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Ploidy level and somatic chromosome number variation in *Agrostis stolonifera*

C. KIK*†‡, Th. E. LINDERS* and R. BIJLSMA†

**Department of Plant Ecology and †Department of Genetics, University of Groningen, Biological Centre, P.O. Box 14, 9750 AA Haren, The Netherlands*

SUMMARY

Agrostis stolonifera is a polyploid complex in which clonal propagation is predominant. Polyploidization often results in the induction of chromosomal aberrations at the mitotic and/or meiotic level. We observed that variation in ploidy level was present in *Agrostis stolonifera*, and that variation in chromosome number within genotypes also occurred. Populations differed in the degree of variation observed. Analysis of the frequency distribution of chromosome numbers per cell among different populations showed a wide range of variation in the inland meadow population, an intermediate level in the polder and salt marsh populations, and a very low level in the sand dune population. The results are discussed in the context of the origin of somatic chromosome number variation.

Key-words: Agrostis stolonifera, clonal plant species, aneusomaty.

INTRODUCTION

Polyploidization is a process which can induce chromosomal aberrations at the meiotic as well as at the mitotic level (Rao & Rao 1977; Van Dijk 1991). Somatic variation of chromosomal origin (aneusomaty) has been reported for, amongst others, *Cardamine pratensis* (Berg 1967), *Poa pratensis* (Speckmann & Van Dijk 1972) and *Orobanche gracilis* (Greilhuber & Weber 1975). Variation at the somatic level might have considerable consequences in clonal perennial plant populations as has been proposed by Hayward & Breese (1968) and Silander (1985). They argued that somatic variation in clonal plant populations allows for rapid genetic changes; favourable changes at the somatic level, which are not directly incorporated into the germ line, can be preserved via clonal replication, whilst deleterious variant ramets can easily be eliminated. The impact of somatic variation in clonal plant populations is, however, still poorly understood (Whitham & Slobodchikoff 1981; Manning 1983; Antolin & Strobeck 1985).

Kik (1989) and Kik *et al.* (1990a,b, 1991, 1992) have recently studied some ecological and genetical aspects of *Agrostis stolonifera* (creeping bent). This grass species is a wide-ranging clonal perennial, which can be found in a multitude of ecologically contrasting habitats. The species forms a polyploid complex in which the occurrence of the cytotypes has been reported, namely a tetraploid ($2n=4x=28$), a pentaploid ($2n=5x=35$) and a hexaploid ($2n=6x=42$) (Björkman 1954). The genome configuration of the tetraploid was

‡Present address: Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, The Netherlands.

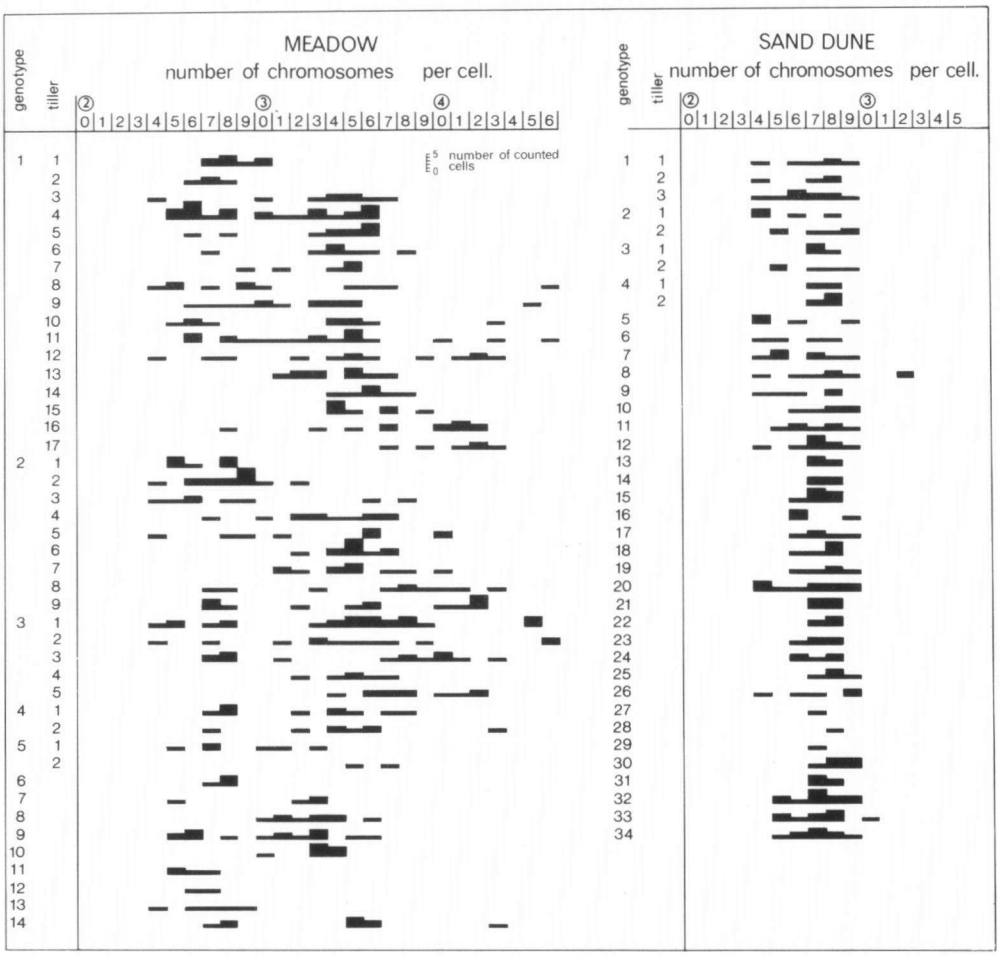

Fig. 1. (a) Chromosome numbers of the meadow and sand dune populations of *Agrostis stolonifera*. In this figure, as in Fig. 1b, genotype refers to an electrophoretically identical group of 'mother plants'. Tiller refers to the tillers collected from the field, which were eventually raised in the greenhouse to 'mother plants'. Chromosome counts (given as histograms) were made from four tillers of one 'mother plant'. Genotypes were identified by electrophoretic analysis of 'mother plants'. The number of 'mother plants' per genotype varied from one (not indicated; see for e.g. meadow genotypes 6–14) to 17 (see meadow genotype 1).

assigned AABB, and the configurations of the penta- and the hexaploid AABBB and/or AAABB, and AAABBB respectively (Jones 1956). Jones suggested that the penta- and hexaploid cytotypes arose through the infrequent production by the tetraploid cytotype of unilateral (ABB and/or AAB) gametes in the case of the pentaploid and unreduced (AABB) gametes in the case of the hexaploid. Björkman (1954) observed that tetraploids predominantly produced euploid gametes, whereas the penta- and hexaploid cytotypes produced no gametes or aneuploid gametes, which makes them functionally sterile. He also established the incidence of B chromosomes. The occurrence of aneuploid plants was reported by Stuckey & Banfield (1946), Juhl (1952) and also Björkman (1954).

In this paper, we report on the determination of the ploidy level of different genotypes originating from four different populations of *Agrostis stolonifera*. We show that

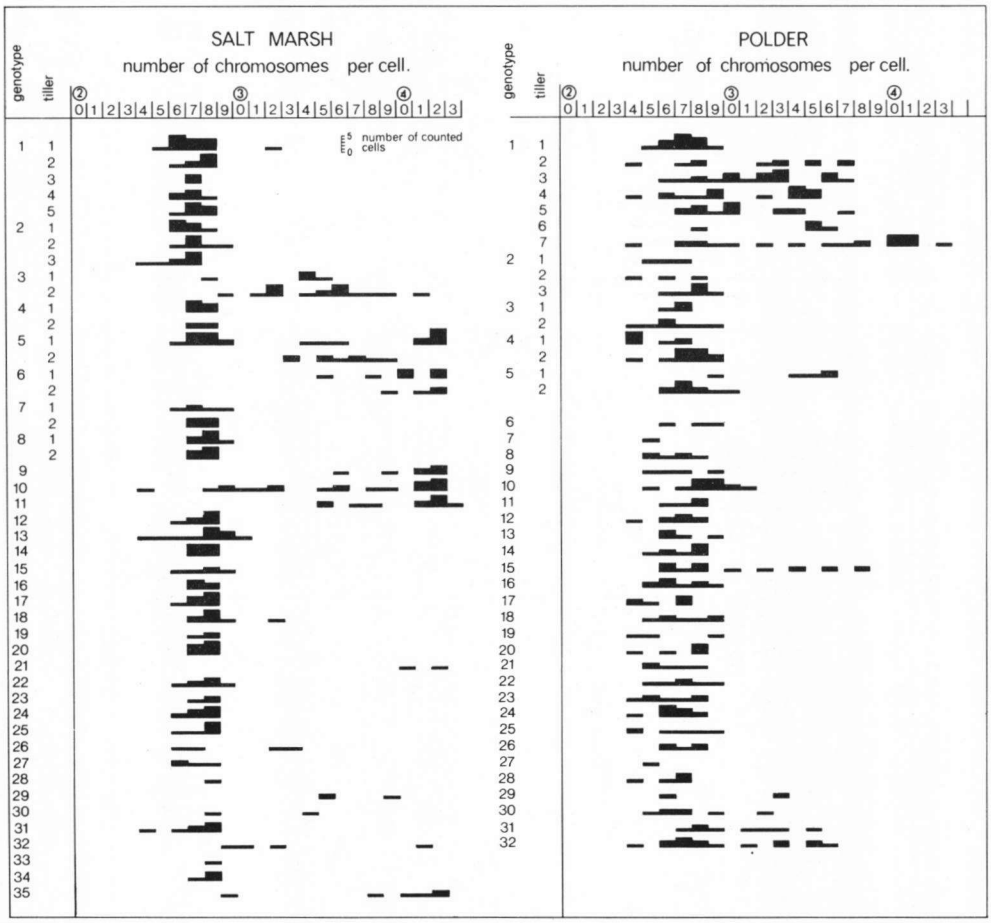

Fig. 1. (b) Chromosome numbers of salt marsh and polder populations of *Agrostis stolonifera*.

variation in chromosome number not only occurs between genotypes but also within genotypes.

MATERIALS AND METHODS

Population samples were taken in four areas: an inland meadow near Zuidlaren (53°04′N, 6°40′E), a salt marsh near Noordpolderzijl (53°62′N, 6°36′E), a polder near Zoutkamp (53°21′N, 6°14′E), and a sand dune on the Waddensea island Schiermonnikoog (53°30′N, 6°15′E). For a detailed description of the study sites see Kik *et al.* (1990a).

Approximately 50 tillers, randomly collected from each site and grown in a heated greenhouse were used as the stock material (*c.* 200 'mother plants'). Isozyme analyses were performed first on 'mother plants' to identify individual genotypes as described previously by Kik *et al.* (1990a).

Chromosome analyses

One week before chromosome counting of a specific 'mother plant', four tillers of this 'mother plant' were brought into hydro-culture using Steiner solution (Steiner 1961).

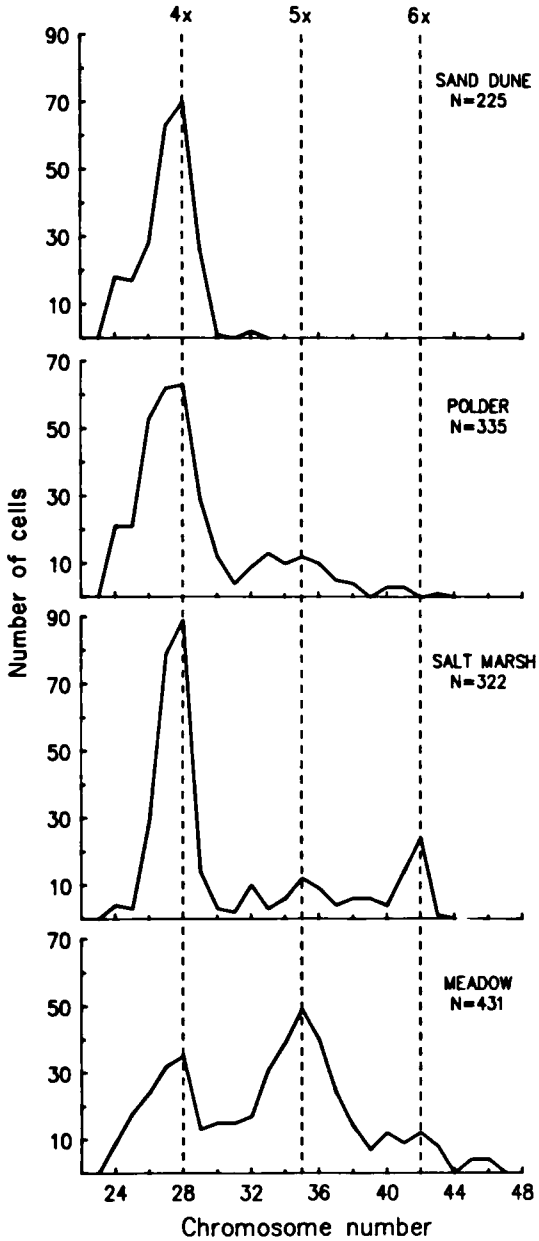

Fig. 2. The frequency distribution (number of counted cells) of chromosome numbers (pooled data) in four populations of *Agrostis stolonifera*. The number of counted cells (N) is indicated.

Yellow-white root tips were selected and were pretreated for 3 h, at room temperature, in a saturated solution of alpha-bromo-naphthalene. They were then fixed in a 100% ethanol: glacial acetic acid mixture (3:1), hydrolized in 5 M HCl for 30–45 min and stained by the Feulgen reaction (Graumann 1953). The root tips were squashed in 45% acetic acid and metaphase stages were used for chromosome counts. Chromosome numbers were

Table 1. Number of tetraploid and non-tetraploid genotypes in populations of *Agrostis stolonifera*

Population	Number of	
	Tetraploids	Non-tetraploids
Meadow	3	10
Salt marsh	22	9
Polder	23	7
Sand dune	31	0

determined mostly from well spread metaphase cells. However, in a few cases, where some overlapping of chromosomes occurred, counting was repeated by the same or a different observer. Due to the amount of work involved, root tips from the four tillers of the same 'mother plant' were not kept separately during these procedures, but were pooled and treated as a single sample.

RESULTS

For all populations, chromosome numbers per cell of each 'mother plant', are shown in Fig. 1. It is clear that the chromosome number per cell is highly variable and covers the range of 24 to 46 chromosomes. Chromosome numbers not only varied between populations or genotypes but also within genotypes and even within 'mother plants'. This was especially true for the inland meadow population (e.g. 'mother plant' 8 of genotype 1 of the inland meadow population showed counts ranging from 24 to 46). This indicated that intraclonal variation in chromosome number is present in the *Agrostis stolonifera* populations.

Counting large numbers of cells (in this case over 1300 cells were scored) was tedious and a number of difficulties were encountered. Firstly, sometimes the number of new root tips and/or metaphases was very small and consequently only very few counts per genotype could be obtained. In the final analyses, only those genotypes from which the chromosome complements of at least three cells had been determined were included. Secondly, metaphase chromosomes were not always nicely spread and precisely arranged in one equatorial plane. The chromosomes of *Agrostis stolonifera* are also relatively long. Given the high number of cells that had to be counted, this may have led to counting errors, and it is to be expected that the actual number of chromosomes is underestimated rather than overestimated. To get an insight into the latter problem, the frequency of each chromosome number per cell was calculated for each population (Fig. 2). For convenience it was assumed that all cell counts were independent. The data reveal a considerable variation among the four populations. Also within a population, a wide range of variation occurred, except in the sand dune population. The sand dune population showed a single peak at the tetraploid chromosome level and the variation around this number was indeed skewed towards lower values. The other populations also showed a peak around the tetraploid chromosome level but, in addition, peaks were observed at higher ploidy levels. The most conspicuous finding was in the inland meadow population, that showed that the highest frequency of cell counts was at the pentaploid chromosome level. The very

variable chromosome number observed even within 'mother plants' complicates a simple grouping of the genotypes into pure ploidy levels. Given the unimodal distribution of the sand dune population we concluded that this population is essentially tetraploid. Based on this assumption we classified the genotypes into two cytotypes. (i) Tetraploids: genotypes with cell counts not exceeding 32 chromosomes (the maximum observed in the sand dune population was taken as the limit). (ii) Non-tetraploids: genotypes with cell counts over 32 chromosomes (Table 1). It is clear that the populations differed considerably in cytotype composition.

DISCUSSION

The most remarkable result is the observed variability in chromosome numbers in *Agrostis stolonifera*, not only within electrophoretically identified genotypes but also within each 'mother plant' (Fig. 1). Chromosome numbers were only determined in root tips, because other parts of the plant generally exhibited low meristematic activities. It is possible that variation in chromosome numbers is not present in other parts of the plant. However, cytogenetical studies on *in vitro* derived plants of other members of the *Gramineae*, e.g. *Triticum durum* (Bennici & D'Amato 1978) and *Hordeum vulgare* (Mix *et al.* 1978) indicated that the variation in chromosome numbers observed in the root apices matched those observed in the shoot apices. Furthermore, it is known that *in vitro* derived plants show a higher degree of chromosome number variation than *in vivo* grown plants (D'Amato 1985). Therefore, it seems reasonable to assume that the variation observed in the roots of *Agrostis stolonifera* reflects those of other parts of the plant.

The allotetraploid (AABB) cytotype of *Agrostis stolonifera* initially arose from interspecific hybridization (Jones 1956). Newly formed penta- and hexaploids from this tetraploid cytotype, may suffer mitotic and meiotic irregularities due to genetic and physiological imbalance. These irregularities, probably caused by non-disjunction, elimination and abnormal spindle formation (Stebbins 1971; Nirmalo & Rao 1984) may lead to elimination of chromosomes and to the production of genetically dissimilar tillers upon which natural selection can act. This process seems to occur in *Agrostis stolonifera* as is illustrated, for example, by the inland meadow genotypes 1, 2 and 3 (Fig. 1). The highly variable chromosome numbers observed even within 'mother plants', suggest that somatic changes in chromosome number occur frequently. Some of this variation in chromosome number can be accounted for by the methods used. However, the rather small variation shown for the sand dune population indicates that this contributes only to a limited extent to the large variation observed for many 'mother plants'. Therefore, somatic changes in chromosome number in *Agrostis stolonifera* seem to be real and to occur especially at higher ploidy levels. The evolutionary implications of somatic variation in chromosome number in ecologically contrasting populations of *Agrostis stolonifera* are discussed in another paper (Kik *et al.* 1992). Although our data are not yet conclusive, both aneuploidy and aneusomaty seem to occur at a high frequency (Fig. 2). These phenomena are thought to be closely related to the process of hybridization and polyploidization (Rao & Rao 1977; Grant 1981) and it has been suggested that aneusomaty also could lead to a return to stable chromosome numbers (Vaarama 1949; Thompson 1962; Koshoo & Narain 1967).

In conclusion, our data show that chromosome number is highly variable in *Agrostis stolonifera* and a high degree of somatic chromosome number variation seems to be present in this species. The observed variation within 'mother plants' suggests that

the number of chromosomes can change rapidly and might be an important factor in evolutionary processes in clonal plant populations.

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