easily detachable and dispersible bulbils. In spite of that, the relationship between clonal integration and shading, which was significant for TIPS in the Pangong area only, persisted also for PICS. These results correspond with the predictions by Pitelka and Ashmun (1985), who expected splitting in the forest understorey, considered as a stressful environment.

In most studies focusing on clonal integration, shading has been considered as a reduction of light intensity by tree and shrub canopies, adversely affecting herbaceous plants of the ground layer. This environment can be considered as stressful, however, in contrast to nutrient-poor environments, such as semi-deserts, arctic tundra and high altitudes in mountains, where nitrogen and phosphorus are limited (Bazilevich and Tishkov 1977), herbaceous plants in the forest understorey are limited by carbon (Zotz et al. 2006). Therefore, even if both environments are stressful, maintenance of clonal integration is costly in the forest understorey only because carbon is the main resource needed to develop and maintain spacers, whereas in open habitats of the arctic tundra, where nitrogen and phosphorus are limited, maintenance of spacers is not a serious problem as the investment of nitrogen and phosphorus into the spacers is relatively low (Körner 1999). This is probably why Pitelka and Ashmun (1985) predicted that clonal splitters should prevail in stressful habitats whereas Jónsdóttir and Watson (1997) reported a prevalence of clonal integrators from stressful environments.

It is very likely that clonal life-history traits are adaptive, but as Fischer and van Kleunen (2001) pointed out, “it is neither clear to which degree this is the case, nor which clonal life-history traits constitute adaptations to which environmental factors”. In this study I used a comparative eco-morphological approach to reveal the relationship between clonal integration and habitat preference. Even if the study area was located in a region usually called a “cold desert” by local ecologists (Chowdhery and Rao 1990), the flora is surprisingly species-rich and includes almost the entire spectrum of life and growth forms known from the Holarctic (Klimeš 2003). Taxonomically this flora is neither depauperate, as all important families and higher taxonomical units of the Holarctic flora, except for exclusively woody taxa, are well represented here (Fig. 2). All these factors make the selected area appropriate for comparative studies focusing on the distribution of plant traits along environmental gradients. In most papers in which a large number of species well

representing the species pool was available, the results obtained from the TIPS and PICS analyses were congruent (Ricklefs 1996). In contrast, in my study most significant results based on TIPS diminished when PICS were applied. This indicates that the observed variation explained by ecological factors is attributable to the phylogenetic relationship between the species. However, as closely related species often share the same clonal integration type (Fig. 2), and clonal integration was evaluated as a dichotomous variable, the power of the test was relatively low. This could be partly responsible for the failure to reject the null hypotheses. To improve the application of the PICS, clonal integration should be evaluated quantitatively, along a metric scale. This is however not easy in a larger species set, as persistence of the physiological connection between ramets is not always congruent with the presence of physical connection. The presence of the physical connection between ramets is also sometimes difficult to prove, especially in turf graminoids (Wilhelm 1995).

As pointed out by Jónsdóttir and Watson (1997), their clonal integration categories are necessarily heterogeneous, as they include plants with a contrasting architecture of below-ground organs, role of vegetative multiplication and reproduction in their life cycle, storage organs, etc. Still, I found that the distribution of clonal integrators and splitters along some environmental gradients was markedly different, and that integrators and splitters are often over-represented in different habitats. The close correlation between clonal integration categories and growth forms on the one hand, and growth forms and phylogenetical relatedness on the other, indicate that in many cases presence of a given set of clonal plant traits may be a necessary consequence of evolutionary history of individual species rather than an adaptation to given environmental conditions.

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