Clone Structure of a 4 Years Old Pasture Population of Perennial Ryegrass (Lolium perenne L.)

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Synopsis


Clone structure of a perennial ryegrass (Lolium perenne L., tetraploid cv. Friend) population was studied using starch gel electrophoresis for a 4-yr-old sward. The sward consisted mosaicly of two patch types, one with distinct stands of L. perenne and one with dense clumps of that. In the patch with distinct stands, two 1 x 1 m² quadrats were placed and determined for the phenotypes or genotypes of all stands by two enzyme systems (PGI and GOT). Of 54 genets detected over two quadrats, nine ones contained two or more ramets which were distributed closely each other. In the densely aggregated patch where it was hard to distinguish ramets, five 25 x 40 cm² quadrats were excavated intact and mapped for the position of the clumps (i.e. ramet aggregation). Electrophoretic analysis showed that the genets tend to form the discrete spatial units, with high density of genet (ca. 132 genets m⁻²). Analysis of the genet composition within 10 large stands specified that six ones consisted of two or more genets. These results suggest that this L. perenne population may be maintained partly by aerial tillering, and that intermixed genets may occur frequently.

Key words: Aerial tillering, Clone structure, Electrophoresis, Lolium perenne, Vegetative reproduction.

Introduction

Most of pasture plants maintain their population size by clonal reproduction such as stolons, rhizomes and haplo-corms⁵,⁶. However, we know relatively little about the features and nature of the clonal reproduction which pasture plants exhibited, and about their responses to impact by different sward management. Understandings of details on the life-history traits related to the clonal reproduction for each species will provide much information for specifying the conditions enabling multi-species coexistence⁵. Thus, elucidating the features of clonal reproduction of pasture plants is meaningful for sward management as well as pasture plant breeding whose objectives have been less considered from the ecological viewpoints.

Lolium perenne has been considered as an excellent species for one component of the sward vegetation, which is also promising for a cool region in Japan. It is characterized as a bunch-type grass with relatively short stature, and has been considered as a strict phalanx species

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without any vegetative reproductive methods enabling the long dispersal\(^6\),\(^11\). It has been, however, often reported that it reproduces by aerial tillers and stolons\(^{1,2,12,13}\). Sawada\(^2\) observed the occurrence of aerial tillers in the \textit{L. perenne} swards in Japan, suggesting its potential contribution for maintenance of its population size.

Recent advances in enzyme electrophoresis techniques has enabled to use enzyme polymorphism as genetic markers in various research areas, which become to popular techniques\(^{24,25}\). There are growing references on isozyme studies of \textit{L. perenne} in which several enzyme systems has been examined\(^{8,10,18,19}\). PGI-2 locus, which is clearly detected on gels and is highly polymorphic (at present four alleles are identified), has been extensively utilized for cultivar identification\(^6,17\) and the studies on inter-specific competition\(^20\). McNeilly and Roose\(^4\) compared the clone structure of diploid \textit{L. perenne} among some swards with different management using PGI-2, GOT-2 and GOT-3 loci. Enzyme polymorphism is, thus, invaluable as genetic markers in analyzing the clone structure of \textit{L. perenne}. The purpose of this paper is to address the following question: What clone structure (i.e. the number and the spatial distributional pattern of genets) developed in a 4-yr-old pasture population of \textit{L. perenne} (tetraploid cv. Friend)?

**Materials and Methods**

1. **Study site**

This study was conducted in the National Grassland Research Institute, Tochigi Prefecture, Japan. A 4-yr-old sward dominated by \textit{L. perenne} (tetraploid cv. Friend) was selected for the study. It was sown September 1986 and has been grazed by cattle since 1987. Details on site were described elsewhere\(^2\).

2. **Sampling methods**

Preliminary field observation showed that \textit{L. perenne} plants inhabited in this sward as following two forms: distinct stands and dense clumps. In other words, the study sward consisted mosaically of these two types of patch. We collected \textit{L. perenne} plants from both types of the patch, site-1 with distinct stands only and site-2 with dense clumps, on 20–24 November 1989. In site-1, two 1×1 m\(^2\) quadrats (QA and QB) were placed. All stands within the quadrats (N=34 and 32 in QA and QB, respectively) were recorded for their position and basal shape, and then collected as several tillers per stand. These samples were brought to the laboratory, transplanted in pots with 10.5 cm diameter, and maintained in an unheated greenhouse of Shizuoka University. Two samples had withered before the electrophoresis, omitted from the analysis. In addition, the genet composition of 10 large stands (ca. 15 cm in basal diameter), which were randomly selected from the sward, were examined to determine if they consisted of multiple genets. These stands were excavated and transplanted in pots with 16 cm diameter.

In site-2, five 25×40 cm\(^2\) quadrats (Q1, Q2, Q3, Q4 and Q5) were randomly placed within the sward. The entire quadrats were carefully excavated, and transplanted in plastic boxes which were placed in the greenhouse. Then after, we recorded the position and basal shape of all clumps (i.e. ramet aggregation) for each quadrat onto a map details. The sample points for the electrophoresis were determined and marked with thin pins on the basis of these maps.
3. Electrophoresis

Preliminary experiment examining 12 enzyme systems (acid phosphatase (ACP), aconitase (ACO), alcohol dehydrogenase (ADH), esterase (EST), glucose-6-phosphate dehydrogenase (G6PDH), glutamate oxaloacetate transaminase (GOT), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6PGD), phosphogluco isomerase (PGI)) showed that two (PGI and GOT) of these were sharply stained and highly polymorphic for tetraploid cv. Friend. These two systems, therefore, were used to distinguish genets. The phenotype of each material was electrophoretically examined at least three times. Additional four systems (ACP, EST, MDH and PGM) were partly used for only the materials with relatively readable activity. In site-2, each 19 to 40 sample points (tillers) were analysed from Q1, Q2, Q3, Q4 and Q5. For 10 large stands, five tillers (one from central position and four from periphery) were selected from each stand and assayed.

Approximately 5 cm of the youngest leaf on a single tiller of each material was ground by mortar and pestle with one drop of extraction buffer (0.5% mercaptoethanol and 0.1 M Tris-HCl (pH7.2)). The extract was absorbed on filter paper wicks (3×8 mm, Whatman No. 3) and placed on the gel. Starch gel electrophoresis was performed as described by ØSTERGAARD et al. but with minor modifications. The gel buffer was : 1 part lithium borate (0.025M LiOH, 0.180M Boric acid) and 9 parts Tris-citrate (0.070M Tris, 0.007M Citric acid) (pH8.1). The tray buffer was : 0.180M Boric acid, 0.025M LiOH (pH8.3). Gels were stained for each enzyme using the methods of WENDEL and WEEDEN.

Genetic interpretation of PGI-2 locus is generally easy for tetraploid cultivars of L. perenne as well as diploid ones, because the products of PGI-2 locus have greatly high resolution. In contrast, interpreting GOT-2 and GOT-3 loci of tetraploid was extremely difficult because of their poor resolution. Therefore, we used PGI-2 genotype and GOT-2 and GOT-3 phenotypes to distinguish the genets. ACP, EST, MDH and PGM were only used to further distinguish the individuals within the groups having identical PGI genotypes and GOT phenotypes.

4. Estimation of clone size

For the genets in QA and QB, the basal area of each genet was estimated from the basal shape of the stands which the genet in question existed. For the genets in Q1, Q2, Q3, Q4 and Q5, the basal area of each clump was firstly estimated from its basal shape, then clump area was divided by the number of sampling points, obtaining each unit area. The unit area was assigned to each genet according to its frequency. Multiple assay of each sample point showed that different genets were sometimes detected at the same points due to tiller dynamics within the quadrats. In such cases, the unit area was subdivided by the number of genets detected and assigned to each genet, providing that these genets intermixed at equal ratio.

Results

1. QA and QB (each 1×1 m² in quadrat size)

A total of 26 and 28 genets were detected in QA (33 stands) and QB (31 stands), respectively.
Of these 54 genets, 9 ones (16.7%) were estimated to consisted of two or more stands, which were located nearly each other (Only QA is shown in Fig.1). Stand number per genet was 1.2 on average pooled over QA and QB, with a maximum of 3.

2. Q1, Q2, Q3, Q4 and Q5 (each 25×40 cm² in quadrat size)

A total of 66 genets (i.e. genet density was 132 genets m⁻²) was detected over 5 quadrats. Genet number per quadrat was 13.2 on average, ranging from 6-21. Genet size was estimated to be 45.0±46.7 cm² (mean± S.D.), ranging from 0.5-286.5 cm². It exhibited L-shaped frequency distribution (Fig. 2), most genets were small : Twenty-eight genets (42.4%) had size smaller than 25 cm², and 51 ones (77.3%) smaller than 50 cm².

Each genet tended to be distributed aggregately (Only Q1 is shown in Fig. 3 to save a space). Fifty-five genets (83.3%) were distributed within a single clump, whereas 11 genets (16.7%) were distributed over two clumps which located nearly each other. Relationship between clump area and the number of genets detected in that clump was positive and significant (r=0.586, df=45, P<0.01). Therefore, there was a tendency that larger clumps contained more genets.

Fig. 1. Clone structure of *Lolium perenne* in quadrat QA (1×1m² in size). Identical numbers indicate the same zymograms for PGI and GOT. Dashed line indicates the putative genet. Letter U means that the stands had withered while grown in a greenhouse, omitted from the analysis. Aerial tillers appearing in November 1989 are also shown on a map.
Fig. 2. Frequency distribution of genet size (i.e. the area estimated to be occupied by a given genet) in the densely aggregated patches of *Lolium perenne*.

Fig. 3. Clone structure and spatial distribution of the micro-scale plant cover (i.e. ramet aggregation) of *Lolium perenne* in quadrat Q1 (25 × 40 cm² in size) located in the densely aggregated patch. Each circle indicates the leaves sampled for the electrophoresis. Identical numbers indicate the same zymograms for PGI and GOT.

3. Genet composition within large stands

Of 10 large stands examined, 6 ones consisted of multiple genets. Two of these had two genets within the stand, and four ones had three genets.

Discussions

1. Ecological significance of aerial tillering

Sawada\(^{22}\) observed the occurrence and establishment of aerial tillers in this *L. perenne* population, suggesting a potential contribution of aerial tillering to maintain its population size. Electrophoretic analysis of *L. perenne* plants from QA and QB demonstrated that a total of nine genets (4.5 ones m\(^{-2}\)) dispersed multiple ramets in a short time (only 4 years). Field observations confirmed that most of these genets dispersed their daughter ramets by aerial
tillers, whereas *L. perenne* plants have the ability to disperse their ramets by the fragmentation of ramets caused by physical pressures as well as by aerial tillers. Aerial tillering seems to be invaluable for the maintenance of *L. perenne* population size, depending on the environmental conditions and genetic feature of the populations.

2. **The nature and development of clone structure of *L. perenne***

What clone structure is likely to develop in *L. perenne* populations? If it reproduces frequently by aerial tillering, it is expected that a group of small daughter ramets is distributed satellite nearby a large parent ramet. In addition, low dispersal distance (4.2–4.8 cm on average) of vegetative propagules strongly leads to the idea that genets are likely to form discrete spatial units but not form the ramet complex in which different genets grow intermixed. The results of this study supported partly this expectation. However, the extent of clonal spread was relatively small, probably because this *L. perenne* population was still in immature state. Analyzing the older populations is needed to test the expectation mentioned above.

HARRIS *et al.* demonstrated that a single genotype spreaded widely in a *L. perenne* sward. McNEILLY and ROOSE showed that clone structure was different among the swards with different management, the extent of clonal spread being greater in the older sward with less intensive management. These results suggest that the genet density will be gradually smaller and a few certain genets will be dominant gradually as time passed. The speed at which a few genets will be dominant may be different among different sward management.

The speed at which a few genets dominate locally may be also considerably different within the same sward. The study sward contained a variety of genet density at micro-scale. Low density (27 genets m$^{-2}$) was found in the patch with *L. perenne* stands only, but high density (132 genets m$^{-2}$) found in the patch with dense clumps. The reason for this result may be as follows: In the patches with *L. perenne* stands, locally light grazing pressure by cattle may rapidly promote to form the stands, and cause severe inter-stand competition, resulting in decreasing genet density. In contrast, such stand formation is not promoted under the heavy grazed condition in which the individual genet size remains to be small, enabling to coexist numerous genets. McNEILLY and ROOSE reported that a roughly managed pasture exhibited only 5 genotypes 0.25 m$^{-2}$, whereas intensively managed swards exhibited the density of 36–43 genotypes. These environmental heterogeneity may occur within the same sward, and a micro-scale variation in genet density may occur within the same sward. The similar phenomenon was observed for *Phleum pratense* (SAWADA, personal observation).

3. **Genet composition within large stands***

Analysis of the genet composition within large stands showed that they frequently consisted of multiple genets. The ramets from different genets grew intermixed to form a single large stand. Also, the clone structure of the dense clumps showed that there were some cases that ramets from different genets grew intermixed to form a single clump. In contrast, there were no data on genet-intermixed stands in QA and QB. These results suggest that some *L. perenne* genets grow intermixed while others grow singly and repeal each other. Similar phenomena have been confirmed for *Phleum pratense* (SAWADA, personal observation). Why this phenomenon occurs? What mechanisms cause this phenomenon? At present,
there is no idea about these questions.

Small sampling size within large stands prevent us to know whether genets form discrete spatial units even within the large stands or whether genets intermingle highly. We do not know whether different genets coexist within the stands for a long time or whether a certain genet becomes to dominate gradually, the others extinct. If some genets exhibit the nature by which promote to the coexistence of multiple genotypes, such genets might be expected to be more suitable genotypes for the swards with intensive management. Further studies on the feature of inter-genet competition will be needed.

4. Genotypic frequency at PGI-2 locus

Are there any differences in the survival rate among PGI-2 genotypes? Hayward et al.\(^9\) compared the genotypic frequency at PGI-2 locus between the seed population and the adult population, showing a significant difference for one strain of *L. perenne*. They concluded that the difference between both populations was caused by either the difference in viability among isozyme genotypes or simply the difference in genetic composition of the seed lots. Rainey et al.\(^3\) and Mitton\(^5\) suggested that there were significant differences in the survival rate among the isozyme genotypes of *L. perenne* and *L. multiflorum*, respectively. Emoto\(^3\) described the genetic composition of the seed population of cv. Friend, which was seeded onto a field in this sward establishment. Therefore, we are able to compare the genotypic composition of the study sward to the seed population using Emoto's data\(^3\). Comparison between them showed a disagreement in the genotypic composition at PGI-2 locus. Our data showed the excess of the genotypes aab and aabc but less aabb and abbc than Emoto's data (Allele description is follows from Hayward and McAdam\(^9\) and Ostergaard et al.\(^8\)). Other genotypes at PGI-2 locus are not presented here due to their low frequencies. Genotypic frequency was aab = 13.3%, aabb = 11.7%, abbc = 9.2%, aabc = 16.7% in our data, whereas aaab = 3.1%, aabb = 35.9%, abbc = 31.3%, aabc = 1.6% in Emoto's data. Although no immediate conclusion is made from this result, this discrepancy at PGI-2 locus might suggest the differences in the survival rate among the genotypes.

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ベレニアルライグラス（*Lolium perenne L.*）の
放牧草地個体群のクローン構造

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要 約

ベレニアルライグラスが空中分けつによってどのように栄養繁殖しているかを明らかにするために、種々のジェネット構造を分析した。ベレニアルライブラスが株化してい
る場所に2個のコドラート（各1×1m²）を置き、その全64株について、PG1-2遺伝子型とGOT-2, GOT-3の表現型を比較した結果、54個（27個/m²）のジェネット（クロン）が検出され、このうち9個のジェネットが2個以上のラメート（株）を分散させていた。これらのラメートはお互いにすぐ近くに分布した。一方、ベレニアルライグラスの密に分布する場所の5個のコドラート（各25×40cm²）を分析した結果、132個/m²のジェネットが存在した。各ジェネットはまとまって分布する傾向があった。他方、10個の大株のクローン構成を分析した結果、6個の株が複数のジェネットから構成されていることが確認された。これらの事実から、ベレニアルライブラスの個体群の維持に空中分けつなが関与していることが明らかになった。

キーワード：栄養繁殖、空中分け、クローン構造、酵素多型、ベレニアルライグラス。

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