Flora 246–247 (2018) 102–108

Contents lists available at ScienceDirect

Flora

journal homepage: www.elsevier.com/locate/flora

DNA-content and chromosome number in populations of *Poa alpina* in the Alps reflect land use history

Florian Spinnler, Jürg Stöcklin*

Institute of Botany, Department of Environmental Sciences, University of Basel, Schönbeinstrasse 6, CH-4056 Basel, Switzerland

ARTICLE INFO

Edited by Alessio Papini

Keywords:
Apomixis
Flow cytometry
Polyploidy
Genetics
Grassland
Swiss Alps

ABSTRACT

Poa alpina, a widespread alpine grass in the temperate and subarctic zone, is a polyploid complex including many cytotypes with a broad range of chromosome numbers. To assess the occurrence and frequency of cytotypes, and to test for effects of past and current land use on their distribution, we measured DNA content using flow cytometry in 543 individuals of *Poa alpina* from agriculturally used grassland parcels and from natural sites in 12 local communities from three cultural traditions in the Swiss Alps. We tested whether geography (local community), affiliation to three cultural traditions, current land use, fertilization, and elevation affected DNA-content of populations. DNA content among individuals was highly variable (2C values ranging 100–600 pg) indicating chromosome numbers between 14 and 69. Cultural tradition, geography (local community), and current land use significantly affected mean DNA-content of *Poa alpina* in populations from agriculturally used parcels. The range of DNA-content and cytotypes was larger in pastures than in meadows, and the distribution of cytotypes differed among cultural traditions. Plants with more chromosomes grew larger in a common garden suggesting higher vigour. Among reproducing plants, an almost equal share was either reproducing by seeds or by bulbils. Seed-producing individuals had a higher DNA-content. Results presented here suggest that human land use is reflected directly in the distribution of cytotypes of different ploidy level and chromosome number. The effect of different cultural traditions is an indication that the current distribution of cytotypes does not only reflect geographic variability and current land use, but also differences in land use in the past that are persisting to the present.

1. Introduction

Alpine meadow-grass (*Poa alpina* L.) is a widespread perennial tuft-forming grass (Hegi, 1935; Hermann, 1956) in high mountains of the northern Hemisphere (Schröter, 1926). In Europe, *Poa alpina* is among the most important fodder plants in alpine grassland and abundant in nutrient-rich pastures and meadows (Hegi, 1935). *Poa alpina* is a polyploid complex including many cytotypes with variable chromosome numbers and common aneuploidy (Müntzing, 1954; Duckert-Henriod and Favarger, 1987). The basic chromosome number of *Poa alpina* is seven. Duckert-Henriod and Favarger (1987) described 18 cytotypes in the Swiss Alps, with a range of chromosomes from 14 to 61, including the more frequent and stable cytotypes with 22, 26, 33 and 37 chromosomes. Studies from North Sweden, from the island of Öland and Gotland in the Baltic Sea found similarly large ranges of chromosome numbers as in Switzerland (Müntzing, 1954). The causes and ecological significance of this diversity are poorly understood.

Multiple hypotheses have been proposed to explain the formation of polyploidy in *Poa alpina* (Gustafsson, 1948; Favarger, 1967; and Stebbins, 1984). The habitat differentiation hypothesis suggests that a high number of cytotypes results from an increasing differentiation of habitats as observed in grassland of the Sheffield region in England (Grime, 1987). The secondary contact hypothesis predicts a high formation of ploidy complexes under the influence of Pleistocene glacial cycles (Gustafsson, 1948; Favarger, 1967; Stebbins, 1984). Under this model, progenitor populations became fragmentated due to the advancement of glaciers; upon glacial retreat, rapid environmental changes and opening of new contact zones caused the differentiated progenitor populations to come into secondary contact and hybridize, inducing polyploidization. Indeed, Brochmann et al. (2004) found that in general, diploids and tetraploids occur in comparable frequencies in boreal and arctic areas, while higher ploidy cytotypes seem to be more successful colonizers after deglaciation. Thus, polyploidization is generally considered as a fast evolutionary process and the many cytotypes of polyploid complexes may be differently adapted.

Mountain grasslands in the Alps have been under long-term human

* Corresponding author.
E-mail address: juerg.stoecklin@unibas.ch (J. Stöcklin).

https://doi.org/10.1016/j.flora.2018.08.002
Received 26 January 2018; Received in revised form 30 July 2018; Accepted 2 August 2018
Available online 04 August 2018
0367-2530/

influence, but in different ways depending on the cultural tradition. Geographic patchiness, and variable abiotic, ecological and human influences affect the distribution of diploids and polyploids (Johnson and Packer, 1965, 1967, 1968; Favarger, 1967; Fischer et al., 2008). Therefore, differences in land use hold a promise to disentangle the factors affecting frequency and distribution of cytotypes in polyploid complexes. For many polyploid complexes, including *Poa alpina*, these factors remain poorly known.

Here, we tested weather *Poa alpina* populations from alpine grasslands in the Swiss Alps differ in their mean DNA content and corresponding ploidy level and chromosome number as a result of geography and different land use history in the past and today. We selected parcels of agriculturally used land in twelve communities representing the three different cultural traditions present in the Swiss Alps ('germanic', 'romanic', 'walser'). For each community, we sampled plants from sites at three elevations, three land use types (grazed pastures, mown meadows, natural sites with unused land), and which were either fertilized or not. In the Alps, the three cultural traditions contributed in the past to high landscape diversity by different land use practices (Bötzing, 2003; Maurer et al., 2006). In each study site (agriculturally used parcels and natural sites), we sampled individual plants of *Poa alpina* and measured their DNA-content. We also assessed the reproductive mode of plants (i.e., through seeds or bulbils) and their vigour by growing the plants in a common garden and harvesting their biomass. We ask weather in *Poa alpina* a different elevation, reproductive mode, and land use history today and in the past affects the distribution of cytotypes with different DNA-content and chromosome number.

2. Material and methods

2.1. Study species

Poa alpina L. (Poaceae) is a circumpolar species in the arctic and alpine zone occurring usually from 1400 to 2500 m a.s.l. in nutrient rich meadows and pastures, but also on pioneer vegetation on scree and in snowbeds (Schröter, 1926; Conert, 1998). Due to its high protein and fat content *Poa alpina* is among the most important fodder grasses in mountain regions (Hegi, 1935). *Poa alpina* is a polyploid complex with highly variable, aneuploid chromosome numbers, ranging from 7 to 42 chromosomes (KEW DNA database). Müntzing (1954), and Favarger (1987) are even reporting as much as 80 chromosomes and 18 different cytotypes in this species. *Poa alpina* occurs with two reproductive modes: either as seed-producing (seminiferous), or as bulbil-producing (pseudo-viviparous) plants. Seed producing plants are facultative apomicts, i.e. seeds are produced either sexually or apomictically (Müntzing, 1933; Steiner et al., 2012). The pseudoviviparous plants reproduce clonally by forming little plantlets (bulbils) instead of seeds that can root and establish easily (Müntzing, 1980; Pierce et al., 2003). By the diversity of cytotypes and the two different mode of reproduction *Poa alpina* is able to successfully occupy a broad ecological niche across a large elevational range (Steiner et al., 2012; Stöcklin and Armbruster, 2016).

2.2. Study areas

We selected individuals of *Poa alpina* from agriculturally used parcels in 12 rural municipalities ('Gemeinde') in the Swiss Alps, and in natural sites within the same local communities (Fig. 1, Maurer et al., 2006). Each local community represents a separate Alpine valley. Four local communities each belong to one of three different cultural traditions (Bötzing, 2003). *The Romanic culture* settled early in alpine valleys after 1800 BCE and was characterized by mixed farming including cattle and growing of cereals. *The Germanic culture* established around 600 CE, when Allemanic people migrated from the north into more humid and cooler regions of the Alps and established a tradition of mainly dairy farming and breeding for meat production. The most recent form of culture was established after 1200 CE, when the *Walser,* allemanic people from the Valais, migrated eastwards and settled at higher elevations and combined a living from farming and trade across alpine passes (Bätzing, 2003*)*. Socio-economic differences among these cultural traditions affecting human land use are still remaining (Pfister, 2004). The socio-economic differences were found to still affect land use diversity in local communities and thereby plant biodiversity at the landscape and the species level (Maurer et al., 2006), as well as genetic microsatellite diversity of *Poa alpina* (Rudmann-Maurer et al., 2007).

2.3. Sampling

Poa alpina was located in parcels of land selected for a biodiversity study in alpine grassland (Maurer et al., 2006). The parcels were situated at three elevational levels in agriculturally used grassland, at the valley bottom (c. 1000 m a.s.l.), at intermediate elevation (c. 1500 m a.s.l.), and in the lower alpine zone above the tree line (c. 2000 m a.s.l.) in each of the 12 local communities. In total, 216 agriculturally used grassland parcels where, according to local farmers and local authorities, the type of land use had not changed in the past, were surveyed for *Poa alpina*. The agriculturally used parcels differed in their combination of current land use (mown or grazed) and were either fertilized or not (see Maurer et al., 2006, and Rudmann-Maurer et al., 2007 for more details on study localities). In addition to agriculturally used parcels, we selected sites with natural occurrences of *Poa alpina* in each local community. Natural sites were found mostly above the climatic treeline (2000–2500 m a.s.l.) on both siliceous and calcareous substrates. Populations were considered as 'natural' when the nearest managed parcel was at least 200 m away and no indication of former land use could be detected. Altogether, for the present study, plants from 71 sites were sampled, including 42 pastures, 13 meadows, and 16 natural sites. The cultural traditions were represented with 19 (Germanic), 22 (Romanic), and 30 (Walser) sites; the current land use with 42 pastures, 13 meadows, and 16 natural sites; and the elevations with 15 (valley bottom), 24 (intermediate) and 32 (alpine zone) sites. Of the 55 agricultural used sites 22 were fertilized according to local farmers.

To measure the intraspecific chromosomal diversity of *Poa alpina,* eight individual plants were randomly selected per site respecting a distance between samples of c. five meters. Plants were excavated in the field during the summer of 2002 and the strongest tillers were planted into plastic pots (7 × 7 cm) and kept in a greenhouse not far from Zürich for multiplication of tillers. Because 25 plants did not survive transplantation, in total 543 individual plants were used in the study, of which 93% represented unique multi-locus microsatellite phenotypes, i.e. different genotypes (Rudmann-Maurer et al., 2007). After three and a half months, several tillers per genotype were available as replicates for the common garden experiment described below. Furthermore, samples from each plant were taken to measure the DNA-content by flow cytometric analysis at the Institute of Biology, Laboratory of Evolutionary Genetics of the University of Neuchatel, Switzerland.

2.4. Chromosome counts

To determine the relationship between DNA-content and chromosome number, 44 randomly selected plants of the study sample were selected to determine chromosome number both microscopically as well as cytometrically (Doležel and Bartoš, 2005). For the microscopic survey, squeezing preparations of root tips on a microscope slide were used. Fine, hairless white root tips were fixed after mitosis had been stopped for 2.5 h with Bromonaphtalene, then root tips and chromosomes were coloured for at least 2–5 weeks with acetic acid (25 ml) to 100 ml methanol (100% denaturated), 25 drops of carmin acetic acid and 2–4 drops of iron acetic acid. Thereafter, the root tips were placed on a paper towel and washed with deionized water. Pectinase (2%) and cellulase (5%) were needed to mellow root tissues for 4.5 h (50 ml deionized water, 2.5 g pectinase, 1 g cellulose). Before squeezing, root