

This article was downloaded by: [78.13.235.189]

On: 21 December 2011, At: 03:12

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Grana

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/sgra20>

# Pollen production in halophytic species of the Chenopodiaceae in a Mediterranean marsh

Francisca Fernández-Illescas<sup>a</sup>, Francisco J. J. Nieva<sup>a</sup>, Belén Márquez-García<sup>a</sup> & Adolfo F. Muñoz-Rodríguez<sup>a</sup>

<sup>a</sup> Departamento Biología Ambiental y Salud Pública, Facultad de Ciencias Experimentales, Universidad de Huelva, Spain

Available online: 19 Nov 2010

To cite this article: Francisca Fernández-Illescas, Francisco J. J. Nieva, Belén Márquez-García & Adolfo F. Muñoz-Rodríguez (2010): Pollen production in halophytic species of the Chenopodiaceae in a Mediterranean marsh, *Grana*, 49:4, 300-307

To link to this article: <http://dx.doi.org/10.1080/00173134.2010.522728>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.tandfonline.com/page/terms-and-conditions>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

## Pollen production in halophytic species of the Chenopodiaceae in a Mediterranean marsh

FRANCISCA FERNÁNDEZ-ILLESCAS, FRANCISCO J. J. NIEVA,  
BELÉN MÁRQUEZ-GARCÍA & ADOLFO F. MUÑOZ-RODRÍGUEZ

*Departamento Biología Ambiental y Salud Pública, Facultad de Ciencias Experimentales, Universidad de Huelva, Spain*

### Abstract

The pollen production per anther has been analysed in ten species of Chenopodiaceae that grow in the salt marshes of the Marismas del Odiel Natural Park (Huelva, south-west Spain). The mean pollen production per anther is 5819, ranged from 580 in *Salicornia ramosissima* to 12 467 in *Salsola vermiculata*, and is related to the size of the anthers. In six of the species, pollen production per anther has been studied in two populations and a strong interaction between species and populations was found. Pollen production of central and lateral flowers has been compared in four species, showing that central flowers produce significantly more pollen than lateral ones. In *Arthrocnemum macrostachyum*, a comparison between the pollen production of upper and lower stamens was performed and no significant differences were found. The pollen/ovule (P/O) ratio has been estimated for each of the species studied for the monoecious species, using the previously calculated male flower/female flower ratio. The monoecious species have the highest P/O ratios, followed by those with hermaphrodite flowers and allogamous reproduction. The lowest P/O ratios were found in *Salicornia ramosissima* (681) and *Suaeda albens* (6025), possibly due to them being autogamous, which is consistent with the annual cycle and ecology of the species. The biggest value was found in the monoecious species *Halimione portulacoides* (88 405). In the remainder of the species, the P/O ratio depends on the density of individuals of each species in the communities.

**Keywords:** Marsh, pollen, P/O ratio, Chenopodiaceae, pollination, reproductive biology

Salt marshes constitute a habitat of great ecological interest and high conservation value and have been the subject of several wide-ranging studies. However, information regarding the reproductive biology of plant species in this environment is sparse. Most studies have been restricted to investigations of the capacity of vegetative reproduction (Long & Mason, 1983; Boorman, 2003) or the ecological factors related to the dispersal and germination of fruits and seeds (Khan & Gul, 2006).

The Chenopodiaceae is one of the most important components of salt marsh vegetation (Long & Mason, 1983; Adam, 1990). The species in this group are mainly anemophilous, although some are dichogamous and diclinous (Connor, 1984; Pendleton et al., 1988; Pendleton et al., 2000). Anemophilous species generally produce large amounts of pollen to

counteract the low efficiency of wind as a pollen vector (Faegri & Van der Pijl, 1979), which gives them high rates of gene flow (Hamrick et al., 1995). However, not all of the species that possess an anemophilous pollination syndrome are allogamous; some species have evolved predominant selfing as sexual strategy (Barrett & Eckert, 1990).

From the point of view of reproductive strategies, the absolute pollen production is not as important as the pollen/ovule (P/O) ratio. The latter was established by Cruden (1977) in order to relate the reproductive system to the ecological preferences of the species. Cruden (2000) also proposed that anemophilous P/O ratios might vary among species according to distance between putative mates, area of the stigma, height of pollen liberation and length of stigmatic receptivity.

Correspondence: A. F. Muñoz-Rodríguez, Departamento de Biología Ambiental y Salud Pública, Facultad de Ciencias Experimentales, Campus El Carmen, Universidad de Huelva, Avda. Fuerzas Armadas, s/n, 21071, Huelva, Spain. E-mail: [adolfo.munoz@dbasp.uhu.es](mailto:adolfo.munoz@dbasp.uhu.es)

(Received 4 March 2010; accepted 28 August 2010)

ISSN 0017-3134 print/ISSN 1651-2049 online © 2010 Collegium Palynologicum Scandinavicum  
DOI: 10.1080/00173134.2010.522728

This study describes the pollen production of ten species of the Chenopodiaceae in the protected Marismas del Odiel Natural Park. The results are discussed in terms of the breeding systems, ecology and population density.

## Materials and methods

### Description of flowers and inflorescences

The details of the flowers and inflorescences in the species of this study are described later (Castroviejo, 1990), in line with the systematic treatment established by Kadereit et al. (2003).

**Subfamily Chenopodioideae, tribe Atripliceae.** — Both *Atriplex halimus* L. and *Halimione portulacoides* (L.) Aellen are shrubs with panicle-like inflorescences with sessile cymose glomerules bearing male and female flowers. The male flowers have five stamens, the female have one pistil with a single ovule.

**Subfamily Salicornioideae, tribe Salicornieae.** — *Salicornia ramosissima* Woods is annual, whereas *Arthrocnemum macrostachyum* (Moric.) K. Koch, *Sarcocornia fruticosa* (L.) A. J. Scott, *Sarcocornia perennis* (Mill.) A. J. Scott and *Sarcocornia alpini* (Lag.) Rivas Mart. are perennial. All have spike-like inflorescences with two opposite cymes in each whorl, each cyme having one central and two lateral hermaphrodite flowers. Each flower has two stamens, one adaxial or upper, and one abaxial or lower, except for *Salicornia ramosissima*, which only had the adaxial stamen in the population studied. The flowers have one pistil with one ovule.

**Subfamily Suaedoideae, tribe Suaedeae.** — *Suaeda albescens* Lázaro Ibiza is annual and herbaceous, *Suaeda vera* Forssk. ex J. F. Gmel. is a shrub. Both produce hermaphrodite flowers in the leaf axils, in cymes with a central flower and, in general, two lateral flowers. Each flower consists of five stamens and one pistil with one ovule.

**Subfamily Salsoloideae, tribe Salsoleae.** — *Salsola vermiculata* L. is a bush with panicle-like inflorescences with a single hermaphrodite flower developed on the axil of a bract. The flowers have five stamens and one pistil with a single primordium.

### Description of the study area

The samples used in this study were collected at four different locations from the tidal marsh in the Marismas del Odiel Natural Park (Huelva, south-west Spain; Figure 1).

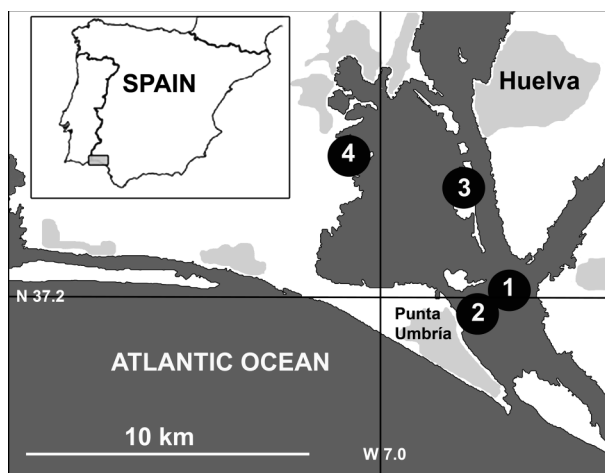


Figure 1. Map with the studied locations.

Both localities 1 and 2 consist of similar vegetation complexes as indicated by Fernández-Illescas et al. (2010a). The lower zone is completely submerged at high tide and consists only of *Sarcocornia perennis* (*Puccinellio maritimae*–*Arthrocnemum perennis* Géhu, 1976). The middle zone is flooded at high tide and houses a community of *Sarcocornia fruticosa* and *Halimione portulacoides* (*Cistancho phelipaeae*–*Arthrocnemum fruticosi* Géhu ex Géhu & Géhu-Franck, 1977). The upper zone is only reached by the highest tides and has a diverse community of halophyte shrubs including *Arthrocnemum macrostachyum*, *Suaeda vera* and *Salsola vermiculata* (*Inula crithmoidis*–*Arthrocnemum macrostachyi* Fontes ex Géhu & Géhu-Franck, 1977). However, when shrubs were cleared from the middle zone, *Salicornia ramosissima* and *Suaeda albescens* could establish (*Suaeda spicatae*–*Salicornietum patulae* Brullo, Furnari ex Géhu & Géhu-Franck, 1984). These two locations differ in their position in the channel through which the tidal flow enters. Locality 2 is more affected by tidal forces and presented a high grade of mechanical perturbation.

Localities 3 and 4 differ in that they are not inundated by tidal flows except in unusual circumstances. Locality 3 is a non-tidal marine deposit with predominant *Atriplex halimus* (*Pegano*–*Salsoletea vermiculatae* Br.-Bl. & O. Bolòs, 1957). Locality 4 lies on the edge of the salt marsh where also fresh water enters, and has mainly *Sarcocornia alpini* and *Arthrocnemum macrostachyum* (*Halimione portulacoidis*–*Sarcocornietum alpini* Rivas-Martínez & Costa, 1984).

### Pollen production per anther and anther size

The methodology follows Cruden (1977) and Tormo et al. (1996). We studied 27 cases (Table I).

In each sample, the number of pollen grains per anther was estimated in five plants. Five mature and closed anthers from different flowers were collected from each plant in order to avoid variations in pollen production due to the position in the plant and size of the flowers (McKone, 1990). The length and width of each anther was measured. The five anthers were placed in a glass tube with 1 ml of distilled water and ground with a glass rod until there were no clusters of pollen visible under a dissecting microscope. Each tube was shaken, five 10 µl drops were extracted from it and arranged separately on slides, and all the pollen grains in each drop were counted under a light microscope. These data were used to estimate the number of pollen grains per anther in each plant.

In each plant ( $n=135$ ) average length $\times$ width was related to the pollen production by using Pearson regression. The reason for not using anther length only derives from the observations of Tormo et al. (1996), who found that the relation between anther length and pollen production is not linear but is quadratic.

#### *Comparison between populations, flower position and anther position*

In six species (*Halimione portulacoides*, *Arthrocnemum macrostachyum*, *Sarcocornia fruticosa*, *Suaeda albenscens*, *Suaeda vera* and *Salsola vermiculata*) pollen production per anther was studied on plants from two different locations (localities 1 and 2). A generalised linear model (GLM) was used for testing differences and interactions between species and locations. To see if there were differences in the pollen production between central and lateral flowers in plants with flowers in cymes (*Salicornia ramosissima*, *A. macrostachyum*, *Suaeda albenscens* and *Suaeda vera*), pollen production per anther was studied in central and lateral flowers separately, and GLM was used for testing differences and interactions between species and flower type. In *A. macrostachyum*, pollen production from the adaxial and abaxial stamens was analysed separately, and GLM was used for testing differences and interactions between locations, flower type and anther position.

#### *Pollen production per flower and P/O ratios*

In each case, the pollen production per flower was estimated from the production of pollen per anther and the number of viable anthers per flower (fully developed and containing mature pollen grains). The latter was estimated on 80 flowers in each population. Hermaphrodite flowers have a single ovule, so the P/O ratio is the same as the pollen production

per flower. In the monoecious species *Atriplex halimus* and *Halimione portulacoides*, the ratio of male and female flowers was estimated. Since female flowers can form in the glomerule after the male flowers and then later start to drop from the glomerule once they are open, the number of flowers of both sexes was counted on 30 glomerules of ten different plants over three consecutive weeks, a period that covers the peak of flowering of both sexes. The P/O ratios were estimated in each case, and the mean for each species was calculated using all the samples. Comparisons of P/O ratios (log transformed data for normalisation) from different species were carried out using analysis of variance (ANOVA).

## Results

### *Pollen production per anther and anther size*

The mean numbers of pollen grains per anther production using all the cases was  $5819 \pm 3239$  with the minimum registered in the lateral flowers of *Salicornia ramosissima* ( $580 \pm 166$ ) and the maximum found in *Salsola vermiculata* in locality 2 ( $12\,467 \pm 1633$ ; Table I). The mean value of anther length ranged from 0.4 mm in the lateral flowers of *Suaeda albenscens* in locality 2 to 1.55 mm of upper anthers in the lateral flowers of *Arthrocnemum macrostachyum* in locality 1. Mean anther length fluctuated from 0.3 mm in the central and lateral flowers of *Salicornia ramosissima* to 0.63 mm of the upper anthers in the central flowers of *A. macrostachyum* in locality 1. Analysis of the samples of all the species showed a positive and significant correlation between the size of the anthers and pollen production ( $r = 0.76$ ;  $p = 0.0000$ ; Figure 2).

### *Comparison between populations, flower position and anther position*

In the six species studied, there was a strong interaction between species and populations (Figure 3; Table II). In *Arthrocnemum macrostachyum*, *Sarcocornia fruticosa* and *Suaeda albenscens*, the production was lower in samples from locality 2. However, the production was lower in *Suaeda vera* samples from locality 1. In the four species in which the effect of flower position was studied, significant differences were found in the pollen production between species and between lateral and central flowers (Table II). Central flowers produced significantly more pollen than lateral ones (Figure 4). In *A. macrostachyum*, there are significant differences between populations and between anther positions (adaxial and abaxial; Table II), and there exists an interaction between populations, flower position and anther position (Figure 5).

Table I. Estimated production of pollen grains per anther, flower and ovule.

| Species                           | Cases |     |      | Anther length (mm) |      | Anther width (mm) |      | Fertile anthers/flower |      | Pollen/flower |       | Male flowers/ovule |    | Pollen/ovule per case |       | Pollen/ovule per species |       |
|-----------------------------------|-------|-----|------|--------------------|------|-------------------|------|------------------------|------|---------------|-------|--------------------|----|-----------------------|-------|--------------------------|-------|
|                                   | Loc   | Pos | Anth | Mean               | SD   | Mean              | SD   | Mean                   | SD   | Mean          | SD    | Mean               | SD | Mean                  | SD    | Mean                     | SD    |
|                                   |       |     |      |                    |      |                   |      |                        |      |               |       |                    |    |                       |       |                          |       |
| <i>Atriplex halimus</i>           | 3     |     |      | 0.51               | 0.04 | 0.52              | 0.05 | 3629                   | 344  | 18144         | 1719  | 2.64               |    | 47900                 | 4537  | 47900                    | 4537  |
| <i>Halimione portulacoides</i>    | 1     |     |      | 0.51               | 0.03 | 0.54              | 0.05 | 4118                   | 480  | 20588         | 2400  | 4.06               |    | 83587                 | 9745  | 88405                    | 9248  |
|                                   | 2     |     |      | 0.55               | 0.05 | 0.57              | 0.04 | 4010                   | 270  | 20048         | 1350  | 4.65               |    | 93223                 | 6278  |                          |       |
| <i>Salicornia ramosissima</i>     | 1     | C   |      | 0.58               | 0.06 | 0.30              | 0.03 | 782                    | 61   | 782           | 61    | 1.00               |    | 782                   | 61    | 681                      | 159   |
|                                   | L     |     |      | 0.59               | 0.03 | 0.30              | 0.00 | 580                    | 166  | 580           | 166   | 1.00               |    | 580                   | 166   |                          |       |
| <i>Sarcocornia fruticosa</i>      | 1     | C   | Up   | 1.27               | 0.11 | 0.61              | 0.02 | 4857                   | 533  | 9616          | 1056  | 1.00               |    | 9616                  | 1056  | 8726                     | 1211  |
|                                   | 2     | C   | Up   | 1.16               | 0.10 | 0.60              | 0.00 | 3918                   | 224  | 7835          | 448   | 1.00               |    | 7835                  | 448   |                          |       |
| <i>Sarcocornia perennis</i>       | 1     | C   | Up   | 0.90               | 0.06 | 0.49              | 0.04 | 3656                   | 447  | 7020          | 859   | 1.00               |    | 7020                  | 859   | 7020                     | 859   |
| <i>Sarcocornia alpini</i>         | 4     | C   | Up   | 1.07               | 0.10 | 0.50              | 0.00 | 4102                   | 597  | 7466          | 1087  | 1.00               |    | 7466                  | 1087  | 7466                     | 1087  |
| <i>Arthrocnemum macrostachyum</i> | 1     | C   | Up   | 1.53               | 0.15 | 0.63              | 0.07 | 8415                   | 1451 | 16268         | 2762  | 1.00               |    | 16268                 | 2762  | 14840                    | 2581  |
|                                   | Low   |     |      | 1.52               | 0.21 | 0.61              | 0.12 | 10071                  | 1312 |               |       |                    |    |                       |       |                          |       |
|                                   | Up    |     |      | 1.55               | 0.07 | 0.60              | 0.00 | 8263                   | 1425 | 14295         | 2421  | 1.00               |    | 14295                 | 2421  |                          |       |
|                                   | L     |     |      | 1.37               | 0.20 | 0.52              | 0.08 | 7981                   | 1476 |               |       |                    |    |                       |       |                          |       |
|                                   | Low   |     |      | 1.44               | 0.14 | 0.53              | 0.06 | 7176                   | 1238 | 14629         | 2159  | 1.00               |    | 14629                 | 2159  |                          |       |
|                                   | Up    |     |      | 1.48               | 0.12 | 0.55              | 0.05 | 7751                   | 996  |               |       |                    |    |                       |       |                          |       |
|                                   | Low   |     |      | 1.42               | 0.08 | 0.51              | 0.02 | 6379                   | 634  | 14168         | 2752  | 1.00               |    | 14168                 | 2752  |                          |       |
|                                   | Up    |     |      | 1.41               | 0.12 | 0.55              | 0.05 | 8078                   | 1494 |               |       |                    |    |                       |       |                          |       |
| <i>Suaeda albescens</i>           | 1     | C   |      | 0.52               | 0.05 | 0.54              | 0.05 | 2639                   | 371  | 6756          | 951   | 1.00               |    | 6756                  | 951   | 6025                     | 818   |
|                                   | L     |     |      | 0.44               | 0.06 | 0.46              | 0.05 | 2338                   | 195  | 5984          | 498   | 1.00               |    | 5984                  | 498   |                          |       |
|                                   | 2     | C   |      | 0.54               | 0.05 | 0.57              | 0.05 | 2341                   | 121  | 6180          | 320   | 1.00               |    | 6180                  | 320   |                          |       |
|                                   | L     |     |      | 0.40               | 0.06 | 0.42              | 0.05 | 1962                   | 218  | 5181          | 576   | 1.00               |    | 5181                  | 576   |                          |       |
| <i>Suaeda vera</i>                | 1     | C   |      | 0.94               | 0.07 | 0.51              | 0.04 | 7715                   | 1402 | 35876         | 6519  | 1.00               |    | 35876                 | 6519  | 34967                    | 10939 |
|                                   | L     |     |      | 0.72               | 0.06 | 0.44              | 0.05 | 5419                   | 843  | 25199         | 3921  | 1.00               |    | 25199                 | 3921  |                          |       |
|                                   | 2     | C   |      | 0.96               | 0.06 | 0.60              | 0.05 | 8422                   | 1602 | 41266         | 7849  | 1.00               |    | 41266                 | 7849  |                          |       |
|                                   | L     |     |      | 0.80               | 0.15 | 0.48              | 0.06 | 7658                   | 3359 | 37526         | 16458 | 1.00               |    | 37526                 | 16458 |                          |       |
| <i>Salsola vermiculata</i>        | 1     |     |      | 1.41               | 0.09 | 0.59              | 0.03 | 12374                  | 1652 | 49742         | 6641  | 1.00               |    | 49742                 | 6641  | 52922                    | 7406  |
|                                   | 2     |     |      | 1.50               | 0.17 | 0.58              | 0.08 | 12467                  | 1633 | 56102         | 7350  | 1.00               |    | 56102                 | 7350  |                          |       |

Abbreviations: Loc, location of sampling point; Pos, position of the flower (C, central; L, lateral); Anth, position of anther in flower (Up, upper anther; Low, lower anther); SD, standard deviation.

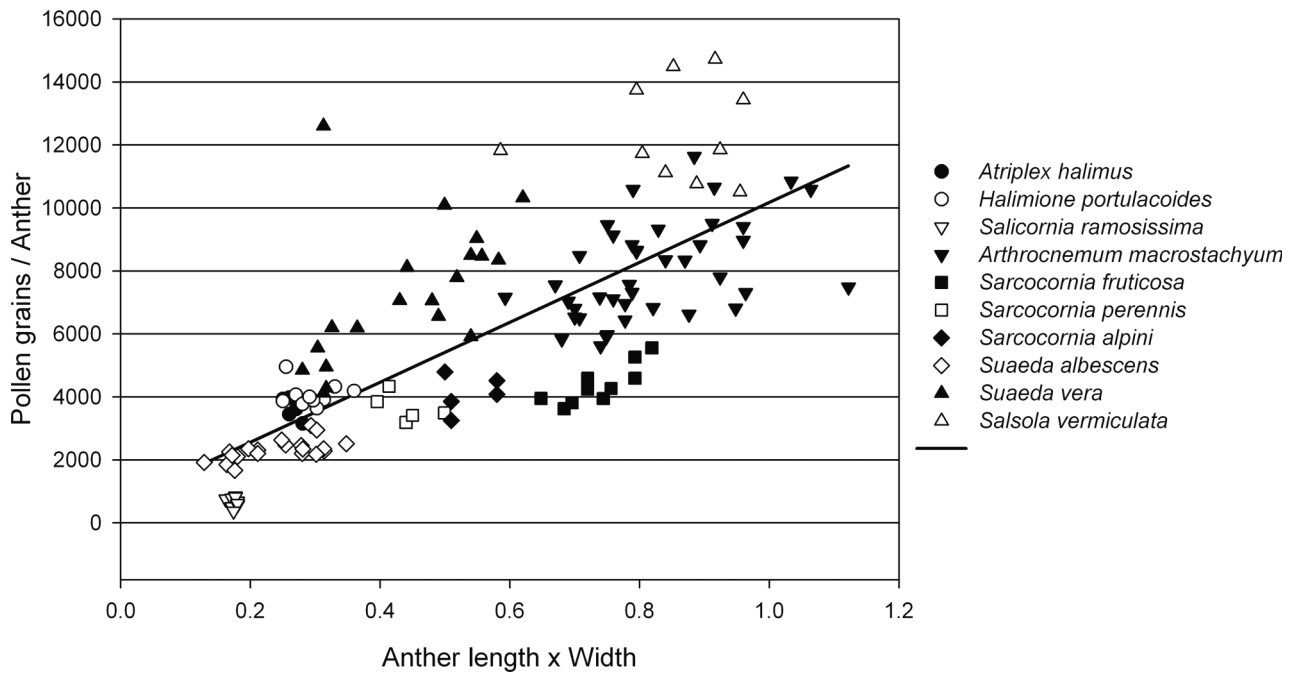


Figure 2. Relation between the size of the anthers (length  $\times$  width) and pollen production per anther in the different samples studied.

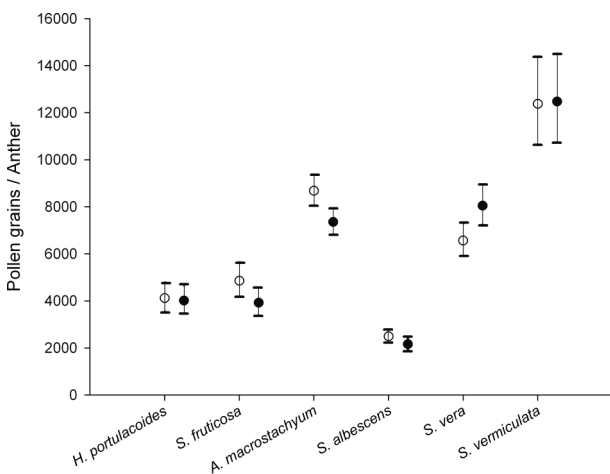


Figure 3. Least squares means (LS means) and their 95% confidence intervals of pollen production per anther for different species and populations (white: locality 1; black: locality 2).

#### Pollen production per flower and P/O ratios

Taking into account the number of anthers per flower in each species and the number of fertile anthers per flower obtained (Table I), the anther viability percentage exceeds 85% in all species, except in *Suaeda albescens*, where it is close to 50%. The latter means that only half of the anthers produce pollen. In monoecious species, the number of male flowers per female flower is higher in *Halimione portulacoides* (4.06 and 4.65) than in *Atriplex halimus* (2.64). The P/O ratios per sample ranged from 580

in lateral flowers of *Salicornia ramosissima* in locality 1 to 93 223 in *Halimione portulacoides* in locality 2 (Figure 6; Table I). *Atriplex halimus*, *Suaeda vera* and *Salsola vermiculata* are second, followed by *Arthrocnemum macrostachyum*, *Sarcocornia fruticosa*, *Sarcocornia perennis*, *Sarcocornia alpini* and *Suaeda albescens* that had a similar P/O ratio, and *Salicornia ramosissima* with the smallest. The P/O ratios were significantly different among the species ( $F = 58.7$ ;  $p = 0.0000$ ; Table III).

#### Discussion

Our study confirms the generally quadratic relationship between size of anthers and pollen production found by other authors (e.g., Subba Redi & Redi, 1986; Tormo et al., 1996). However, some species are above the regression line, while others are below it (Figure 2). This may be due to the size differences of pollen grains between species. According to data in Candau (1987), the species above the regression line (i.e. *Atriplex halimus*, *Halimione portulacoides*, *Suaeda vera* and *Salsola vermiculata*) have smaller pollen grains ( $<22 \mu\text{m}$ ) than the rest of the species.

In each of the cases studied, the variation in pollen production per anther among the different individuals is comparable to that found in similar studies (Stanton & Preston, 1988; McKone, 1990) with coefficients of variation (relationship between the deviation and the mean) between 5.2% and 43.9% and a mean value of 14.6%. Pollen production per

Table II. *F*-Statistics and significance levels (*p*) resulting from GLM analysis summarising the effects of locality, flower position and anther position. Only significant values are shown.

| Species included                                                                                                                                                                 |                                                              | <i>F</i> | <i>p</i> |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|----------|----------|
| <i>Halimione portulacoides</i> , <i>Sarcocornia fruticosa</i> , <i>Arthrocnemum macrostachyum</i> , <i>Suaeda albescentis</i> , <i>Suaeda vera</i> and <i>Suaeda vermiculata</i> | Species                                                      | 207.02   | 0.0001   |
|                                                                                                                                                                                  | Population                                                   | —        | —        |
|                                                                                                                                                                                  | Species $\times$ population                                  | 3.94     | 0.0027   |
| <i>Salicornia ramosissima</i> , <i>Arthrocnemum macrostachyum</i> , <i>Suaeda albescentis</i> and <i>Suaeda vera</i>                                                             | Species                                                      | 539.69   | 0.0001   |
|                                                                                                                                                                                  | Flower position                                              | 15.80    | 0.0002   |
|                                                                                                                                                                                  | Species $\times$ flower position                             | —        | —        |
| <i>Arthrocnemum macrostachyum</i>                                                                                                                                                | Population                                                   | 14.09    | 0.0007   |
|                                                                                                                                                                                  | Flower position                                              | —        | —        |
|                                                                                                                                                                                  | Anther position                                              | 6.63     | 0.0149   |
|                                                                                                                                                                                  | Population $\times$ flower position                          | —        | —        |
|                                                                                                                                                                                  | Population $\times$ anther position                          | —        | —        |
|                                                                                                                                                                                  | Flower position $\times$ anther position                     | —        | —        |
|                                                                                                                                                                                  | Population $\times$ flower position $\times$ anther position | 4.41     | 0.0438   |

anther differs between the two populations; the data showed some inter-population variability that may be related to differences in environmental conditions affecting pollen production (Delph et al., 1997; Guardia & Belmonte, 2004).

Flower position appears to be an important factor for pollen production; central flowers produce more than lateral, and as a result, they have a larger P/O ratio. In *Arthrocnemum macrostachyum*, the lower anthers produce more pollen than the upper. This difference varies with flower position and population.

The mean P/O ratio of the populations studied (29677) is similar to that found by Cruden (2000) for anemophilous species (22150), although some authors estimated this relationship to be above 1 000 000 (Ackerman, 2000). We found large differences in P/O ratio between the species, which suggests that factors other than the pollination system also affect these ratios. These differences might be explained in various ways.

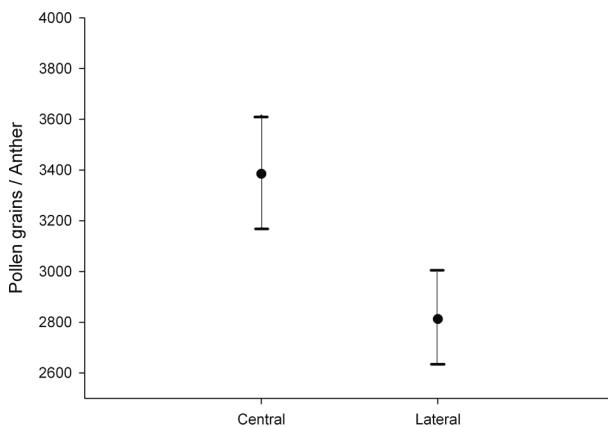


Figure 4. Least squares means (LS means) and their 95% confidence intervals of pollen production per anther for central and lateral flowers.

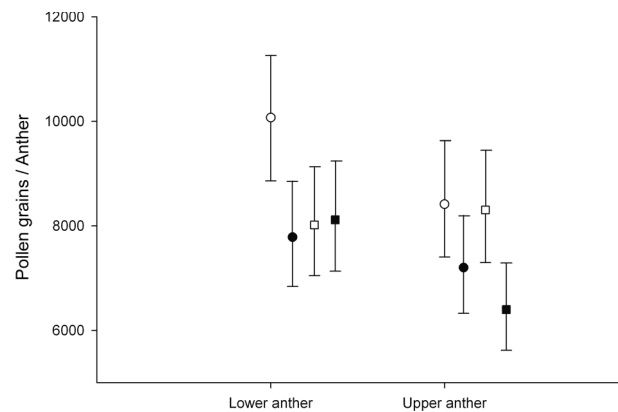
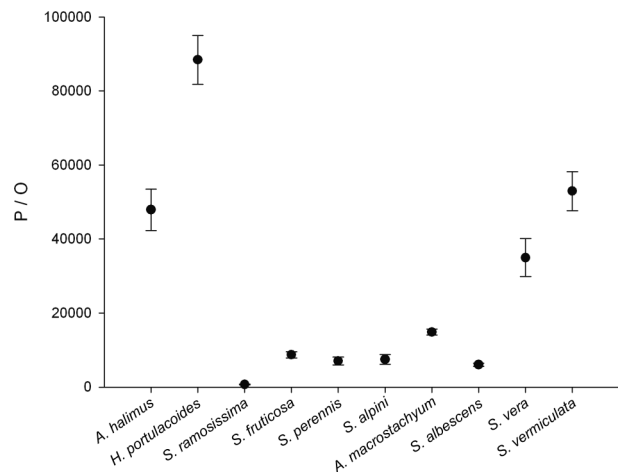
Figure 5. Least squares means (LS means) and their 95% confidence intervals of pollen production of lower and upper anthers of *Arthrocnemum macrostachyum* for different flower positions (circle: central; square: lateral) and populations (white: locality 1; black: locality 2).

Figure 6. Means and their 95% confidence intervals of P/O ratios for each species.

Table III. Comparison between P/O ratios (log transformed) of the species studied (ANOVA).

|                                       | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|---------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| <i>Atriplex halimus</i> (1)           |     |     |     |     |     |     |     |     |     |
| <i>Halimione portulacoides</i> (2)    | *** |     |     |     |     |     |     |     |     |
| <i>Salicornia ramosissima</i> (3)     | *** | *** |     |     |     |     |     |     |     |
| <i>Sarcocornia fruticosa</i> (4)      | *** | *** | *** |     |     |     |     |     |     |
| <i>Sarcocornia perennis</i> (5)       | *** | *** | *** | —   |     |     |     |     |     |
| <i>Sarcocornia alpini</i> (6)         | *** | *** | *** | —   | —   |     |     |     |     |
| <i>Arthrocnemum macrostachyum</i> (7) | *** | *** | *** | *** | *** | *** |     |     |     |
| <i>Suaeda albescens</i> (8)           | *** | *** | *** | *** | —   | —   | *** |     |     |
| <i>Suaeda vera</i> (9)                | —   | *** | *** | *** | *** | *** | *** | *** |     |
| <i>Salsola vermiculata</i> (10)       | —   | *** | *** | *** | *** | *** | *** | *** | *** |

\* $p < 0.05$ ; \*\* $p \leq 0.01$ ; \*\*\* $p < 0.001$ .

Observations on pollen viability in the populations studied here indicated that in the tribe *Salicornieae* on average 80% of pollen is viable, in the tribe *Atripliceae* this is close to 65%, and in the tribes *Salsoleae* and *Suaedeae* it is around 50% (Fernández-Illescas et al., 2010b). *Atriplex halimus* and *Halimione portulacoides* are, moreover, monoecious and have high P/O ratios, whereas all other species studied possess hermaphrodite flowers and have much lower P/O ratios (except *Salsola vermiculata*). This agrees with observations by Cruden (2000). Finally, *Salicornia ramosissima* is autogamous and had the lowest P/O ratio of all. Its flowers possess a single stamen with a small anther, which comes in direct contact with the receptive stigma (Dalby, 1962; Davy et al., 2001).

The predominance of autogamy has been demonstrated in molecular studies, where it was observed that outbreeding rates in some populations were lower than 1% (Noble et al., 1992). The autogamy in this annual colonising species could be an advantage in the colonisation of bare soils (Richards, 1986), where the plant usually forms virtually mono-specific communities that can be very dense (Ellison, 1987; Davy et al., 2001). *Suaeda albescens* is also annual, grows in the same habitats, and has a similar ecology and colonising behaviour (Davy et al., 2001; Boorman, 2003). This species also shares habitat in communities of *Suaeda spicatae*–*Salicornietum patulae* but without reaching densities like those of *Salicornia ramosissima*. *Suaeda albescens* has features that could be interpreted as a tendency towards autogamy, namely the second-lowest P/O ratio of the study and the drastic reduction in the number of functional

anthers. Both *Salicornia ramosissima* and *Suaeda albescens* produced more pollen in the stamens of central flowers than of lateral flowers. In addition, some authors (Dalby, 1962; Ungar, 1979) have observed that *Salicornia* seeds formed from central flowers are larger than those from lateral flowers, and there were even differences in the germination requirements between the two types of seeds (Ellison, 1987).

In the species of the tribe *Salicornieae*, dichogamy is a common characteristic in the increase in the rate of cross-fertilisation (Connor, 1984). With exception of *Salicornia ramosissima*, the P/O ratios were found to be within the range for allogamous species (Cruden, 1977). But although a higher pollen production should be expected for all species because they are anemophilous (Faegri & Van der Pijl, 1979), the three investigated *Sarcocornia* species seem to have relatively low values (Cruden, 2000), whereas the pollen production of *Arthrocnemum macrostachyum* is more in accordance with its reproductive system. According to Cruden (2000), this might be related to population densities: pollen production is usually higher for species in which the individuals grow as a rule at larger distances from each other. In agreement with their relatively low pollen production, the *Sarcocornia* species grow in highly restricted habitats with extensive coverage and little diversity (Fernández-Illescas et al., 2010a). *Sarcocornia perennis* occupies the lower levels of the salt marshes, where it acts as a coloniser (Davy et al., 2006) and dominates the *Puccinellio maritimae*–*Arthrocnemetum perennis* (Rivas-Martínez et al., 1980) community. *Sarcocornia fruticosa* occupies a mid-position in communities of *Cistancho phelipaeae*–*Arthrocnemetum fruticosi* in high coverage and dominating together with *Halimione portulacoides* (Rivas-Martínez et al., 1980). *Sarcocornia alpini* is dominant in communities of the *Halimione portulacoides*–*Sarcocornietum alpini* (Fernandes-Lousa et al., 1999). In contrast, *Arthrocnemum macrostachyum* and the other allogamous species with hermaphrodite flowers (*Salsola vermiculata* and *Suaeda vera*) as well as the two monoecious species (*Atriplex halimus* and *Halimione portulacoides*) inhabit a wider range of communities, generally with lower coverage and with a greater diversity of species, which results in a greater distance between individuals, and therefore a higher P/O value.

# Acknowledgements

The authors are grateful to the institution and staff of the Marismas del Odiel Natural Park, with special thanks to Enrique Sánchez-Gullón for his help and

collaboration, and to Jacinto Román for his assistance with GLM analyses.

## References

- Ackerman, J. D. (2000). Abiotic pollen and pollination: ecological, functional, and evolutionary perspectives. *Plant Systematics and Evolution*, 222, 167–185.
- Adam, P. (1990). *Saltmarsh ecology*. Cambridge: Cambridge University Press.
- Barrett, S. C. H. & Eckert, C. G. (1990). Variation and evolution of mating systems in seed plants. In S. Kawano (Ed.), *Biological approaches and evolutionary trend in plants* (pp. 229–254). New York: Academic Press.
- Boorman, L. A. (2003). *Saltmarsh review. An overview of coastal saltmarshes, their dynamic and sensitivity characteristics for conservation and management*. Peterborough: Joint Nature Conservation Committee Report 334.
- Candau, P. (1987). Chenopodiaceae. In B. Valdés, M. J. Díez & I. Fernández (Eds), *Atlas polínico de Andalucía Occidental* (pp. 103–105). Utrera: Universidad de Sevilla.
- Castroviejo, S. (1990). Chenopodiaceae. In S. Castroviejo, M. Lainz, G. López-González, P. Montserrat, F. Muñoz-Garmendia, J. Paiva & L. Villar (Eds), *Flora iberica Vol. II* (pp. 476–552). Madrid: Real Jardín Botánico.
- Connor, H. E. (1984). Gynodioecism in *Sarcocornia quinqueflora* (Salicorniaceae) in New Zealand. *New Zealand Journal of Botany*, 22, 433–439.
- Cruden, R. W. (1977). Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. *Evolution*, 31, 32–46.
- Cruden, R. W. (2000). Pollen grains: why so many? *Plant Systematics and Evolution*, 222, 143–165.
- Dalby, D. H. (1962). Chromosome number, morphology and breeding behaviour in the British *Salicorniae*. *Watsonia*, 5, 150–162.
- Davy, A. J., Bishop, G. F. & Costa, C. S. B. (2001). *Salicornia* L. (*Salicornia pusilla* J. Woods, *S. ramosissima* J. Woods, *S. europaea* L., *S. obscura* P. W. Ball & Tutin, *S. nitens* P. W. Ball & Tutin, *S. fragilis* P. W. Ball & Tutin and *S. dolichostachya* Moss). *Journal of Ecology*, 89, 681–707.
- Davy, A. J., Bishop, G. F., Hossman, H., Redondo-Gómez, S., Castillo, J. M., Castellanos, E. M., Luque T. & Figueroa, M. E. (2006). Biological flora of the British Isles: *Sarcocornia perennis* (Miller) A.J. Scott. *Journal of Ecology*, 94, 1035–1048.
- Delph, L. F., Johannsson, M. H. & Stephenson, A. G. (1997). How environmental factors affect pollen performance: ecological and evolutionary perspectives. *Ecology*, 78, 1632–1639.
- Ellison, A. M. (1987). Effects of competition, disturbance, and herbivory on *Salicornia europaea*. *Ecology*, 63, 576–586.
- Faegri, K. & Van der Pijl, L. (1979). *The principles of pollination ecology*. Oxford: Pergamon Press.
- Fernandes-Lousa, M., Costa, J. C., Capelo, J. H., Pinto-Gomes, C. & Neto-Silva, C. (1999). Overview of the vegetation and landscape of the lower Algarve (southern Portugal); siliceous ecosystems, schist, sandy substrata, dunes and saltmarshes. *Itinera Geobotanica*, 13, 137–147.
- Fernández-Illescas, F., Nieva, F. J. J., Silva, I., Tormo, R. & Muñoz, A. F. (2010a). Pollen production of Chenopodiaceae species at habitat and landscape scale in Mediterranean salt marshes: an ecological and phenological study. *Review of Palaeobotany and Palynology*, 161, 127–136.
- Fernández-Illescas, F., Cabrera, J., Nieva, F. J. J., Márquez-García, B., Sánchez-Gullón, E. & Muñoz-Rodríguez, A. F. (2010b). Production of aborted pollen in marsh species of *Chenopodiaceae*: evidence of partial male sterility in *Suaeda* and *Salsola* species. *Plant Systematics and Evolution*, 288, 167–176.
- Guardia, R. & Belmonte, J. (2004). Phenology and pollen production of *Parietaria judaica* L. in Catalonia (NE Spain). *Grana*, 43, 57–64.
- Hamrick, J. L., Godt, M. J. W. & Sherman-Broyles, S. L. (1995). Gene flow among plant populations: evidence from genetic markers. In P. G. Hoch & A. G. Stevenson (Eds), *Experimental and molecular approaches to plant biosystematics* (pp. 215–232). St Louis, MO: Missouri Botanical Garden.
- Kadereit, G., Borsch, T., Weising, K. & Freitag, H. (2003). Phylogeny of Amaranthaceae and Chenopodiaceae and the evolution of C4 photosynthesis. *International Journal of Plant Sciences*, 164, 959–986.
- Khan, M. A. & Gul, B. (2006). Halophyte seed germination. In M. A. Khan & D. J. Weber (Eds), *Eco-physiology of high salinity tolerant plants* (pp. 11–30). Dordrecht: Springer.
- Long, S. P. & Mason, C. F. (1983). *Saltmarsh ecology*. Glasgow: Blackie & Son Ltd.
- McKone, M. J. (1990). Characteristics of pollen production in a population of New Zealand snow-tussock grass (*Chionochloa pallens* Zotov). *New Phytologist*, 116, 555–562.
- Noble, S. M., Davy, A. J. & Oliver, R. M. (1992). Ribosomal DNA variation and population differentiation in *Salicornia* L. *New Phytologist*, 122, 553–565.
- Pendleton, R. L., McArthur, E. D., Freeman, D. C. & Blauer, A. C. (1988). Heterodichogamy in *Grayia brandegei*: report from a new family. *American Journal of Botany*, 75, 267–274.
- Pendleton, R. L., Freeman, D. C., McArthur, E. D. & Sanderson, S. C. (2000). Gender specialization in heterodichogamous *Grayia brandegei* (Chenopodiaceae): evidence for an alternative pathway to dioecy. *American Journal of Botany*, 87, 508–516.
- Richards, A. J. (1986). *Plant breeding systems*. London: George Allen & Unwin Ltd.
- Rivas-Martínez, S., Costa, M., Castroviejo, S. & Valdés, E. (1980). Vegetación de Doñana (Huelva, España). *Lazararo*, 2, 5–190.
- Subba Redi, C. & Redi, N. S. (1986). Pollen production in some anemophilous angiosperms. *Grana*, 25, 55–61.
- Stanton, M. L. & Preston, R. E. (1988). Ecological consequences and phenotypic correlates of petal size in wild radish, *Raphanus sativus* (Brassicaceae). *American Journal of Botany*, 75, 528–539.
- Tormo, R., Muñoz, A., Silva, I. & Gallardo, F. (1996). Pollen production in anemophilous trees. *Grana*, 35, 38–46.
- Unger, I. A. (1979). Seed dimorphism in *Salicornia europaea* L. *Botanical Gazette*, 140, 102–108.