GENETIC VARIABILITY WITHIN *CAREX SEMPERVIRENS* TUSsockS OF CONTRASTING VITALITY

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Tentative evidence indicates that a discrete tussock may not be genetically uniform, especially if its vitality is reduced. Using molecular markers, we studied the genetic composition of eight vigorous and eight weak tussocks of *Carex sempervirens* (cover $\geq 75\%$ and $\leq 35\%$, respectively) to elucidate the relationship between tussock vigor and genetic variability. Four *C. sempervirens* tussocks harbored more than one genotype. However, all four variable tussocks were weak, and the additional genotypes occurred only at the edge of the tussock base. The formation of a genetically variable tussock of *C. sempervirens* is best explained by a seedling or small tussock being incorporated by a large, expanding tussock that eventually surrounds it. The vitality-related pattern was most likely caused by the greater intraspecific competition both inside and around the vigorous tussocks. We conclude that the formation of a tussock may involve more than one seedling and that tussocks, therefore, may not a priori be treated as genetic individuals.

Keywords: clonal diversity, clonal plants, phalanx growth form, small-scale genetic variability, tussock formation.

Introduction

Tussock-forming graminoids are distributed worldwide (Briske and Derner 1998) and play a substantial role in many ecosystems, including wetlands, tundra, and high-altitude grasslands (Chapin et al. 1979; Jonsdottir and Watson 1997; Klimes et al. 1997; Korner 1999). These species represent the typical phalanx form of clonal growth (Lovett-Doust 1981), which is characterized by the compact spatial arrangement of tillers within a genetic individual (i.e., genet; van Groenendaal and de Kroon 1990; de Kroon and van Groenendaal 1997; Briske and Derner 1998).

Tussocks of perennial graminoids commonly consist of separate tiller groups (Briske and Anderson 1990; Welker et al. 1991; Lord 1993; Wilhalm 1995). In a study involving 103 tussocks of 24 tussock-forming perennial grasses, Wilhalm (1995) found that each tussock consisted of 2–35 separate tiller groups. Similarly, using an isotopic tracer, Dodd and van Amburg (1970) reported that a tussock of *Andropogon scoparius* consisted of many tiller groups, each acting as a unique physiological individual. Generally, it is believed that the separate tiller groups result from fragmentation of a genet because of aging or disturbances such as trampling and grazing and that they are genetically identical (Dodd and van

Amburg 1970; Butler and Briske 1988; Wilhalm 1995). However, the tiller groups may also result from the establishment and clonal expansion of different seedlings, so that a tussock is genetically variable.

There are three major processes by which a genetically variable tussock may be generated, namely, coincidental aggregation, incorporation by radial expansion, and infiltration by seeds. First, genetic variability within tussocks may originate from the accidental aggregation of two or more seedlings (or small tussocks) growing next to each other. Second, in the process of radial expansion, a large tussock may integrate or incorporate but, at least in the beginning, not outcompete and eliminate seedlings or small tussocks growing near the edge of its base. Third, seedlings of the tussock species may establish themselves and survive within a large tussock. Studies have shown that, frequently, various plant species can grow within tussocks of graminoid species such as *Molinia caerulea* (Rutter 1955), *Eriophorum vaginatum* (Fetcher 1985), *Ischaemum aristatum* var. *glaucum* (Yabe 1985), *Carex stricta* (Lord and Lee 2001), *Carex nudata* (Levine 1999, 2000, 2001), and *Carex sempervirens* (Yu et al. 2004). In a streamside community in northern California, for instance, more than 60 different species colonize the tussocks of *C. nudata*, one tussock containing up to 20 different species (Levine 1999, 2000, 2001). Similarly, in a subalpine grassland in the Swiss National Park, 68 different vascular species were found inside tussocks of *C. sempervirens*, with up to 19 species in a single tussock, attaining a cumulative cover of up to 102% (F.-H. Yu, B. Krüsi, M. Schütz, J. J. Schneller, and O. Wildi, unpublished data). It is, therefore,

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reasonable to assume that seedlings of the tussock species may also establish themselves within tussocks.

Under field conditions, the vitality of large tussocks may vary considerably (Gatsuk et al. 1980; Briske and Derner 1998), with weak tussocks having a canopy cover of, for instance, less than 15%, compared with close to 100% in vigorous tussocks (F.-H. Yu and B. Krüsi, personal observations). Intraspecific competition between the well-developed tussocks and the new seedlings is very likely to be much higher in a vigorous tussock than in a weak one. We therefore hypothesize that the chance to find new genets is higher in weak tussocks than in similar-sized vigorous ones. Although numerous studies have investigated the genotypic uniformity of clonal plants (reviewed by Ellstrand and Roose [1987]; McLellan et al. [1997]), most were done on a much larger scale, i.e., on the population level. To our knowledge, the genetic identity of tillers within tussocks of graminoids has rarely been assessed.

Carex sempervirens Vill. (evergreen sedge) forms well-delineated tussocks with more or less circular bases and occurs frequently in the mountain ranges of central and southern Europe (Hess et al. 1967). In a previous study conducted in a subalpine grassland, we observed tussocks of *C. sempervirens* with basal diameters of up to 49 cm and tussock bases rising up to 13 cm above the soil surface. We also found many different vascular species growing in the tussocks (F.-H. Yu et al., unpublished data).

In this study, we specifically address the following questions: (1) Are large tussocks of *C. sempervirens* formed by one or several genets; i.e., are they genetically uniform or variable? (2) If they are genetically variable, is there a pattern with regard to tussock vitality; i.e., are weak tussocks more variable than vigorous ones? (3) What are the spatial patterns of the different genets within the genetically variable tussock; i.e., do the additional genets occur anywhere within the tussock or only in specific positions, e.g., at the edge or in the center? (4) Which of the above-mentioned mechanisms, i.e., coincidental aggregation, incorporation by radial expansion, and infiltration by seeds, best explains our findings?

Material and Methods

Sampling Design

The study area is an abandoned subalpine pasture, 10.7 ha in size and ranging in altitude from 1920 to 1980 m a.s.l., currently dominated by *Carex sempervirens*. The pasture is located in the Swiss National Park in the Engadine Valley of the Central Alps. On an area of ca. 100 m × 100 m, we selected in August 2004 eight weak and eight vigorous tussocks of *C. sempervirens* with a basal diameter of more than 25 cm that were, in addition, well delineated and clearly separated from other tussocks. The weak and vigorous tussocks differed in many vitality-related traits, such as number of inflorescences (3.6 vs. 48.9), canopy cover (24.8% vs. 89.3%), and number of vascular plant species growing on the tussock base (14.6 vs. 8.5), but not in size (basal diameter: 31.4 vs. 30.4 cm; table 1). The difference in vitality was confirmed by a greenhouse experiment where tillers from weak tussocks produced much less biomass and fewer leaves than tillers originating from vigorous tussocks (F.-H. Yu, M. Schütz, B. Krüsi, J. J. Schneller, and O. Wildi, unpublished data).

For sampling, we used a 30 × 30-cm metal frame consisting of 36 quadrats, each 5 cm × 5 cm in size. We assigned a specific number to each of the 36 quadrats so that we knew the relative sampling position within a tussock. For sampling, the center of the frame was positioned in the center of the base of the selected tussock (fig. 1a). In each quadrat where *C. sempervirens* tillers were present, one or two interconnected tillers of *C. sempervirens* were taken. For quadrats completely located on the tussock base (quadrats 8–11, 14–17, 20–23, and 26–29 in the case of a 30-cm-diameter tussock; fig. 1a), we took tillers from the center of the quadrats. For quadrats at the edge of the tussock base (quadrats 1–7, 12–13, 18–19, 24–25, and 30–36 in the case of a 30-cm-diameter tussock; fig. 1a), however, we collected tillers from the edge of the tussock. If the basal diameter was between 30 and 35 cm, the outermost quadrats were extended to encompass the edge of the tussock base, and the tillers were collected at the very edge. Because of the differences in vitality and thus

Table 1
Characteristics of the Studied Tussocks of *Carex sempervirens*

Trait	Weak tussocks				Vigorous tussocks				<i>t</i>
	Mean	SE	Maximum	Minimum	Mean	SE	Maximum	Minimum	
Basal diameter (cm)	31.4	0.4	34	30	30.4	0.8	34	28	1.08 ns
Basal height (cm)	8.1	0.5	10	6	9.8	0.4	12	8	2.47*
Maximum canopy diameter (cm)	47.6	1.7	55	40	67.8	1.8	76	62	8.28***
MCD/BD	1.5	0.1	1.3	1.8	2.2	0.1	1.9	2.5	8.16***
Maximum canopy height (cm)	19.1	1.2	24	15	30.4	1.2	37	27	6.72***
Average canopy height (cm)	15.0	0.9	20	12	23.7	0.7	27	21	7.52***
No. <i>Carex sempervirens</i> inflorescences	3.6	1.6	13	0	48.9	7.6	85	31	5.81***
Canopy cover of <i>C. sempervirens</i> (%)	24.8	2.7	35	15	89.3	3.3	100	75	15.23***
Cover of all species (%)	73.1	3.9	95	60	94.6	2.1	100	85	4.87***
Species richness	14.6	1.0	18	9	8.5	0.8	12	5	4.63***

Note. MCD/BD = maximum canopy diameter/basal diameter. Species richness = number of vascular species other than *C. sempervirens*. Footnotes indicate significance of the *t*-tests between means (*df* = 14); ns, *P* > 0.05.

* *P* < 0.05.

*** *P* < 0.001.

Fig. 1 a, Sampling design; b–d, genetically uniform tussocks; e–h, genetically variable tussocks. V = vigorous tussock; W = weak tussock. The size of each quadrat is 5 cm × 5 cm. The circle represents the border of the tussock base. Empty quadrats mean that there was no tiller of *Carex sempervirens*. Shaded quadrats stand for the additional genotypes.

the number of empty quadrats, sample size varied among the tussocks, ranging from 26 to 36 (e.g., fig 1b–1h). The samples were dried with silica gel and kept at room temperature until DNA extraction.

DNA Extraction and RAPD-PCR

Total genomic DNA was extracted using DNeasy Plant Mini Kit (QIAGEN). We used three Operon oligonucleotide primers (decamers OPA-4, OPD-2, and OPD-18; QIAGEN) to assess the genotypic pattern of tussocks. In a previous study, the three primers produced 36 interpretable polymorphic markers, and each of the 300 different tillers, sampled from different tussocks, proved to be of a different genotype (F.-H. Yu et al., unpublished data). DNA amplification was performed in Biometra T1 Thermocycler (Biolabo Scientific Instruments), with 12.5 μ L of PCR reaction mixture composed of 1 $\times$ PCR buffer, 0.2 mM of each dNTP, 0.4 μ M primer, 2.0 μ M MgCl₂, 0.7 U *Taq* DNA polymerase (Sigma), and 5–10 ng of DNA template. The reaction was run for an initial 2 min at 94°C, followed by 40 cycles of 15 s at 94°C, 45 s at 39°C, and 90 s at 72°C, and then ended with a cycle of 6 min at 72°C and 5 min at 4°C (Weising et al. 2005). The amplification products were separated by electrophoresis on 1.5% agarose gels with 1 $\times$ TBE (Tris-borate-EDTA) buffer, and stained with ethidium bromide. Images were obtained using the Syngene imaging device (Biolabo Documentation System).

Detection of New Genets

Because of the high variability, RAPD is suitable for distinguishing between different genotypes (genets) of a clonal plant (Steinger et al. 1996). However, RAPD is sensitive to slight changes in reaction conditions (Weising et al. 2005).

To minimize this problem, we took the following measures: (1) For each primer, we made a master mixture for all the samples of a given tussock, containing all reaction components except the DNA template. Ten microliters of the master mixture was then poured into each reaction container and 2.5 μ L DNA template was added. (2) PCR of these samples was conducted using the same thermocycler and during the same period of time. (3) The resulting PCR products were separated on the same gel or on two parallel-running gels. Once different genotypes were detected within a tussock, DNA of the sample of the additional genotype was re-extracted, PCR was repeated together with that of at least six different samples of the dominant genotype, and the products were run again in the same gel (see fig. 1). If the sample with DNA re-extraction still proved to be a new genotype, the tussock was treated as genetically variable.

Results and Discussion

Each of the 16 tussocks studied was dominated by a different genotype (genet). Twelve tussocks were genetically uniform (e.g., fig. 1b–1d), whereas each of the other four contained two genotypes (fig. 1e–1h). Of the two genotypes in each of the four genetically variable tussocks, one (hereafter termed “dominant genet”) was present in the majority of the quadrats, whereas the other (hereafter called “additional genet”) occurred in only one (fig. 1f–1h) or four quadrats (fig. 1e). Recent studies have shown that the separation into several tiller groups within a tussock is a common phenomenon in tussock-forming perennial graminoids (Butler and Briske 1988; Williams and Briske 1991; Wilhalm 1995). The same holds true for *Carex sempervirens* tussocks (J. J. Schneller, personal observation). It is generally believed that the separate tiller groups result from the fragmentation of

an originally interconnected genet (Butler and Briske 1988; Williams and Briske 1991; Wilhalm 1995). The findings of our study, however, suggest that they may also originate from different seedlings.

All the genetically variable tussocks were weak ones, whereas all the vigorous tussocks were genetically uniform (figs. 1, 2), even though the number of inflorescences and

thus seed production was much greater in the vigorous than in the weak tussocks (table 1). These findings agree with the hypothesis that the chance of finding new genotypes is higher in weak than in vigorous tussocks. The observed vitality-related pattern was most likely caused by the greater intra-specific competition both inside and around the vigorous tussocks, as expressed, e.g., by size and cover of the canopy

Fig. 2 RAPD genotypes within tussocks of *Carex sempervirens*. *a*, Vigorous tussock 4 (V-4), with only one genotype A; *b*, weak tussock 2 (W-2), with a dominant genotype B and an additional genotype C; *c-e*, three weak tussocks (W-4, W-6, and W-8), with a dominant genotype (D, F, or H) and a single occurrence of an additional genotype (E, G, or I). The primer used was OPA-4, but the other two primers, i.e., OPD-2 and OPD-18, showed the same deviations from the dominant genotypes. 1st = DNA extracted at the first time; 2nd = DNA re-extracted.

of *C. sempervirens* (table 1). Because of the extremely high canopy cover in the vigorous tussocks (table 1), which is closely positively related to tiller density (F.-H. Yu and B. Krüsi, personal observation), intraspecific competition between the dominant genet and potential seedlings was likely too severe to allow new genets of *C. sempervirens* to be established either in the center or at the edge of the tussock base. In addition, seedling establishment immediately outside the tussock base but still under the tussock canopy is most likely much more difficult in the case of vigorous tussocks, e.g., because of shading (table 1). On the other hand, proximity to a large tussock may also increase the chances for survival of *C. sempervirens* seedlings (i.e., facilitation; Levine 1999), because large tussocks are avoided by large herbivores, such as red deer. Even then, however, chances that seedlings and small tussocks are outcompeted by the nearby large tussock are much higher with vigorous than with weak tussocks. To what extent the vigor of the tussocks affects establishment and survival of competing plants is, for instance, also illustrated by the significantly reduced number of vascular species (other than *C. sempervirens*) present in vigorous tussocks (table 1).

Another possible explanation for the observed pattern could be that vigorous and weak tussocks grew at different rates or represented different life stages of *C. sempervirens* tussocks, e.g., as described by Gatsuk et al. (1980) for tussock grasses. Thus, weak tussocks might be older than similar-sized vigorous tussocks. Consequently, one could argue that weak tussocks should be genetically more diverse than vigorous ones because there was more time to incorporate additional genets. In particular, this would be relevant if genetic diversity had originated from infiltration by seeds. However, there is evidence that the basal diameter of senescent tussocks of *C. sempervirens* is shrinking (B. Stüssi and B. Krüsi, personal observation), which would exclude incorporation by radial expansion as a possible process to generate genetic variability in weak tussocks.

All additional genets were found at the edge of the tussocks (fig. 1e–1h). This pattern corresponds best to the mechanism of incorporation where a large, expanding tussock eventually surrounds and incorporates a seedling or small tussock that had once started to grow outside but near the base of the large tussock. In coincidental aggregation of two

genets of similar strength and age, one would expect a more balanced presence of the two genets. In infiltration of a large tussock by seeds, one would expect to find some of the additional genets also near the center of the large tussock. The observed pattern indicates that, if seeds fall inside or around tussocks, the seedlings (new genets) may establish and survive only near the edge of large, weak tussocks. This may result from the massive amount of litter accumulated in the center of such tussocks. The litter accumulated in the up-to-13-cm-tall tussock base may effectively prevent the establishment of *C. sempervirens* seedlings mechanically and possibly also chemically (i.e., accumulation of toxic substances) in and near the center, even in weak *C. sempervirens* tussocks. The long persistence of *C. sempervirens* at a given microsite may also, for instance, have led to the depletion of certain macro- and micronutrients.

Conclusions

This study is the first to investigate genetic diversity within a tussock. We conclude that the formation of a tussock may involve more than one seedling. Consequently, tussocks, especially large, weak ones, may not a priori be treated as genetic individuals in ecological and evolutionary studies. We suggest that, without assessing genotypic uniformity, questions on, e.g., tiller regulation and clonal integration within a tussock should be addressed with caution, especially if large, weak tussocks are considered. Regarding the strategy of *Carex sempervirens*, we hypothesize that the combination of vegetative and generative reproduction may render the population more resilient. Survival of new genets in and around large, weak tussocks of *C. sempervirens* may help maintain its population density and diversity and thus may help increase the chances for the continued dominance of the species.

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Literature Cited

- Briske DD, VJ Anderson 1990 Tiller dispersion in populations of the bunchgrass *Schizachyrium scoparium*: implications for herbivory tolerance. *Oikos* 59:50–56.
- Briske DD, JD Derner 1998 Clonal biology of caespitose grasses. Pages 106–135 in G Cheplick, ed. *Population biology of grasses*. Cambridge University Press, Cambridge.
- Butler JL, DD Briske 1988 Population structure and tiller demography of the bunchgrass *Schizachyrium scoparium* in response to herbivory. *Oikos* 51:306–312.
- Chapin FS III, K van Cleve, MC Chapin 1979 Soil temperature and nutrient cycling in the tussock growth form of *Eriophorum vaginatum*. *J Ecol* 67:169–189.
- de Kroon H, J van Groenendaal, eds 1997 *The ecology and evolution of clonal plants*. Backhuys, Leiden.
- Dodd JD, GL van Amburg 1970 Distribution of ^{134}Cs in *Andropogon scoparius* Michx. clones in two native habitats. *Ecology* 51:685–689.
- Ellstrand NC, ML Roose 1987 Patterns of genotypic diversity in clonal plant species. *Am J Bot* 74:123–131.
- Fetcher N 1985 Effects of removal of neighboring species on growth, nutrients, and microclimate of *Eriophorum vaginatum*. *Arct Alp Res* 17:7–17.
- Gatsuk LE, OV Smirnova, LI Vorontzova, LB Zaugolnova, LA Zhukova 1980 Age states of plants of various growth forms: a review. *J Ecol* 68:675–696.
- Hess HE, E Landolt, R Hirzel 1967 *Flora der Schweiz und angrenzender Gebiete*. Birkhäuser, Basel.
- Jonsdottir I, M Watson 1997 Extensive physiological integration: an adaptive trait in resource-poor environments? Pages 109–136 in

- H de Kroon, J van Groenendaal, eds. The ecology and evolution of clonal plants. Backhuys, Leiden.
- Klimes L, J Klimesova, R Hendriks, J van Groenendaal 1997 Clonal plant architecture: a comparative analysis of form and function. Pages 1–29 in H de Kroon, J van Groenendaal, eds. The ecology and evolution of clonal plants. Backhuys, Leiden.
- Korner C 1999 Alpine plant life: functional plant ecology of high mountain ecosystems. Springer, New York.
- Levine JM 1999 Indirect facilitation: evidence and predictions from a riparian community. *Ecology* 80:1762–1769.
- 2000 Species diversity and biological invasions: relating local process to community pattern. *Science* 288:852–854.
- 2001 Local interactions, dispersal, and native and exotic plant diversity along a California stream. *Oikos* 95:397–408.
- Lord JM 1993 Does clonal fragmentation contribute to recruitment in *Festuca novae-zelandiae*? *N Z J Bot* 31:133–138.
- Lord LA, TD Lee 2001 Interactions of local and regional processes: species richness in tussock sedge communities. *Ecology* 82: 313–318.
- Lovett Doust L 1981 Population dynamics and local specialization in a clonal perennial (*Ranunculus repens*). I. The dynamics of ramets in contrasting habitats. *J Ecol* 69:743–755.
- McLellan AJ, D Prati, O Kaltz, B Schmid 1997 Structure and analysis of phenotypic and genetic variation in clonal plants. Pages 185–210 in H de Kroon, J van Groenendaal, eds. The ecology and evolution of clonal plants. Backhuys, Leiden.
- Rutter AJ 1955 The composition of wet-heath vegetation in relation to the water table. *J Ecol* 43:507–543.
- Steinger T, C Korner, B Schmid 1996 Long-term persistence in a changing climate: DNA analysis suggests very old ages of clones of alpine *Carex curvula*. *Oecologia* 105:94–99.
- van Groenendaal J, H de Kroon 1990 Clonal growth in plants: regulation and function. SPB Academic, The Hague.
- Weising K, H Nybom, K Wolff, G Kahl 2005 DNA fingerprinting in plants: principles, methods, and applications. CRC, London.
- Welker JM, DD Briske, RW Weaver 1991 Intraclonal nitrogen allocation in the bunchgrass *Schizachyrium scoparium* Hubb.: an assessment of the physiological individual. *Funct Ecol* 5:433–440.
- Wilhelm T 1995 A comparative study of clonal fragmentation in tussock-forming grasses. *Abstr Bot* 19:51–60.
- Williams DG, DD Briske 1991 Size and ecological significance of the physiological individual in the bunchgrass *Schizachyrium scoparium*. *Oikos* 62:41–47.
- Yabe K 1985 Distribution and formation of tussocks in Mobaratsumi Marsh. *Jpn J Ecol* 35:183–191.
- Yu F-H, M Schütz, B Krüsi, JJ Schneller, O Wildi 2004 Succession affects the diversity-area relationships in *Carex sempervirens* tussocks. Page 146 in A Otte, D Simmering, L Eckstein, N Hölzel, R Waldhardt, eds. Eco-complexity and dynamics of the cultural landscape. Proceedings of the 34th Annual Conference of the Ecological Society of Germany, Austria, and Switzerland. Gesellschaft für Ökologie, Berlin.