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CYTOTAXONOMIC STATUS AND MORPHOLOGICAL CHARACTERISATION OF SALICORNIA DOLICHOSTACHYA AND SALICORNIA BRACHYSTACHYA

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SUMMARY

Morphological studies on a number of field populations of *Salicornia europaea* agg. were made.

Contrary to other studies two species were distinguished: *Salicornia dolichostachya* with 2n = 36 and *Salicornia brachystachya* with 2n = 18 (respectively *S. procumbens* agg. and *S. europaea* agg. in Flora Europaea (TUTIN et al. 1964). A cyto-taxonomical study under laboratory conditions showed that the morphological characteristics used to distinguish the two species in the field were functional.

A demographic pilot study showed a different distribution of the two species over the salt marsh.

1. INTRODUCTION

"Botanists of the highest authority differ in opinion respecting the specific distinctions of the British Salicorniae" (BAXTER 1839, cited in DALBY 1962). Judged by the number of papers on the taxonomy of *Salicornia,* confusion over this matter still exists. *Salicornia* was described in the 19th edition of the Flora van Nederland (VAN OOSTSTROOM 1977) as a monospecific genus, although the author states that the species – *Salicornia europaea* L. – is likely to represent a complex of more than one species. In the new edition of this flora (VAN DER MEYDEN et al. 1983), two species of *Salicornia* are distinguished (viz. *S. dolichostachya* Moss. and *S. brachystachya*.G. F. W. Meyer, respectively reported as *S. procumbens* agg. and *S. europaea* agg. in Flora Europaea (TUTIN et al. 1964)). Although VANDENBERGHE (1890) considered the morphological differences to be merely phenotypic, most authors describe more than one species. In a number of studies the annual *Salicorniae* are divided into three taxa: one taxon with one-flowered cymes and 18 chromosomes (*S. pusilla*), and two taxa with three-flowered cymes of which one has 18 chromosomes (*S. europaea*-group) and the other 36 (*S. procumbens*-group) (Ball, in TUTIN et al. 1964, SCOTT 1977). In some studies the latter two taxa are subdivided again into a number of species (BALL & TUTIN 1959, CONTANDRIOPOULOS 1968, GROUZIS et al. 1976, 1977, and

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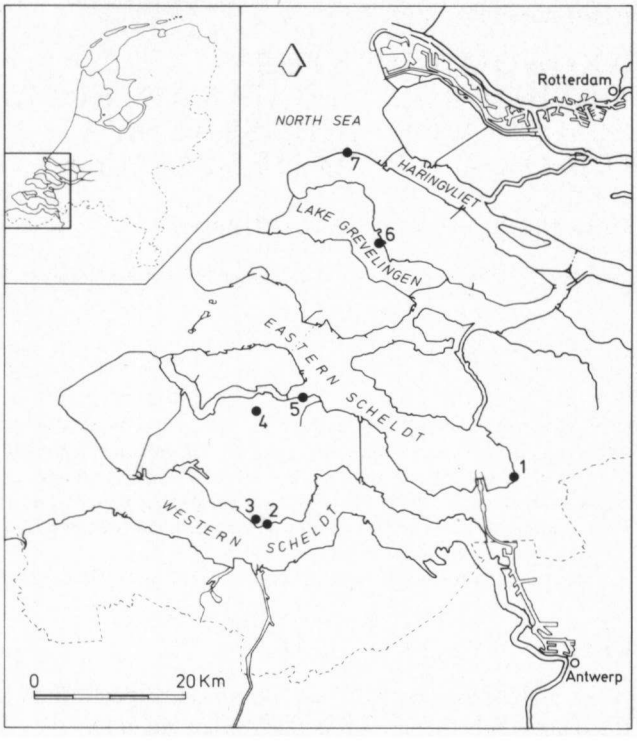

Fig. 1. Map of the southwest part of the Netherlands with the locations where *Salicornia* populations were sampled.
1 = salt marsh south of Bergen op Zoom (2 populations).
2 = salt marsh near Ellewoutsdijk (2 populations).
3 = Inlaag 1887: a low lying saline marshy area inside the sea dike (2 populations).
4 = brackish creek south of Wolphaartsdijk (1 population).
5 = salt marsh north of Wilheminadorp (1 population).
6 = Nature reserve "Slikken van Flakkee" on the banks of the saline lake Grevelingen (1 population).
7 = salt marsh of nature reserve "De Kwade Hoek" (4 populations).

KNOERR 1968) or into a complex of subspecies, varieties, forma or paramorphs (BAKKER et al. 1966, BINET & LANGLOIS 1961, DALBY 1962, KÖNIG 1939, LANGLOIS 1961a,b, PARRIAUD 1971). On the Iberian peninsula only *Salicorniae* with 2n = 18 were found (CASTROVIEJO & COELLO 1980). From this very confusing number of papers, in which a host of different names is used, it was decided to incorporate in our research project on the population dynamics of *Salicornia* spp. some taxonomical and morphological investigations to see which taxonomic index we should use.

The taxonomic index eventually chosen should provide a good key, which uses characteristics that are applicable in the field without any destructive sampling. The present study deals with a morphological investigation on the *Salicor-*

niae in the salt marsh south of Bergen op Zoom (number 1 in *fig. 1*), together with some demographic studies and a (cyto-) taxonomical study in other marsh areas (nrs. 2-7 in *fig. 1*) to help the interpretation of the findings of the field investigation.

The underlying study argues for the taxonomic division of the genus into two species.

2. MATERIALS AND METHODS

For the morphological and demographic study six vegetation zones can be distinghuished in the salt marsh south of Bergen op Zoom (*fig. 2*). In these vegetation zones a number of quadrats were marked out, most of them along two transects. All quadrats measured 1 × 1 m. The quadrats were recorded at varying time intervals during the growing season using a frame with a 5 × 5 cm grid within each 5 × 5 cm² square. Germination, mortality and a number of morphological characteristics for each individual were recorded.

For cytotaxonomical investigations three representative plants from each population being studied were dug up and taken to the laboratory, just before seed ripening. The location and number of populations studied are shown in *fig. 1*. The plants were identified using a number of characteristics described

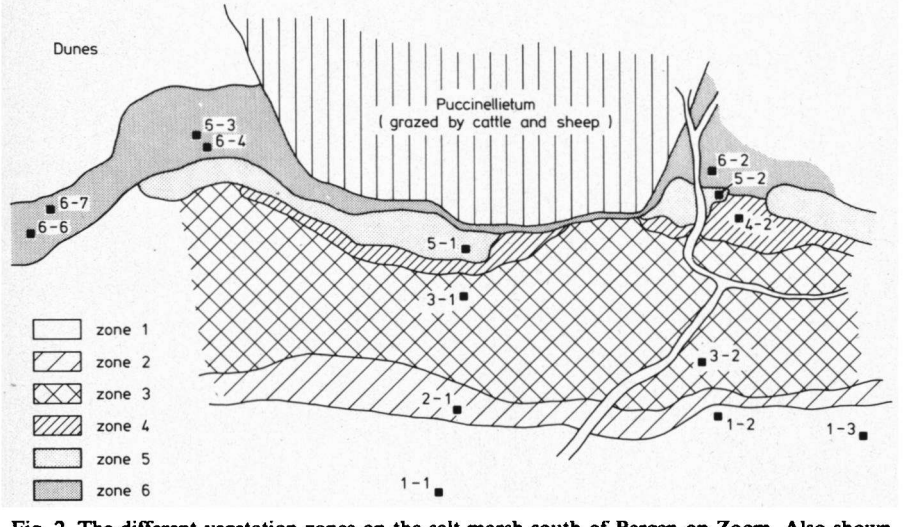

Fig. 2. The different vegetation zones on the salt marsh south of Bergen op Zoom. Also shown is the location of the quadrats (■).

Zone 1: sparse vegetation of *Zostera marina var. stenophylla, Zostera noltii*, small tufts of *Spartina anglica* and *Salicornia europaea.*

Zone 2: vegetation dominanted by larger *Spartina anglica* tussocks.

Zone 3: vegetation dominated by *Spartina anglica* and *Aster tripolium.*

Zone 4: vegetation dominated by *Puccinellia maritima*, with *Spartina anglica* and *Aster tripolium.*

Zone 5: vegetation dominated by *Limonium vulgare, Plantago maritima, Triglochin maritima.*

Zone 6: beach-line zone dominated by *Elytrigia pungens.*

by KÖNIG (1960), *viz.* branching pattern, length of the inflorescence, and seed morphology, supplemented with some others, described in the present paper. After ripening the seeds, collected per plant, were sown in trays on a mixture of gardening peat and sand in a ratio of 6 to 1. The trays were placed in a glasshouse at 20 °C during the day and at 10 °C during the night. Additional illumination with mercury vapour lamps was used to keep the light intensity at about 20,000 lux. The photoperiod lasted 12 hours and the relative humidity was around 80%. After the development of the first segment, ten plants were randomly selected and placed on a 0.5 N aerated Hoagland nutrient solution. The salinity of the nutrient solution was gradually increased from 0 to 9‰ Cl^-. A number of morphological characteristics were measured by the time the flowers were completely developed, *i.e.* when the pollen was released. Macro photographs were made of the top inflorescence of the main axis of the parent plant taken from the field. On this photographs the segment and the flower ratio were measured. The segment ratio is the ratio between the width in the middle and at the base of the central segment of the top inflorescence. The flower ratio is the ratio between the length and width of the central flower (*fig. 3A*). From three individuals taken from the parent population the ratio segment/stele was measured in a way explained in *fig. 3B*.

The recorded characteristics of the daughter plants are tabulated in *fig. 8*. For the chromosome counts, roots tips were collected and immediately fixed

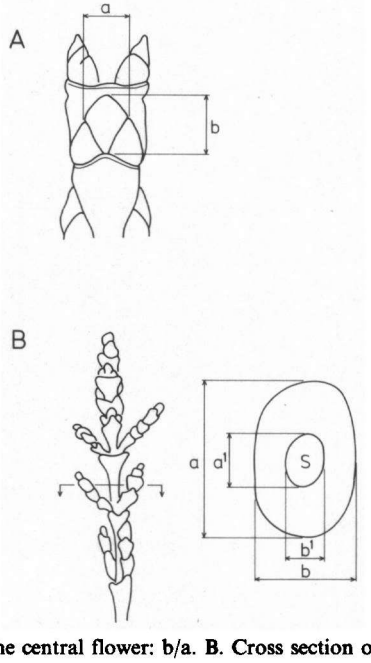

Fig. 3. A. Flower ratio of the central flower: b/a. B. Cross section of a generative segment. The segment chosen is the segment before the last fully developed segment. Ratio segment/stele: $a \times b - a^1 \times b^1 / a^1 \times b^1$.

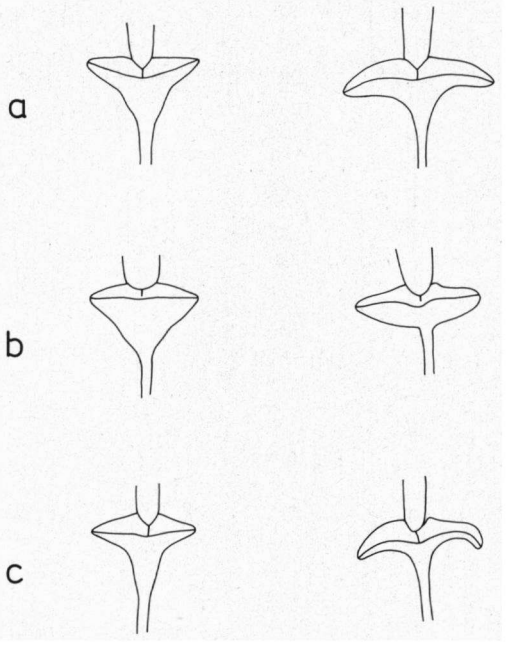

Fig. 4. Shape of the cotyledons of *Salicornia dolichostachya* (left) and *Salicornia brachystachya* (right) under normal turgor (a), extreme turgor (b) and no turgor (c).

with Karpechenko fluid. The root tips were then embedded in paraffin wax. After extraction from the paraffin wax the root tips were stained with haematoxylin, according to Heidenhain and finally embedded in Turtox resin.

3. RESULTS

3.1. Field observations and ecology

The morphological characteristics of the individuals under investigation were recorded during the whole study. It appeared that the seedlings were easy to distinguish: the abaxial side of the cotyledons of the preliminary called *S. dolichostachya* seedling formed an obtuse angle with the hypocotyl, while the angle for socalled *S. brachystachya* seedlings was perpendicular to acute, depending on the succulence of the seedlings (*fig. 4*). This feature, not described by KÖNIG (1960), seemed a rather constant characteristic. Studies by Koutstaal (unpublished) and Verhoef – Allan (unpublished) on populations in other saline areas confirmed this.

During the period of vegetative growth the two species were not easy to distinguish. The segments of *S. brachystachya* were slightly widened towards the top in the shape of a funnel, while the segments of *S. dolichostachya* were more cylindrical. The leaves that form the segments were less fused at the top than those of *S. dolichostachya*. This made the top rim of the *S. brachystachya* seg-

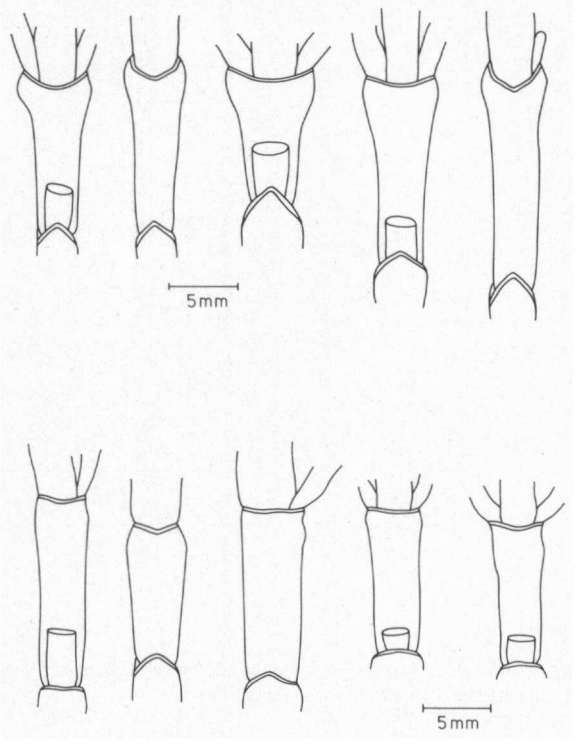

Fig. 5. Segments of *Salicornia brachystachya* (top) and *Salicornia dolichostachya* (bottom). Note the incurvated rim of the segments of *S. brachystachya* and the funnel shape.

ments more deeply curved than that of *S. dolichostachya* (*fig. 5*). The segments of *S. brachystachya* were in cross section under moderate succulence slightly ellipsoid while those of *S. dolichostachya* were circular. Under little succulence segments from both species appeared funnel-shaped and ellipsoidal, while under extreme succulence both species had thick barrel-shaped segments, difficult to distinguish apart. As soon as the inflorescences developed, the species could be distinguished very easily again. The cross section of the fertile segments of *S. brachystachya* was distinctly ellipsoid, while that of *S. dolichostachya* was circular. The fertile segments of *S. brachystachya* were slightly bulging in the middle or at the top of the segment, while the fertile segments of *S. dolichostachya* were more cylindrical. In the axillary cymes formed by the three flowers of *S. brachystachya,* the bract of the middle flower is larger than the bracts of the two other flowers, while the three flower bracts of *S. dolichostachya* were much more equal in size. The bract of the middle flower of the latter species was tapering, while that of *S. brachystachya* had a rounded top (*fig. 6*), a feature already described by DUVAL-JOUVE (1868).

All these characteristics, although variable, were usable to distinguish both species in the field. For the demographic study described in this paper they served

Fig. 6. Generative segments of *Salicornia brachystachya* (left) and *Salicornia dolichostachya* (right). Note the difference in size of the flower bracts of one cyme of *S. brachystachya* as compared with those of *S. dolichostachya* and the tapering top of the bract of the middle flower of *S. dolichostachya*.

equally well, especially when every individual was mapped and followed through its complete life cycle. Difficulties in distinguishing both species in their vegetative state did therefore not occur. Two differences between the species were found that could not be used in the field: 1. The root system of *S. dolichostachya* had a primary root with thickened branches while *S. brachystachya* had not. The dry weight of the total root system of the latter species was, however, on an average higher. 2. The seedlings of *S. brachystachya* were sometimes bright

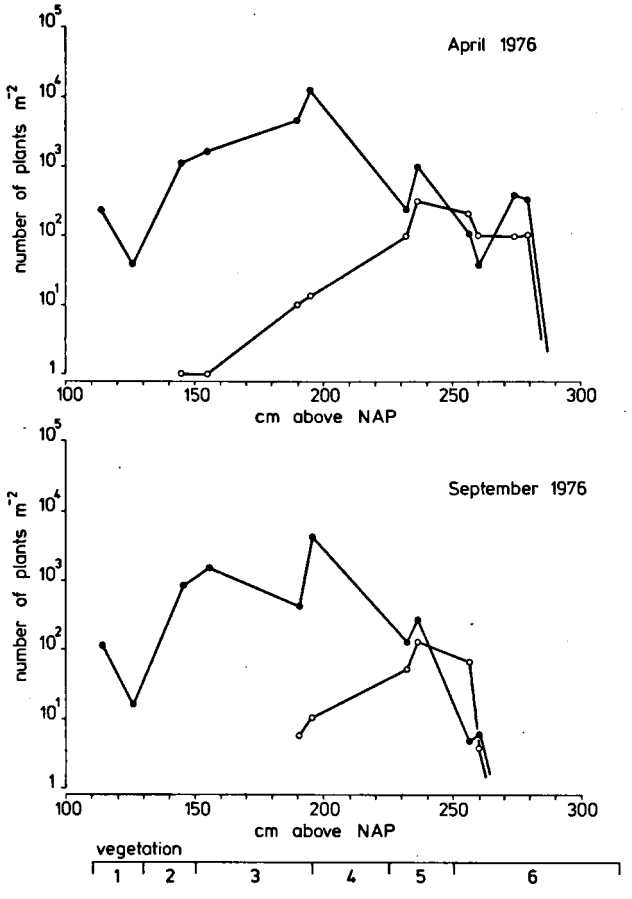

Fig. 7. Number of plants of *Salicornia dolichostachya* (●) and *Salicornia brachystachya* (○) in plots of 1 m^2 situated at various heights above N.A.P. (Dutch Ordnance Level) in the salt marsh south of Bergen op Zoom (Each point in the graph is the value in one plot).

red, which colour could return at the end of the growing season; *S. dolichostachya* plants turned at the end of the growing season light green to yellow. The plants of *S. brachystachya* were in general of a darker green colour than the *S. dolichostachya* plants.

Fig. 7 depicts the densities of *S. brachystachya* and *S. dolichostachya* plants in the plots laid out in the various vegetation zones, arranged according to their height above N.A.P. (Dutch Ordnance Level). The highest density of *S. dolichostachya* plants was reached at a level lower than that of *S. brachystachya*. This difference existed throughout the growing season. The complete dissappearrance of plants of both species above 260 cm above N.A.P. was due to the fact that these individuals emerged from seeds washed up in the beach-line. The plant debris on which they emerged dried out very quickly at neap tide periods causing

wilting and death of the seedlings. In another paper this demographic work will be discussed in greater detail (BEEFTINK et al., in preparation).

3.2. Greenhouse experiments

The results of the morphological and cytotaxonimical study on various *Salicornia* populations are summarized in *fig. 8*. Only the length of the anthers appeared to be distinctly different for both *Salicornia* species. All other characteristics measured showed an overlap. The number of chromosomes measured was either 18 or 36; other numbers did not occur in this study.

All 29 plants with 18 chromosomes were grown from seeds from parent plants identified as *S. brachystachya* following the key of KÖNIG (1960) extended with some other characteristics previously described.

In all but three cases had the plants with 36 chromosomes been grown from seeds from parent plants identified as *S. dolichostachya*. These three plants were identified as *S. brachystachya*.

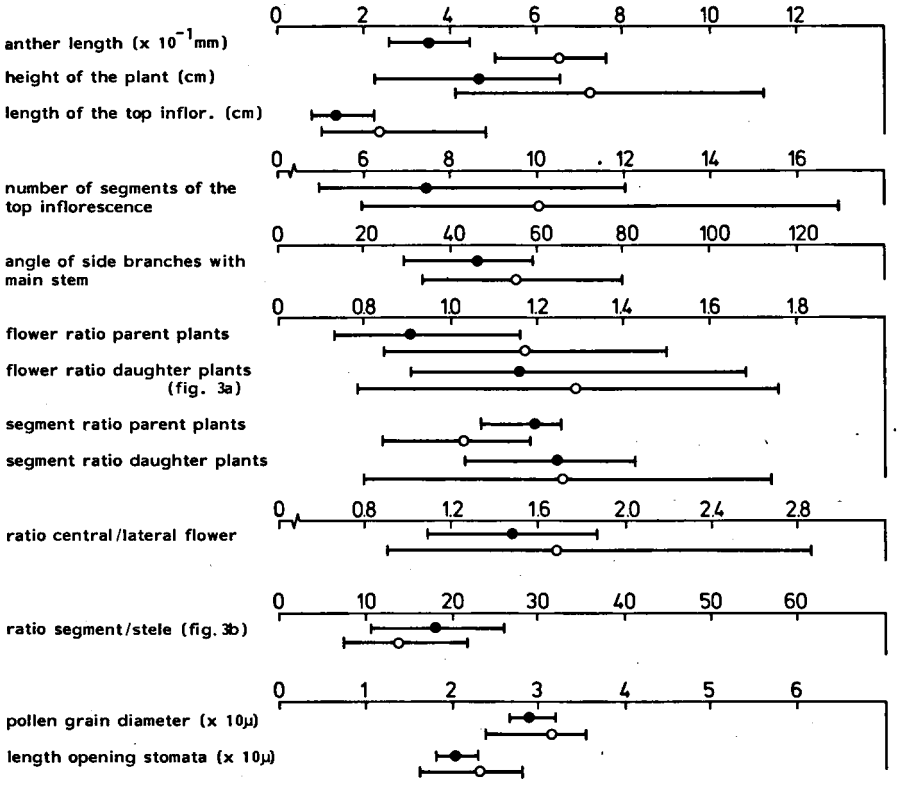

Fig. 8. Mean of measured values of a number of morphological characteristics of *Salicornia* plants with 18 (●) and 36 (○) chromosomes. The horizontal bars depict the span of the values.

4. DISCUSSION

In the southwest of the Netherlands annual *Salicornia* plants occur with 18 and 36 chromosomes. This is also the case in other places along the European coasts (BALL & TUTIN 1959, CONTANDRIOPOULOS 1968, CRISTOFOLINI & CHIAPELLA 1970, KÖNIG 1939, LAUSI 1969, NANNFELDT 1955, PARRIAUD 1971). Although the morphological characteristics overlap to a great extent it is possible – especially in the seedling and in the generative stage – to tell the two taxa apart. The chromosome counts conform to a large extent the usefulness of the other characteristics used for identification: only in 3 out of 84 cases a wrong identification was made. These were individuals from non-tidal salt marshes where the morphological characteristics are more difficult to separate, probably due to certain environmental parameters (e.g. high salinity). The length of the anthers appears to be the most distinct difference between the two taxa. BALL & BROWN (1970) came to the same conclusion while studying the *Salicornia* population of the Dee estuary.

Other authors also came to the conclusion that only two taxa with three-flowered cymes occur in the same area: one tetraploid and one diploid (Ball, in TUTIN et al. 1964, KNOERR 1968, KÖNIG 1939, NANNFELDT 1954) or at most three (BINET & LANGLOIS 1961, GILLNER 1960, GROUZIS et al. 1976, LANGLOIS 1961a).

Especially the paper of BALL & BROWN (1970) indicates clearly that an elaborate taxonomy of *Salicornia* as proposed by BALL & TUTIN (1959) and Ball (in TUTIN et al. 194) is of little use in one location.

However, the present study of populations of different localities shows a considerable amount of variation between individuals of one taxon (*fig. 8*) which could be ascribed to phenotypic or geographic difference (WILKON-MICHALSKA 1985). It is probably more plausible that a local group of indidivual plants is a line. The diploid plants especially may have cleistogamy where the anthers stay inside the flower. This could also explain the difference found in different localities with very different proportions of diploid and tetraploid taxa. This coincides with the findings of CRISTOFOLINI & CHIAPELLA (1970), who worked on the chemotaxonomy of *Salicornia*, and concluded in their paper that, chemotaxonomically spoken, the diploid individuals belong to one single taxonomic unit and the tetraploid individuals to another.

In the present study on *Salicornia* we like to distinguish only two taxa: the diploid species *S. brachystachya* and the tetraploid species *S. dolichostachya*. *S. dolichostachya* is the species that has its highest density in the lower marsh whereas *S. brachystachya* reaches its highest density in the zone dominant by *Triglochin maritima, Limonium vulgare* and *Plantago maritima*.

However, in Britain the situation in the various salt marshes seems much more complex as has been described by JEFFERIES et al. (1981), JEFFERIES & GOTTLIEB (1982) and Davy (pers. comm.) whereby up to seven species are distinguished: four diploid and three tetraploid. But the taxonomic index used in the Flora Europaea (TUTIN et al. 1964) is too much based on the British situation which

is apparently rather different from the salt marshes in the southwest Netherlands.

Our study, concluding to distinguish two species, showed that the diploid species was in general more variable than the tetraploid species. The former species might even be considered as a species aggregate. Recent studies on the differences in the morphology of *S. brachystachya* found in the salt marsh and found in certain places in saline grassland (e.g. on the dutch island of Schiermonnikoog) could justify a division into two taxa *S. brachystachya* ssp. *ramosissima* and *S. brachystachya* ssp. *europaea* respectively (Schat, in preparation).

The occurrence of a tetraploid species besides a diploid one illustrate that also in the salt-marsh ecosystem polyploidy is an important process governing speciation. Other salt-marsh genera in which polyploidy is common are *Suaeda* (HARA 1969), *Puccinellia* (SØRENSEN 1953), *Spartina* (MOBBERLY 1956), and *Limonium* (ERBEN 1978).

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