OIKOS 105: 461–470, 2004

# Intraclonal variation in defence substances and palatability: a study on *Carex* and lemmings

**Kari Anne Bråthen, Jep Agrell, Dominique Berteaux and Ingibjörg S. Jónsdóttir**

Bråthen K. A., Agrell, J., Berteaux, D. and Jónsdóttir, I. S. 2004. Intraclonal variation in defence substances and palatability: a study on *Carex* and lemmings. – Oikos 105: 461–470.

Clonal sedges consist of integrated ramets at different development stages. Many of these sedges are important food for herbivores, yet differences in herbivore preferences and defence allocation between ramet development stages have not previously been evaluated. In this study we investigated intraclonal ramet variation in level of plant defence and nutrient compounds and intraclonal ramet preferences by lemmings (*Lemmus trimucronatus*) in field samples of a rhizomatous sedge (*Carex stans*). Plant defence was measured as the level of proteinase inhibitor activity (PIA) and the ratio of PIA to soluble plant proteins (SPP), whereas plant nutrients were measured as the level of soluble plant sugars (SPS) and SPP. Flowering ramets generally had a higher content of defence compared to vegetative ramets, which is consistent with the optimal defence theory predicting that defence compounds are allocated to the ramet stage of the highest fitness value. Compared to vegetative ramets, the flowering ramets had a lower content of SPP and a higher content of SPS. The lemmings showed preference differences between the ramet development stages, and to a large extent the ramet content of defence compounds and nutrient compounds covaried with these preferences in the predicted way. This study shows that defence allocation between ramet development stages of the clonal sedge *Carex* conforms to predictions of the optimal defence theory.

*K. A. Bråthen, Inst. for Biology, Univ. of Tromsø, N-9037 Tromsø, Norway (kari.brathen@ibg.uit.no). – J. Agrell, Dept of Animal Ecology, Ecology Building, Lund Univ., SE-223 62 Lund, Sweden – D. Berteaux, Québec Univ. at Rimouski, 300 allée des Ursulines, Rimouski (Québec) G5L 3A1 Québec, Canada. – I. S. Jónsdóttir, UNIS, P.O. Box 156, NO-9171 Longyearbyen, Norway.*

Several clonal plants have the ability to maintain physiological connections between vegetatively reproduced individuals (reviewed in Klimeš et al. 1997 and in Jónsdóttir and Watson 1997). These connections integrate individuals (ramets) at different development stages, each of which potentially can become an independent individual. The integration allows the ramets to share resources (Ashmun et al. 1982, Jónsdóttir and Callaghan 1988, 1990, Jónsdóttir et al. 1996, Hutchings and Wijesinghe 1997, Marshall and Price 1997), and to be more efficient in resource acquisition (Alpert and Stuefer 1997). Each ramet may add to the prosperity of the clone, either in terms of resource acquisition, growth or reproduction. Still, the contribution of an individual ramet to the integrated physiological unit (IPU, sensu Watson and Casper 1984, Watson 1986) changes through its development from being newly reproduced until it reaches the flowering stage. Resources are therefore expected to be allocated between ramets within an integrated unit depending on the ramet development stage.

Similarly, within an IPU ramets of different development stages should have different contents of defence substances. Optimal defence theory predicts that a plant

Accepted 3 October 2003


ISSN 0030-1299

allocates defence substances between plant parts or tissues to increase plant fitness (McKey 1974, Rhoades 1979). Within a plant, a combination of the fitness value, the risk of herbivory and the cost of the defence of a given plant part or tissue determine the allocation of defence substances to that plant part or tissue (Rhoades 1979). Moreover, the allocation of defence substances is not static, but changes continuously with the development of the plant (McKey 1974). However, in the application of the optimal defence theory, there are more options to the allocation of defence substances within clonal plants compared to that within non-clonal plants. Because, in addition to differential fitness value of plant parts within individual ramets, clonal plants with physiological integration between ramets most likely differentiate between integrated ramets at different development stages.

Rhizomatous sedges are important forage for herbivores, particularly in alpine and arctic tundra environments. These sedges usually consist of several interconnected and physiologically integrated ramets, and it takes a few years for each ramet to complete a monocarpic life cycle (Jónsdóttir et al. 1996). Seedling recruitment is infrequent, and vegetative propagation is the prevalent form for reproduction in these sedges (Carlsson and Callaghan 1990b, Jónsdóttir 1995, Stenström 1999, Tolvanen and Henry 2000), causing most young plants to be vegetatively recruited ramets. Because herbivory is unpredictable in the alpine and arctic tundras, the defence content of rhizomatous sedges should not be constitutive, but rather of an inducible character (Adler and Karban 1994). Also, optimal defence allocation is less likely to evolve when plants are forage to a diversity of herbivores, as the plant in such situations is less likely to have success from its defence (Adler and Karban 1994, Jokela et al. 2000). However, the rhizomatous sedges should show optimal defence allocation that is effective towards arvicoline rodents. Rhizomatous sedges are highly palatable to these herbivores (Batzli and Lesieutre 1991), and may thus experience a tremendous grazing impact in years when arvicoline rodents reach peak densities.

Proteinase inhibitors are among the secondary plant chemicals that have received considerable interest in recent years for their potential to reduce herbivory. Proteinase inhibitors (PI) are substances that can cause growth depression and pancreatic hypertrophy in monogastric mammalian herbivores, and their toxicity depends on the level of dietary proteins (reviewed by Gallaher and Schneeman 1984). Ramets of the clonal sedge *Carex bigelowii* have shown an increase in the ratio of proteinase inhibitor activity (PIA) to soluble plant proteins (SPP) in response to simulated grazing (Seldal et al. 1994), and similar levels of PIA have been found in *Carex stans* (Seldal, pers. comm.). *Carex aquatilis,* a close relative of *C. stans* (Murray 1994) has SPP as the dominant leaf storage form of nitrogen (Chapin and Shaver 1988). Because lemmings do not eat *Carex* individuals with higher levels of PIA to SPP (Seldal et al. 1994), the proteinase inhibitors seem to act as an efficient plant defence against lemmings (Karban and Baldwin 1997). No attempt has yet been made to distinguish between development stages of ramets in terms of their concealed defence compounds.

Apart from defence, food preferences by arvicoline rodents are likely to correlate with plant content of nutrient compounds like proteins and sugars, as has been shown for other herbivores (Jones and Roberts 1991, Jensen 1993) as well as to methoxybenzoxazolinone (6-MBOA, a compound found after wounding in several rhizomatous tundra graminoids, Negus and Berger 1998). However, the allocation of nutrient compounds, i.e. plant primary metabolites, between plant parts, may rarely covary with that of the defence compounds, i.e. secondary metabolites (Zangerl et al. 1997). The allocation of nutrient compounds between ramet development stages may govern the herbivore preferences until defence is induced.

We measured intraclonal levels of PIA, SPP and soluble plant sugars (SPS) in the rhizomatous sedge *Carex stans* Drej., sometimes considered as *C. aquatilis* Whalenb. ssp. *stans* (Drej.) Hult. (Murray 1994), and examined to what extent the development stages of the ramets affected the feeding behaviour of a small mammal herbivore, the brown lemming (*Lemmus trimucronatus* Richardson) which uses *Carex* as one of its major food plants (Batzli and Jung 1980). More specifically we addressed the following questions: (1) do ramet development stages differ in leaf content of PIA, SPP and SPS; (2) does the content of these substances change with phenology; (3) does the occurrence of defence or nutrient compounds depend on whether the ramets have been previously grazed; (4) do lemmings discriminate between ramets of different development stages; and (5) do these ramet preferences covary with the level of defence or nutrient compounds?

## Material and methods

The sampling of ramet development stages from populations of *Carex* and the ramet preference trials with lemmings were conducted during the Swedish "Tundra Northwest 1999" expedition at three Arctic sites: North Yukon National Park (69° 25.23′N, 139° 36.33′W, August 3rd 1999), North Banks Island (73° 37.34′N, 115° 52.13′W; August 10th 1999) and South Devon Island (74° 33.35′N, 82° 47.93′W; August 25th 1999). Bioassays were performed on board the ship with brown lemmings caught in North Yukon National Park.

## Sampling of *Carex* for quality analysis and lemming bioassays

At each site a vegetation unit dominated by *C. stans* was selected for the sampling of ramet development stages, where also the plant phenology and marks from previous grazing were measured to account for the possible source of variation introduced by these factors. The sampled populations were on mesic soil on either level or gentle south sloping terrain. We identified three stages of *Carex* ramets: A ramet was considered "young" if no dead leaves were attached to its shoot base, "old" if it had produced green leaves the previous year and was still vegetative, and "flowering" if it had produced an inflorescence in the sampling year. Within a $30 \times 6$ m grid young, old or flowering ramets were sampled every third meter along the long side of the grid for quality analysis, giving a total of ten samples of each ramet type. Each sample was packed in aluminium foil and stored on dry ice in a field thermos. For the preference trials with lemmings at least eight pieces of turfs with all ramet types present were sampled at random within the same grid. Each turf (approximately $20 \times 20$ cm in area) was dug up with 10–15 cm deep soil attached to prevent harm to the rhizomes. The turfs were brought on board the ship, where they were used in ramet preference trials within 24 hours.

## Analysis of plant quality

We analysed proteinase inhibitor activity (PIA), soluble plant proteins (SPP) and soluble plant sugars (SPS) in the leaves of the sampled ramets. In the flowering ramets the content of defence was not measured in the reproductive structures themselves, i.e. not in the flowers or fruits, but in the leaves. We have thus assumed that the entire flowering ramet including its vegetative parts (leaves) is a sexually reproducing part to the clone. The leaves of all ramet stages were freeze-dried and homogenised in a ball mill at top speed for 3 min. Some samples were pooled in order to get enough material for analysis, resulting in five to eight samples of each ramet type per site.

Neutral extracts were made from the freeze-dried leaves (10 mg per 1 ml of a 40 mM TRIS-HCl buffer, pH 8.1, with 2% w/v Triton X-100). SPP was measured by the protein-dye binding method (Bradford 1976), using bovine serum albumin as a standard (Bio-Rad standard II). The extracts were further analysed for PIA in a standard assay on trypsin (Sigma) using Nα-benzoyl-DL-arginine-p-nitroanilide (BApNA) as chromogenic substrate (Bergmeyer and Gawehn 1974). The hydrolysis of the ester was followed spectrophotometrically at 405 nm for 3 min. PIA was calculated using Trypsin inhibitor (Sigma type I-S) as a standard. The ratio between PIA and SPP was calculated because the toxic effect of trypsin inhibitors is dependent upon the level of dietary proteins (Gallaher and Schneeman 1984).

SPS were extracted from the freeze-dried leaves in 90% ethanol at 60°C for 5 min (modified from Farrar 1993). The extracted SPS was colored using 0.5% phenol (MERCK) and concentrated sulphuric acid ($H_2SO_4$) and quantified spectrophotometrically at 485 nm according to the methods of Sturgeon (1990) and Dubois et al. (1956). The leaf content of SPS was calculated using sucrose (BDH Laboratory Supplies) as standard.

## Measures of phenology and previous grazing

Phenological measurements on both old vegetative and flowering stages were conducted within ten $50 \times 50$ cm plots at each site, positioned at random at every third meter along the long side of the grid.

Because the leaves of *C. stans* stay attached for some years after they wither, they allow a comparison between previous and current leaves as a measure on vegetative phenology (Jónsdóttir and Bråthen 1999). Every plot was divided into four quadrates, and in each quadrat the old vegetative ramet closest to the center was chosen. The lengths of the longest current year's and previous year's leaves attached to the ramet were measured and mean values for each plot were calculated. The leaves wither from the top as they age, and a leaf was considered as representative for the current year if less than 25% of its length had withered. The vegetative phenology (Pv) corresponds to the relative difference between the two lengths, and a phenological estimate comparable for all sites was achieved by the formula:

$$Pv = CL/PL$$

where CL is the length of the longest current year leaf and PL the length of the longest previous year leaf.

The development of the female flower was used as an indication of the flowering phenology (Pf). The levels were 1: closed, 2: open, 3: stigmas receptive, 4: stigmas withered, and 5: seeds developed. Mean flowering phenology of all the flowering ramets of *Carex stans* in each plot was calculated using the formula:

$$Pf = \sum_{le=1}^{5} le \times (n_{le}/n_t)$$

Where le is level, $n_{le}$ the number of flowering ramets at a given level and $n_t$ the total number of flowering ramets.

For comparison of the vegetative and the flowering phenology, their scales were transformed into relative scales by dividing the level of phenology of a given plot with the maximum level of phenology achieved for each respective ramet stage. The phenology of the young vegetative ramets were assumed to follow that of the old vegetative ramets, therefore no separate measure of phenology was performed for this development stage.

We also quantified the proportion of ramets previously grazed within each of the ten plots at all three sites. All ramets with traces from previous grazing and all undamaged ramets were registered. The proportion of previously grazed ramets was quantified independently for each ramet development stage.

Because most of the samples on plant quality data were pooled over two plots, the same plots were pooled for the data on both phenology and previous grazing.

### Lemming bioassays

Lemmings (*Lemmus trimucronatus*) were trapped with Sherman live traps placed in mesic habitat in North Yukon National Park. Eight adult individuals (four males and four females) were caught and transported to the laboratory on board the ship. There the lemmings were housed individually in laboratory cages ($60 \times 40 \times 20$ cm) supplied with a 5 cm layer of wood shavings, with cotton, dried grass and mosses as nest material. The lemmings were provided with water and food ad lib (laboratory chow, crushed oats, apple and hay, primarily *Phleum pratense* and *Festuca pratensis*). Temperature in the lab ranged between 10–15°C, and the light regime followed outdoor conditions.

Bioassays examining lemming preferences for different *Carex* ramets were performed in the laboratory in cages identical to the ones housing the lemmings. These clean cages were covered with 2 cm of wood shavings and one artificial nest made of cotton. Immediately before a preference test was initiated, surplus ramets on the *Carex* turfs to be used were trimmed away, leaving two ramets of each of the three ramet development stages. The turf was placed in one end of the cage, and the preference test was initiated by the release of one lemming into the nest at the opposite end. The lemming was then continuously observed for 30 min during which the time spent feeding on the different ramet stages was registered to the closest second. After 30 min the food preference trial was terminated if the animal had spent a minimum of 60 s feeding on the *Carex* ramets. If this was not the case observation continued for another 30 min. In order to avoid depletion of one food type a trial was terminated if the animal consumed all the above ground parts of one ramet stage. A trial was also terminated if all above ground ramets of one stage were cut down, hereby avoiding any preference bias between vertical and horisontal plant material. In both cases the data collected up until the termination of a trial was included in the analyses as long as the animal had spent a minimum of 60 s feeding on the *Carex* ramets. Each lemming was tested only once on a *Carex* turf from a specific site.

The amount of biomass consumed per second was independent of ramet development stage (Agrell and Berteaux, unpubl.), and consequently the percent feeding time allocated to a specific ramet stage shows the relative preference for this development stage.

### Statistical analysis

Plant quality data was analysed by a mixed general linear model (GLM) with ramet development stage as fixed factor and site as random factor (i.e. 'block'), including phenology and previous grazing as covariates. Each defence and nutrient substance was analysed separately and the most parsimonious model was selected using the best subsets procedure of StatSoft (2001) and the Akaike information criterion (AIC, Burnham and Anderson 1992). To explore how the predictor variables were related, GLM ANOVA and correlation analyses were performed. Ramet preference data were analysed by mixed GLM with ramet development stage and lemming gender as fixed factors and site as random factor. All variables showed normal distribution except the variable previous grazing, which was arcsine transformed prior to analysis to meet the assumption of normality. Where appropriate, significant test results were followed by Fisher's LSD post hoc test for identification of important contrasts (Zar 1996).

All calculations were performed using the statistical software StatSoft (2001).

## Results

### Relationships between predictor variables for plant quality

The predictor variables for plant quality were all significantly related to each other. Whereas the phenology of the plants differed between sites independent of ramet development stage (ANOVA $F_{(2, 33)} = 53.71$, $p < 0.001$; Fig. 1), the proportion of previously grazed ramets differed between sites in interaction with ramet development stage (ANOVA interaction $F_{(4, 45)} = 7.43$, $p < 0.001$; Fig. 1). The stage of phenology and the proportion of previously grazed ramets were correlated ($r = 0.58$, $p < 0.001$, $n = 54$). When correlation analysis were run separately for each ramet development stage this relationship was tighter (flowering ramets $r = 0.76$, $p < 0.001$, $n = 18$, old ramets $r = 0.83$, $p < 0.001$, $n = 18$, young ramets $r = 0.83$, $p < 0.001$, $n = 18$). The tight relationship between the phenology and previous grazing limited the ability to distinguish the separate effect of either on plant quality, and hence they were included as covariates in the analysis of plant quality one at a time.

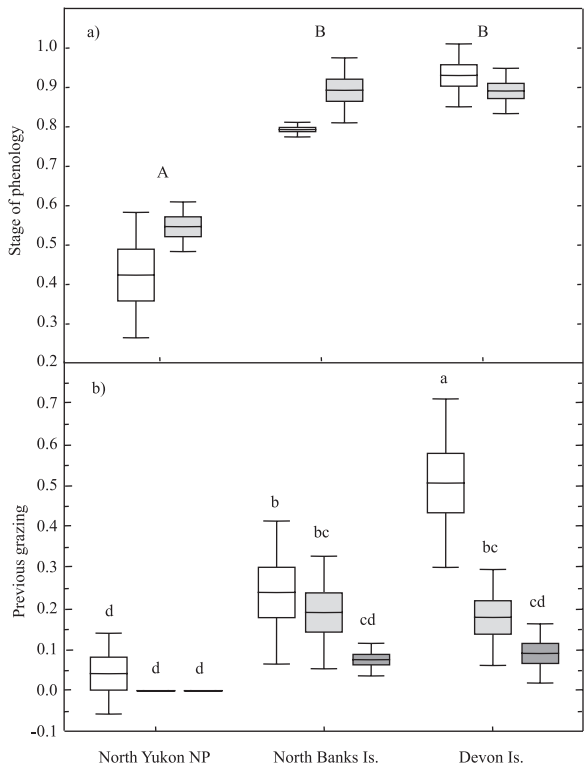


Fig. 1. Phenology and previous grazing of *Carex stans* at three sites in the Canadian arctic: a) level of vegetative and flowering phenology and b) proportion of ramet development stages with traces from previous grazing in the field. Box plots refer to mean value, ±SE and ±95% CI. Different letters indicate significant difference between a) sites and b) ramet developmental stages within and between sites at $p < 0.05$ (LSD post hoc test). Flowering, old vegetative and young vegetative ramets are represented by open, light and dark grey boxes respectively.

### Plant quality variation between ramet development stages

The ramet development stages differed in all measured aspects of plant quality, that is, in both of the defence aspects (the proteinase inhibitor activity [PIA] and the ratio of PIA to soluble plant proteins [SPP]), and both of the nutrient compounds (SPP and the soluble plant sugars [SPS]).

Generally the flowering ramets had higher PIA and PIA:SPP ratio than both young and old vegetative ramets, whereas the vegetative ramets did not differ in their PIA and PIA:SPP ratio content (Table 1, Fig. 2). Leaf concentration of SPP showed the opposite pattern, with flowering ramets having lower content than both young and old vegetative ramets (Table 1, Fig. 3). Moreover, SPP content differed between young and old vegetative ramets. The SPS was higher in the flowering ramets, whereas there again was no difference between the old and the young vegetative ramets (Table 1, Fig. 3). Irrespective of ramet development stage, the contents of PIA:SPP, SPP and SPS differed between sites (Table 1, Fig. 2, 3). The ramet content of SPS was however best explained with a model including phenology as covariate, when site no longer had significant effect (Table 1, Fig. 3). The variation in ramet content of PIA was equally well explained by a model involving only ramet development stage and a model involving only previous grazing, independent of ramet development stage (Table 1, Fig. 2, 3). Clearly, the flowering ramet had the highest content of PIA (Fig. 2) and had the most marks from previous grazing (Fig. 1), but the positive correlation between ramet content of PIA and previous grazing was weak (Table 1). The ramet content of PIA:SPP and SPP was neither affected by phenology nor by previous grazing (Table 1).

### Plant quality and lemming preference

The bioassays demonstrated that lemming preferences (i.e. % feeding time allocated to a ramet stage) varied between ramet development stages, and that the preferred ramet development stage differed according to site (GLM interaction between site and ramet development stage $F_{(4, 57)} = 9.03$, $p < 0.001$). Flowering, old vegetative and young vegetative ramets were preferred at the first, second and third site respectively (Fig. 4). Adding lemming gender as a factor did not explain more of the variance, indicating that there was no difference in preference for ramet development stages between female and male lemmings (GLM interaction between site, sex and ramet development stage $F_{(4, 48)} = 0.60$, $p = 0.667$).

All of the four aspects of plant quality covaried with the lemming preferences. When comparing plant quality of ramet stages at a given site with the corresponding lemming preferences, ramet stages with high levels of PIA and PIA:SPP ratios were least preferred by lemmings (Fig. 2), at least at the two sites North Banks Is. and Devon Is. where levels of PIA and PIA:SPP were the most variable between ramet stages. In contrast, ramet stages with high levels of SPP were preferred by the lemmings (Fig. 3), whereas the relationship between SPS content of ramets and lemming preference was both positive and negative (Fig. 3). Thus, for the most part there were correspondences within sites between the ramet stage food quality and the ramet stage preferences displayed by the lemmings.

## Discussion

This study shows that *Carex stans* allocates defence and nutrient substances depending on ramet development stage. Previous studies on clonal plant species have shown that differences in defence substance allocation depend on plant sex (Elmqvist et al. 1988, Hjältén 1992) and development stage (Danell et al. 1987), but whether

Table 1. Results of mixed GLM on the effect of ramet development stage and site on leaf ramet content of proteinase inhibitor activity (PIA), soluble plant proteins (SPP), ratio of PIA to SPP (PIA:SPP) and soluble plant sugars (SPS). The covariates phenology and previous grazing in the field were included in the model one at a time. Where one of the covariates has effect on a plant quality substance, its correlation or partial correlation with the substance is shown. Site was the random factor in the analysis. Only predictor variables that were chosen by model selection are included in the table.

| Variables | df | Effect | MS | F | p | R |
|---|---|---|---|---|---|---|
| PIA | | | | | | |
| with phenology as covariate (AIC = 108.12) | | | | | | |
| Ramet stage | 2 | Fixed | 1.48 | 3.60 | 0.034 | |
| Error | 51 | | 0.41 | | | |
| with previous grazing as covariate (AIC = 108.54) | | | | | | |
| Previous grazing | 1 | Fixed | 2.00 | 4.74 | 0.034 | 0.29 |
| Error | 52 | | 0.42 | | | |
| PIA:SPP | | | | | | |
| Ramet stage | 2 | Fixed | 0.0030 | 9.21 | < 0.001 | |
| Site | 2 | Random | 0.0020 | 5.98 | 0.005 | |
| Error | 45 | | 0.0003 | | | |
| SPP | | | | | | |
| Ramet stage | 2 | Fixed | 7282 | 10.20 | < 0.001 | |
| Site | 2 | Random | 24340 | 34.10 | < 0.001 | |
| Error | 49 | | 714 | | | |
| SPS | | | | | | |
| with phenology as covariate (AIC = 469.16) | | | | | | |
| Ramet stage | 2 | Fixed | 3974.0 | 12.70 | < 0.001 | |
| Site | 2 | Random | 780.4 | 2.50 | 0.093 | |
| Phenology | 1 | Fixed | 3630.5 | 11.60 | 0.001 | 0.44 |
| Error | 48 | | 312.9 | | | |
| with previous grazing as covariate (AIC = 479.78) | | | | | | |
| Ramet stage | 2 | Fixed | 1143.2 | 3.00 | 0.059 | |
| Site | 2 | Random | 7540.4 | 19.80 | < 0.001 | |
| Previous grazing | 1 | Fixed | 367.0 | 0.96 | 0.331 | 0.14 |
| Error | 48 | | 380.9 | | | |

these differences appeared intraclonally was not considered. Thus, intraplant differences in allocation of defence compounds have been confirmed by studies on biennial plants (reviewed by Hamilton et al. 2001), while intraclonal differences in allocation of defence compounds are first confirmed in this study.

The defence content was overall higher in the flowering compared to the vegetative ramets. Thus, *C. stans* conforms to predictions of the optimal defence theory (McKey 1974, Rhoades 1979). Similar allocation of defence compounds has been found within individuals of the biennal plant *Pastinaca sativa*, with the reproductive structures having higher content of defence compounds than the leaves (Zangerl and Rutledge 1996). *P. sativa* also showed a combination of constitutive and inducible defence of the involved defence compound (Zangerl and Rutledge 1996). Similarly, *C. stans* may keep both constitutive and inducible defence as indicated by both the relatively high levels of proteinase inhibitor activity (PIA) in plants with no signs of previous grazing (at the North Yukon NP site), and by the positive correlation between ramet content of PIA and previous grazing. Apart from PIA, the ramet content of PIA to SPP, was also markedly higher in the flowering ramets. The flowering ramets thus had not only the overall highest content of defence substances, but also an overall higher toxicity of the defence substance (Gallahar and Schneeman 1985).

Implicit in the theory of optimal defence lays the assumption that defence affects herbivore preference (Rhoades 1985). Thus, assuming that *Carex* defence investment largely determines lemming preference among ramet development stages, the preference for a given ramet stage should coincide with when its loss represents the lowest cost to the clone. Moreover, it may not always be the vegetative ramets that have the lowest cost to the clone because clonal plants can gain fitness from both clonal growth and from sexual reproduction (Pan and Price 2002). In this study a low preference for the young ramets was found during early phenological levels (i.e. at the first sites). This seems beneficial to a clone as it relieves the herbivore pressure on young ramets until they have established their own roots and storage reserves. Indeed, young ramets within an IPU have the support of the older ramets (Jónsdóttir and Callaghan 1989, 1990), in contrast to seedlings which have no such support and for the most part are found to be more palatable than the adults (Fenner et al. 1999, Karban and Thaler 1999). We also found a decreased preference for the flowering ramet as phenology proceeded (i.e. at the last sites). This coincided with the production of seeds, which represents a nutrient cost to clones of *Carex* (Carlsson and Callaghan 1990a). If the nutrient investment in the flowering ramet increases as the seeds develop, the benefit of protection increases. Such increase in defence levels along with the develop-

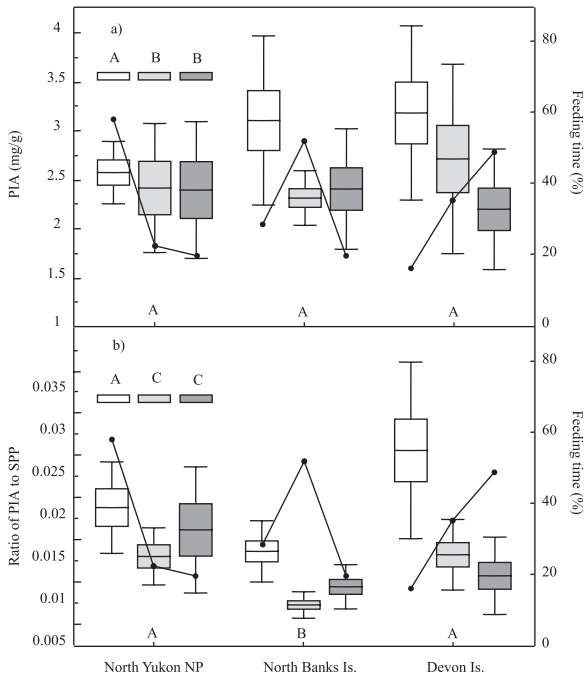


Fig. 2. Plant defence compounds in ramet development stages of *Carex stans* at three sites in the Canadian arctic: a) proteinase inhibitor activity (PIA) and b) ratio of PIA to soluble plant proteins (SPP). Box plots refer to mean value, ± SE and ±95% CI. Different letters indicate significant difference between sites or ramet developmental stages at $p < 0.05$ except between A and C for which $p < 0.005$ (LSD post hoc test). Flowering, old vegetative and young vegetative ramets are represented by open, light and dark grey boxes respectively. Solid line shows the lemming preferences between ramet development stages at each site (Fig. 4 for details).

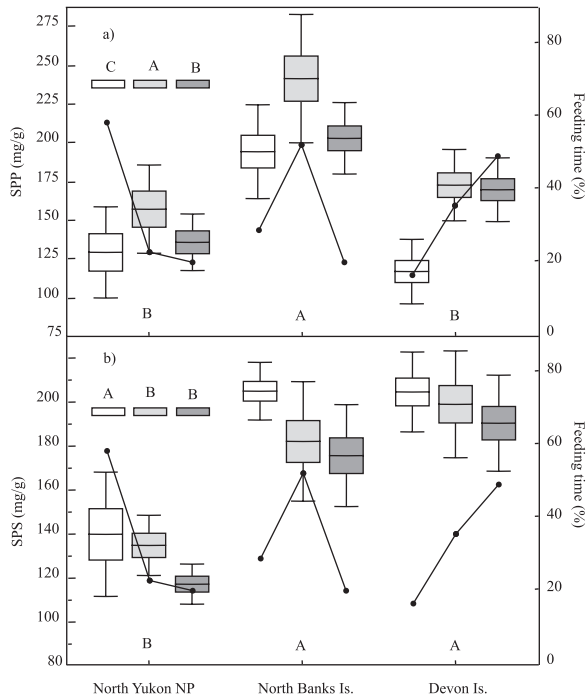


Fig. 3. Plant nutrient compounds in ramet development stages of *Carex stans* at three sites in the Canadian arctic: a) soluble plant proteins (SPP) and b) soluble plant sugars (SPS). Box plots refer to mean value, ±SE and ±95% CI. Different letters indicate significant difference between sites or ramet developmental stages at $p < 0.05$ except between A and C for which $p < 0.005$ (LSD post hoc test). Flowering, old vegetative and young vegetative ramets are represented by open, light and dark grey boxes respectively. Solid line shows the lemming preferences between ramet development stages at each site (Fig. 4 for details).

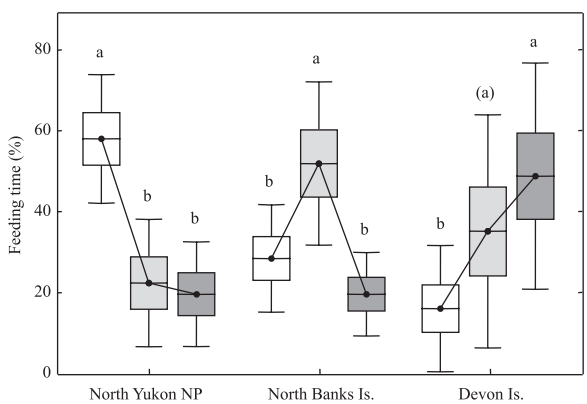


Fig. 4. Lemming preferences (% feeding time) for ramet development stages of *Carex stans* sampled at three sites in the Canadian arctic. Box plots refer to mean value, ±SE and ± 95% CI. Different letters indicate significant difference in % feeding time within sites at $p < 0.05$ except between (a) and b for which $p = 0.06$ (LSD post hoc test). Flowering, old vegetative and young vegetative ramets are represented by open, light and dark grey boxes respectively.

ment of reproductive structures has previously been demonstrated (Zangerl et al. 1997).

In this study the preferences displayed by lemmings did not always correspond to the content of defence and nutrient substances. For instance, at the first site the flowering ramet was by far the most preferred development stage despite its slightly higher defence content. At the two last sites the flowering ramet was no longer the preferred development stage, consistent with the fact that its content of defence compounds was higher than that of the vegetative ramets. Thus, results are complex with respect to the effect of defence content on the observed lemming preferences. Similarly, herbivore performance did not correspond to the predicted effects of PIA content in *Glycine max* where other quality aspects were believed to be involved (Underwood et al. 2002). In *C. stans*, none of the nutrient compounds could fully explain lemming preferences. Possibly there are plant quality variables other than the ones examined in this study, which further explain the lemming feeding preferences.

Differential feeding by lemmings on *Carex* ramets may have larger effects in years of high lemming density, changing the relative proportions of different ramet stages in sedge populations. Indeed, lemming fluctuations have been suggested to cause cyclicity in flowering (Andersson and Jonasson 1986, Järvinen 1987, Jónsdóttir et al. 2000b). However, besides lemmings there are other herbivores like muskox, caribou and hare that also hold *C. stans* as a favoured food plant (Klein and Bay 1994). Different herbivores may respond differently to the defence of a plant (Karban and Agrawal 2002) and different herbivores may dominate at different sites. For instance, lemmings were the dominating mammalian herbivore at North Yukon NP, where they were estimated to account for 99% of the herbivore consumption, while muskoxen had the main impact at both North Banks Island and Devon Island, accounting for 97% of the total herbivore consumption (C. Krebs and D. Berteaux, unpubl.). Whereas lemmings are susceptible to PIA because they are monogastric (Gallaher and Schneeman 1984), the muskox, being a ruminant, is probably less responsive to PIA (Baintner and Pongor 1984, but see Cheeke and Palo 1995). The arctic sedge *C. stans* thus most likely experiences differential selection pressures from its herbivores. According to Jokela et al. (2000) differential selection pressures within a plant population restrain coevolution with any one of the herbivores, rather favouring evolution of other adaptations like tolerance. Indeed, indications of tolerance to grazing have been shown in both *Carex aquatilis* var. *stans* (Raillard and Svoboda 1999, Tolvanen and Henry 2000, Tolvanen et al. 2001) and in *Carex bigelowii* (Jónsdóttir 1991, Bråthen 2003). Despite this, both *Carex* species do also show ability to induce defence (Seldal et al. 1994, this study), although the defence efficiency seems variable. Rhizomatous *Carex* plants probably have both defensive strategies because neither of them is efficient enough under the variable conditions prevailing in arctic and alpine environments. *Carex* plants can experience a 250-fold difference in herbivore density between years (Batzli et al. 1980), but herbivore population densities may also be stable or show fluctuations over larger time scales. Thus, to meet the challenge imposed by different mammalian herbivores with widely different grazing regimes, the dual ability to induce defence and to tolerate grazing can be advantageous.

The potential ability of *Carex* to influence ramet development stage preferences by certain herbivores, and thereby their composition of sexual and vegetative ramets, have consequences for the demography and fitness of the clonal plant (Wikberg 1995). Indeed, in clonal sedge species like *Carex*, where individual genets may reach very old ages (Jónsdóttir et al. 2000a), such influence of grazing effects on ramet stage composition in one year can have pronounced effects on fitness in later years. The true benefit of evolved adaptations towards grazing in a clonal species like *Carex* may thus be difficult to spot in short term studies like the present one where the ultimate benefits potentially are years into the future.

*Acknowledgements* – The plant sampling and ramet preference trials were conducted during the Swedish "Tundra Northwest 1999" expedition in the Canadian Arctic. We thank the Swedish Polar Secretariat for logistic support. Göran Högstedt kindly helped with registrations in the field and he, Ivar Folstad and Rolf A. Ims gave valuable comments on the manuscript. Financial support was given by the Norwegian Research Council (KAB), The Royal Physiographic Society in Lund (JA), the Canadian Circumpolar Institute (DB) and the Swedish Natural Science Research Council (ISJ).

## References

Adler, F. R. and Karban, R. 1994. Defended fortresses or moving targets? Another model of inducible defences inspired by military metaphors. – Am. Nat. 144: 813–832.

Alpert, P. and Stuefer, J. F. 1997. Division of labour in clonal plants. – In: de Kroon, H. and van Groenendael, J. M. (eds), The ecology and evolution of clonal plants. Backhuys Publishers, pp. 137–154.

Andersson, M. and Jonasson, S. 1986. Rodent cycles in relation to food resources on an alpine heath. – Oikos 46: 93–106.

Ashmun, J. W., Thomas, R. J. and Pitelka, L. F. 1982. Translocation of photoassimilates between sister ramets in two rhizomatous herbs. – Ann. Bot. 49: 403–415.

Baintner, K. and Pongor, S. 1984. Effects of feeding raw soybean flour on the digestion of the adult sheep. – Acta. Vet. Hung. 32: 205.

Batzli, G. O. and Jung, H. G. 1980. Nutritional ecology of microtine rodents: resource utilization near Atkasook, Alaska. – Arc. Alp. Res. 12: 483–499.

Batzli, G. O. and Lesieutre, C. 1991. The influence of high-quality food on habitat use by Arctic microtine rodents. – Oikos 60: 299–306.

Batzli, G. O., White, R. G., MacLean, S. F. et al. 1980. The herbivore-based trophic system. – In: Brown, J., Miller, P. C., Tieszen, L. L. et al. (eds), An arctic ecosystem. The coastal tundra at Barrow, Alaska. US/IBP Synthesis Series, Dowden, Hutchinson and Ross, Inc. PA, pp. 335–410.

Bergmeyer, H. U. and Gawehn, K. 1974. Methods in enzymatic analysis. – Academic Press.

Bradford, M. M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. – Anal. Biochem. 72: 248–254.

Bråthen, K. A. 2003. Arctic and alpine plants and their interactions with herbivores – from individuals to the community. – Ph D thesis, Univ. of Tromsø, Norway.

Burnham, K. P. and Anderson, D. R. 1992. Data-based selection of an appropriate biological model: the key to modern data analysis. – In: McCullough, D. R. and Barrett, H.R. (eds), Wildlife 2001 populations. Elsevier Science, pp. 16–30.

Carlsson, B. Å. and Callaghan, T. V. 1990a. Programmed tiller differentiation, intraclonal density regulation and nutrient dynamics in *Carex bigelowii*. – Oikos 58: 219–230.

Carlsson, B. Å. and Callaghan, T. V. 1990b. Effects of flowering on the shoot dynamics of *Carex bigelowii* along an altitudinal gradient in Swedish Lapland. – Oikos 58: 219–230.

Chapin, F. S. III and Shaver, G. R. 1988. Differences in carbon and nutrient fractions among arctic growth forms. – Oecologia 77: 506–514.

Cheeke, P. R. and Palo, R. T. 1995. Plant toxins and mammalian herbivores: co-evolutionary relationships and antinutritional effects. – In: Journet, M., Grenet, E., Farce, M.-H. et al. (eds), Recent developments in the nutrition of herbivores. Proc. IVth Int. Symp. Nutrition of Herbivores. INRA Editions, Paris, pp. 437–456.
Danell, K., Elmqvist, T., Eriksson, L. et al. 1987. Are there general patterns in bark-eating by voles on different shoot types from woody plants? – Oikos 50: 396–402.
Dubois, M., Gilles, K. A., Hamilton, J. K. et al. 1956. Colorimetric method for determination of sugars and related substances. – Analyt. Chem. 28: 350–356.
Elmqvist, T., Ericson, L., Danell, K. et al. 1988. Latitudinal sex ratio variation in willows, *Salix* spp., and gradients in vole herbivory. – Oikos 51: 259–266.
Farrar, J. F. 1993. Carbon partitioning. – In: Hall, D. O., Scurlock, J. M. O., Bolhár-Nordenkampf, H. R. et al. (eds), Photosynthesis and production in a changing environment: a field and laboratory manual. Chapman and Hall, pp. 232–246.
Fenner, M., Hanley, M. E. and Lawrence, R. 1999. Comparison of seedling and adult palatability in annual and perennial plants. – Funct. Ecol. 13: 546–551.
Gallaher, D. and Schneeman, B. O. 1984. Nutritional and metabolic response to plant inhibitors of digestive enzymes. – In: Friedman, M. (ed.), Nutritional and toxicological aspects of food safety. Plenum Press, pp. 299–320.
Hamilton, J. G., Zangerl, A. R., DeLucia, E. H. et al. 2001. The carbon-nutrient balance hypothesis: its rice and fall. – Ecol. Lett. 4: 86–95.
Hjältén, J. 1992. Plant sex and hare feeding preferences. – Oecologia 89: 253–256.
Hutchings, M. J. and Wijesinghe, D. K. 1997. Patchy habitats, division of labour and growth dividends in clonal plants. – Trends Ecol. Evol. 12: 390–394.
Järvinen, A. 1987. Microtine cycles and plant production: what is cause and effect? – Oikos 49: 352–357.
Jensen, S. P. 1993. Temporal changes in food preferences of wood mice (*Apodemus sylvaticus* L.). – Oecologia 94: 76–82.
Jokela, J., Schmid-Hempel, P. and Rigby, M. C. 2000. Dr. Pangloss restrained by the Red Queen – steps towards a unified defence theory. – Oikos 89: 267–274.
Jones, E. L. and Roberts, J. E. 1991. A note on the relationship between palatability and water-soluble carbohydrates content in perennial ryegrass. – Irish J. Agric. Res. 30: 163–167.
Jónsdóttir, I. S. 1991. Effects of grazing on tiller size and population dynamics in a clonal sedge (*Carex bigelowii*). – Oikos 62: 177–188.
Jónsdóttir, I. S. 1995. Importance of sexual reproduction in arctic clonal plants and their evolutionary potential. – In: Callaghan, T. V., Oechel, W. C., Gilmanov, T. et al. (eds), Global change and arctic terrestrial ecosystems. Ecosystem report 10, European Commission, DG XII, Luxemburg, pp. 81–88.
Jónsdóttir, I. S. and Callaghan, T. V. 1988. Interrelationships between different generations of interconnected tillers of *Carex bigelowii*. – Oikos 52: 120–128.
Jónsdóttir, I. S. and Callaghan, T. V. 1989. Localized defoliation stress and the movement of $^{14}$C-photoassimilates between different tillers of *Carex bigelowii*. – Oikos 54: 211–219.
Jónsdóttir, I. S. and Callaghan, T. V. 1990. Intraclonal translocation of ammonium and nitrate nitrogen in *Carex bigelowii* Torr. ex Schwein. using $^{15}$N and nitrate reductase assays. – New Phytol. 114: 419–428.
Jónsdóttir, I. S. and Watson, M. A. 1997. Extensive physiological integration: an adaptive trait in resource-poor environments? – In: de Kroon, H. and van Groenendael, J. M. (eds), The ecology and evolution of clonal plants. Backhuys Publishers, pp. 109–136.
Jónsdóttir, I. S. and Bråthen, K. A. 1999. Can plant population biology solve the mysteries for Arctic tundra ecology? – In: Grönlund, E. (ed.), Polarforskningssekretariatet. Årsbok, pp. 71–75.
Jónsdóttir, I. S., Callaghan, T. V. and Headley, A. D. 1996. Resource dynamics within clonal plants. – Ecol. Bull. 45: 53–64.
Jónsdóttir, I. S., Augner, M., Fagerström, T. et al. 2000a. Genet age in marginal populations of two clonal *Carex* species in the Siberian Arctic. – Ecography 23: 402–412.
Jónsdóttir, I. S., Stenström, A., Fagerström, T. et al. 2000b. Population variation in clonal sedges along the arctic coast of Eurasia: effects of climate, weather and lemmings. – In: Stenström, A. From pollination to variation – reproduction in arctic clonal plants and the effects of simulated climate change. Ph-D Thesis. Botanical Institute, Göteborg Univ., Sweden.
Karban, R. and Baldwin, I. T. 1997. Induced responses to herbivory. – The Univ. of Chicago Press.
Karban, R. and Thaler, J. S. 1999. Plant phase change and resistance to herbivory. – Ecology 80: 510–517.
Karban, R. and Agrawal, A. A. 2002. Herbivore offense. – Annu. Rev. Ecol. Syst. 33: 641–664.
Klein, D. R. and Bay, C. 1994. Resource partitioning by mammalian herbivores in the high arctic. – Oecologia 97: 439–4.
Klimeš, L., Klimešova, J., Hendriks, R. et al. 1997. Clonal plant architecture: a comparative analysis of form and function. – In: de Kroon, H. and van Groenendael, J. M. (eds), The ecology and evolution of clonal plants. Backhuys Publishers, pp. 1–29.
Marshall, C. and Price, E. A. C. 1997. Sectorality and its implications for physiological integration. – In: de Kroon, H. and van Groenendael, J. M. (eds), The ecology and evolution of clonal plants. Backhuys Publishers, pp. 79–107.
McKey, D. 1974. Adaptive patterns in alkaloid physiology. – Am. Nat. 108: 305–320.
Murray, D. F. 1994. Floristics, systematics, and the study of arctic vegetation – a commentary. – J. Veg. Sci. 5: 777–780.
Negus, N. C. and Berger, P. J. 1998. Reproductive strategies of *Dicrostonyx groenlandicus* and *Lemmus sibiricus* in high-arctic tundra. – Can. J. Zool. 76: 391–400.
Pan, J. J. and Price, J. S. 2002. Fitness and evolution in clonal plants: the impact of clonal growth. – Evol. Ecol. 15: 583–600.
Raillard, M. C. and Svoboda, J. 1999. Exact growth and increased nitrogen compensation by the arctic sedge *Carex aquatilis* var. *stans* after simulated grazing. – Arc. Antarc. Alp. Res. 31: 21–26.
Rhoades, D. F. 1979. Evolution of plant chemical defence against herbivores. – In: Rosenthal, G. A. and Janzen, D. H. (eds), Herbivores. Their interaction with secondary plant metabolites. Academic Press, pp. 3–54.
Rhoades, D. F. 1985. Offensive-defensive interactions between herbivores and plants: their relevance in herbivore popualtion dynamics and ecological theory. – Am. Nat. 125: 205–238.
Seldal, T., Andersen, K. and Högstedt, G. 1994. Grazing-induced proteinase inhibitors: a possible cause for lemming population cycles. – Oikos 70: 3–11.
StatSoft, Inc. 2001. STATISTICA 6.0 (data analysis software system). www.statsoft.com.
Stenström, A. 1999. Sexual reproductive ecology of *Carex bigelowii*, an arctic-alpine sedge. – Ecography 22: 305–313.
Sturgeon, R. J. 1990. Monosaccharides. – In: Dey, P.M. & Harborne, J.B. (eds), Methods in plant biochemistry Vol. 2. Carbohydrates. Academic Press, London, pp. 1–38.
Tolvanen, A. and Henry, G. H. R. 2000. Population structure of three dominant sedges under muskox herbivory in the high Arctic. – Arc. Antarc. Alp. Res. 32: 449–455.
Tolvanen, A., Schroderus, J. and Henry, G. H. R. 2001. Demography of three domiant sedges under contrasting grazing regimes in the high Arctic. – J. Veg. Sci. 12: 695–670.

Underwood, N., Rausher, M. and Cook, W. 2002. Bioassay versus chemical assay: measuring the impact of induced and constitutive resistance on herbivores in the field. – Oecologia 131: 211–219.
Watson, M. A. 1986. Integrated physiological units in plants. – Trends Ecol. Evol. 1: 119–123.
Watson, M. A. and Casper, B. B. 1984. Morphogenetic constraints on patterns of carbon distribution in plants. – Annu. Rev. Ecol Syst. 15: 233–258.
Wikberg, S. 1995. Fitness in clonal plants. – Oikos 72: 293–297.
Zangerl, A. R. and Rutledge, C. E. 1996. The probability of attack and patterns of constitutive and induced defence: a test of optimal defence theory. – Am. Nat. 147: 599–608.
Zangerl, A. R., Green, E. S., Lampman, R. L. et al. 1997. Phenological changes in primary and secondary chemistry of reproductive parts in wild parsnip. – Phytochemistry 44: 825–831.
Zar, J. H. 1996. Biostatistical analysis. – Prentice Hall.