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# The clonal structure of *Festuca rubra* in adjacent maritime habitats

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## SUMMARY

The clonal structure of *Festuca rubra* L. was studied in three locations: dune base, dune top and sand ridge, in the beach plain on the Frisian island of Schiermonnikoog by means of electrophoretic analyses. The zymograms of four enzymes of 100 plants sampled 30 cm apart in a 2·7-m square grid in each location were compared. Large differences in clonal structure were found between the three sites. The sand ridge site appeared to be dominated by one clone, whereas the dune base site consisted of at least 35 clones. A site on top of an embryo-dune was intermediate with at least 30 clones. Morphological analyses agreed with these conclusions.

*Key-words:* clonal structure, differentiation, *Festuca rubra*, population.

## INTRODUCTION

In the past decades many studies have dealt with differentiation between closely adjacent plant populations. In these studies, concerned with both natural (McNeilly 1968; Glaszmann *et al.* 1982; Pollard & Briggs 1984) and theoretical (Jain & Bradshaw 1966; Dickinson & Antonovics 1973) situations, much attention has been paid to the interplay of selection and gene flow. Reproductive processes play an important role in this context. Differentiation may include differences in clonal structure. Silander (1979) and Gray *et al.* (1979) found large differences between the clonal structures of adjacent stands of *Spartina patens* (Ait.) Muhl. and *Puccinellia maritima* (Hudson) Parl. Although the mating system is often discussed in studies on small-scale differentiation, much less attention is paid to vegetative reproduction. Pollen and gene flow are often studied in detail, whereas migration by vegetative proliferation in perennial plant species is neglected, although the influence of the latter process on the genetical composition of the existing population can be enormous.

*F. rubra* is a rhizomatous, perennial plant species with a wide ecological amplitude, showing differentiation through adaptations to a range of environmental factors (Rozema *et al.* 1978; Karataglis 1980). Differentiation between adjacent stands of *F. rubra* has been established (Rhebergen 1985; Rhebergen & Nelissen 1985) on the Frisian island of Schiermonnikoog. The investigations of Harberd (1961) and Harberd & Owen (1969) have provided clear evidence of differentiation in clonal structure between populations, with clone sizes varying from a few centimetres to over 200 m. The present study aims to investigate the clonal structure of adjacent *F. rubra* stands in contrasting habitats.

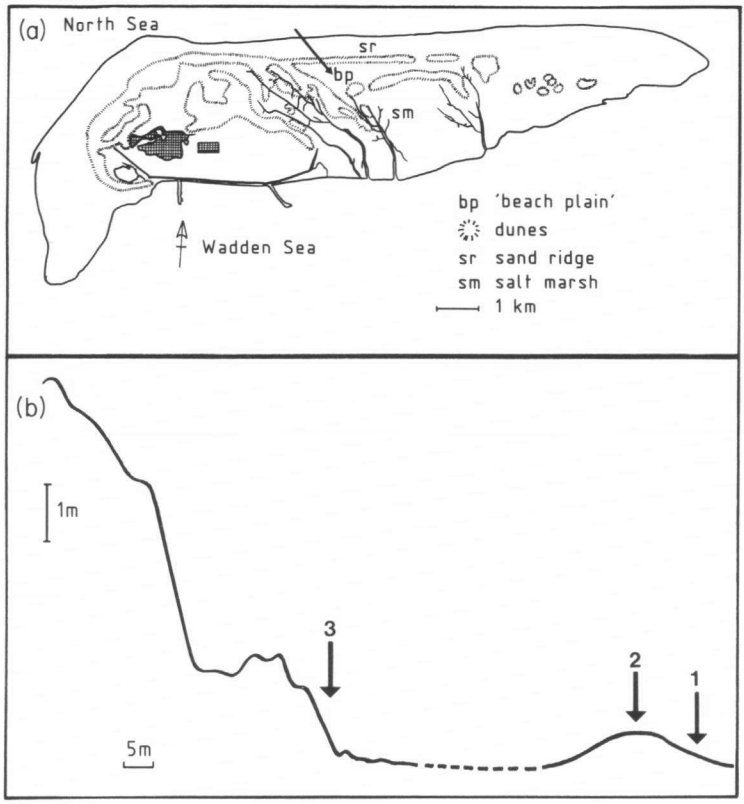


**Fig. 1.** (a) Schiermonnikoog. Arrow indicates study site. (b) Profile showing the position of three locations under study.

## MATERIALS AND METHODS

### *Sampling*

The study area, a coastal plain known as the 'beach plain', is located on the eastern part of the Frisian island of Schiermonnikoog (latitude 53°29′, longitude 6°12′) (Fig. 1). A detailed description of the 'beach plain' can be found in Rozema (1978). Plants were sampled at three different locations. Location 1 (dune base) was situated at the base of one of the low embryo-dunes in the 'beach plain' (near bench mark 8.200); this site represents the most saline and most frequently flooded habitat in which *F. rubra* occurs in this region. The adjacent Location 2 (dune top) was situated 50 cm above Location 1 on top of the same dune, whereas Location 3 (dune top) was chosen at the base of a sand ridge that separates the 'beach plain' from the North Sea beach.

In each location plants (individuals) were sampled using as 2·7-m square grid which was divided into 30 × 30 cm squares. Single tiller ramets of plants found at each of the 100 intersections were collected and grown in a glasshouse at 20 ± 3°C, 60 ± 10% relative humidity and with a 50 W $m^{-2}$ light supplement (12 h day) in square PVC pots (7 × 7 × 8 cm) filled with a commercial garden soil/sand mixture (1:1). After 3 months each individual was divided into two equal portions, each transplanted into 11-cm diameter PVC pots filled with a garden soil/sand mixture (1:1). One portion was used for isozyme analysis, the other for morphological analysis.

*Isozyme analysis*

For isozyme analysis one complete tiller without roots from each individual was homogenized with insoluble polyvinylpyrrolidon (Polyclar AT) in cold 0·1 mol $l^{-1}$ K-phosphate buffer (pH 7·3) containing $10^{-2}$ $mol^{-1}$ DTT (dithiothreitol). The homogenate was centrifuged at 15 000 r.p.m. for 20 min at 4°C. The supernatant was used for further analysis. Isozymes were separated using vertical slab-gel electrophoresis (DESAGA) with a 6% acrylamide gel. The two buffers used in this system were: a Tris-glycine electrophoresis buffer pH 8·3 and a Tris-HCl gel buffer pH 8·9 (Maurer 1971). The supernatant was applied to the gel after 30 min pre-electrophoresis at a constant current of 50 mA. The run started with a constant current of 50 mA (30 min), followed by 2–1 1/2 h at 100 mA at 4°C.

Gels were stained for alcohol dehydrogenase (ADH), using the method of Brown *et al.* (1978), NADH-dehydrogenase (NADH-DH), in the manner of Menken (1980), 6-phosphogluconate-dehydrogenase (6-PGDH), following Shaw & Prasad (1970), and peroxidase (PO), according to Verkleij *et al.* (1980).

Plants that showed vague bands for one or more of the enzymes were omitted from the analysis. The clonal structure within each location was determined from the zymograms of these four enzymes. The genotype of the individuals could not be determined, as *F. rubra* is a polyploid species.

*Morphological analysis*

The results of the isozyme analysis were used to select individuals for the morphological analysis. From each location three groups, each group of three individuals belonging to a single clone (based on zymogram comparisons), and three groups, each group of three randomly chosen individuals, were used to study the morphological resemblance between clonal material. Leaf length and number of tillers were assessed after 4-months growth in the greenhouse.

## RESULTS

*Isozyme analysis*

The results showed large differences in clonal distribution between the sample areas in the three locations, assuming that individuals with identical zymogram patterns and the same code number within one location, belong to the same clone (Fig. 2). Although the structural difference between dune base and sand ridge locations was quite pronounced, no unique zymograms were found in the sand ridge location. The differences in clone sizes are illustrated in Fig. 3. Location 3 from the sand ridge was dominated by one clone, whereas the dune base location consisted of at least 35 small clones. Clone sizes in location from the dune top varied between small and intermediate, resulting in about 30 clones.

*Morphological analysis*

Variation in quantitative morphological characters can provide an indication of resemblance within groups. In the present study the standard error was used as a measure of variation. If we generalize the results: for the dune base and dune top locations the variation for leaf length and number of tillers was smaller in Groups 1, 2 and 3, each containing individuals belonging to the same clone, than in Groups 4, 5 and 6 (Table 1). This would indicate a greater resemblance between plants in groups containing individuals of the same clone compared to randomly produced groups.

Thus, although the morphological analysis was limited for practical reasons, its results are clearly supporting the evidence provided by the isozyme analysis.

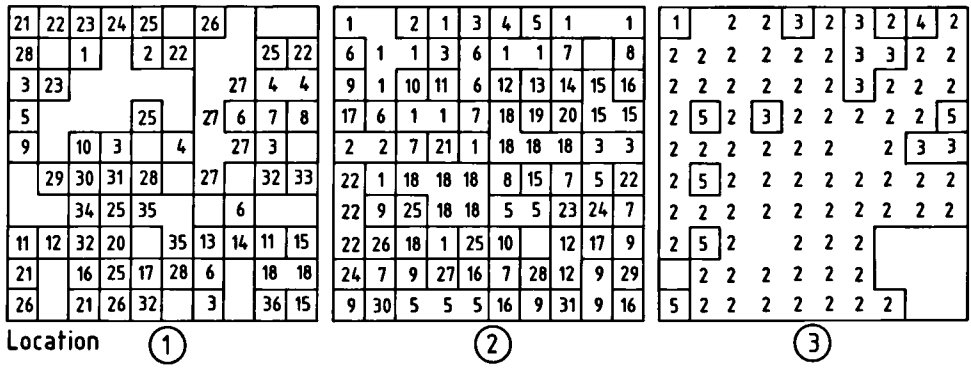


**Fig. 2.** Diagrams showing clonal structure within the three locations. (1: base of embryo-dune; 2: top of embryo-dune; 3: sand ridge). Each square represents one tiller of *F. rubra* collected in a grid. Identical numbers within one location indicate similar zymograms for four enzymes (O). There is no relation between numbers at different locations. Empty squares indicate plants with vague zymograms, omitted from the analysis.

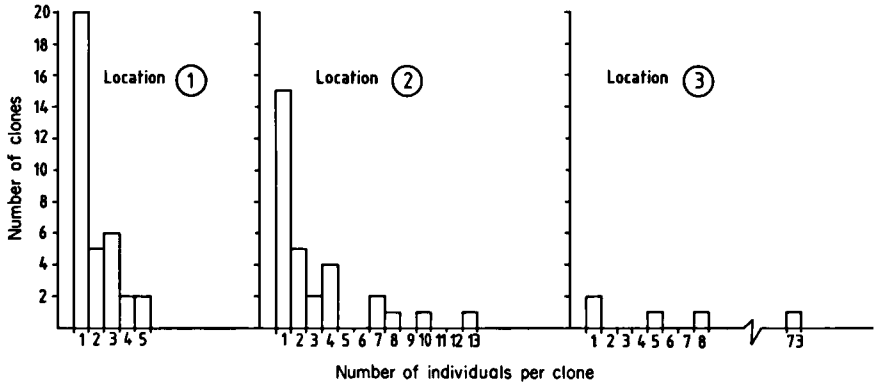


**Fig. 3.** Frequency of clone size measured as number of individuals per clone, in each of the three locations.

## DISCUSSION

Zymograms have only infrequently been used to study clonal structure in plant populations, even though isozymes allow simple allelic comparisons between individuals, particularly in diploids. Polyploids require more laborious analyses to reveal the genetic background of the zymograms, which may explain the relatively small amount of electrophoretic studies in this successful group of plant species (for example, Lumaret & Valdeyron 1978; Hancock & Bringhurst 1981). In the present study, the genetic basis of the *F. rubra* isozyme patterns was not determined. This means that phenotypes distinguished on a relatively small number of loci were compared. This method, therefore, does not differ in principal from a qualitative, morphological analysis. Differences in zymograms between individuals indicate that they belong to different clones. The opposite (same zymogram, same clone) may be true in general but not in all cases, because of the small amount of loci involved and the complexity of the isozyme patterns of polyploids, where apparently identical zymograms may be coded by different alleles (Lumaret & Valdeyron 1978). Thus this method probably underestimates the number of distinct

**Table 1.** Leaf length and tiller number of six groups of individuals for the three locations. The first three groups consisted of three individuals belonging to the same clone based on zymogram comparisons. The last three groups consisted of three randomly chosen individuals. Means ± standard error

| Location | Group | Leaf length (cm) | Tiller number |
|---|---|---|---|
| 1 | 27a | 46·8 ± 0·9 | 30·0 ± 7·5 |
| | 27b | 38·2 ± 2·1 | 21·3 ± 4·8 |
| | 27c | 35·2 ± 6·9 | 20·3 ± 8·8 |
| | 3 | 32·7 ± 7·1 | 21·0 ± 8·7 |
| | 8 | 39·7 ± 4·0 | 25·3 ± 8·8 |
| | 32 | 36·3 ± 3·7 | 31·3 ± 12·0 |
| 2 | 18a | 44·7 ± 3·6 | 14·7 ± 0·9 |
| | 18b | 56·3 ± 3·0 | 14·7 ± 4·4 |
| | 18c | 58·0 ± 2·7 | 14·3 ± 5·8 |
| | 1 | 62·0 ± 7·8 | 16·7 ± 4·7 |
| | 5 | 56·7 ± 6·5 | 8·7 ± 1·3 |
| | 16 | 49·8 ± 4·4 | 21·3 ± 9·4 |
| 3 | 2a | 57·3 ± 2·7 | 8·7 ± 3·7 |
| | 2b | 58·0 ± 4·3 | 17·0 ± 2·1 |
| | 2c | 49·3 ± 6·2 | 11·7 ± 2·9 |
| | 1 | 58·3 ± 1·3 | 10·3 ± 2·0 |
| | 4 | 56·2 ± 1·3 | 16·0 ± 2·7 |
| | 5 | 48·7 ± 3·8 | 8·0 ± 2·0 |

clones. For the same reason comparison of zymograms between locations is less relevant. Only the combination of close proximity of the plants and identical zymograms point out that these plants probably belong to the same clone.

Morphological data are more often used to establish clonal structures than electrophoretic data: for instance, in the well-known studies of Harberd (1961) and Harberd & Owen (1969) on *F. rubra*. However, Wu *et al.* (1975) showed remarkable differences in clonal structure between tolerant and non-tolerant populations of *Agrostis stolonifera* using electrophoretic techniques and morphological analyses. Only minor discrepancies between the conclusions based on both methods were established, indicating that both techniques give a reliable estimation of the clonal structure. In the present study the vegetative morphological characters were studied in a short-term experiment; it was not possible to study variation at the flowering and fruiting stage. The results of the limited quantitative analysis of vegetative morphology did, however, agree with the conclusions based on the electrophoretic data.

The results (presented in Figs 2 and 3) indicate pronounced differences in clonal structure between the sand-ridge site and the site at the base of an embryo-dune. The clonal structure at the top of the embryo-dune was intermediate. Similar observations were made by Silander (1979) for *Spartina patens* in more or less comparable habitats. Gray *et al.* (1979) found differences in clonal structure between a grazed and an ungrazed population of *Puccinellia maritima*, whereas no differences were found between upper and

lower parts of the same salt marsh population. A random spatial distribution of genetic individuals was also found in populations of *Alnus incana* ssp. *rugosa*, which differed in size, age and history (Huenneke 1985). McNeilly & Roose (1984) reported differences in genotype distribution in *Lolium perenne* L. between pastures of different ages and subjected to contrasting management regimes. Kik (1987) observed a large difference in clone variability between a meadow population of *Agrostis stolonifera*, on the one hand, and a salt marsh polder and sand dune populations on the other. The low number of large clones in the meadow population could be explained by a relatively strong selection for competitive ability and the absence of generative propagation in this population. In the present study there is no obvious difference in age between plants from the three locations. The sand ridge was established in the early sixties and it is likely that the population under study dates from this period. The reported differences in clonal structure have a strong genetic basis, as similar results were obtained after growing plants for 2 years in a common garden (Rhebergen 1985) and are probably the result of selective processes. Anderson & Taylor (1979) showed that *F. rubra* plants from mobile dunes were adapted to sand accretion through vigorous tillering and production of new roots. The long leaves of these plants give a higher area for photosynthesis than the shorter (but more numerous) leaves of *F. rubra* plants from fixed dunes in periods of high rates of accretion. Thus, an explanation is found for the extensive clonal growth and longer leaves of *F. rubra* plants in the sand-ridge location.

The differences found in this study are the result of differences in reproductive processes which, in turn, they influence. Clones several metres across, as found in the present study, or more (up to 200 m, Harberd 1961), may hinder cross-fertilization as pollen flow in *F. rubra* on Schiermonnikoog is restricted to a few metres (Rhebergen 1985). Auquier (1977) found high rates of selfing in *F. rubra* subsp. *arenaria* (which occurs in dune habitats, as at the sand-ridge Location 2) and low rates for *F. rubra* subsp. *litoralis* (which is found in salt marshes, as at Location 1 from the dune base). The author found no relation between self-fertility and potential gene flow. He emphasized the importance of self-fertility when colonizing new habitats.

Plants from the sand ridge (compared with plants from the base of the embryo-dune) show a greater vegetative spread, produce more florets (Rhebergen 1985) and possibly have a higher rate of selfing (Auquier 1977). Thus it seems that the resources of plants from the more or less open dune habitat are more directed towards reproduction, whereas in plants from the closed salt marsh habitat a greater portion of plant resources is directed towards individual survival. In addition, clonal competition could decrease the number of clones in this population (Kik 1987). These results agree with the conclusions of Silander & Antonovics (1979), concerning *Spartina patens*.

It is obvious that the results of the present investigations and similar studies published in the literature (Gray *et al.* 1979; Wu *et al.* 1975, 1979) should prompt the population biologist to take care when sampling vegetatively reproducing individuals and comparing the genetic structure of populations.

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