Pollen–Stigma Interference in Two Gynodioecious Species of Lamiaceae with Intermediate Individuals

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- **Background and Aims** Intermediate individuals (perfect flowers with very high degree of pollen abortion) in a gynodioecious plant species are very rare. A study is made of male–female relationships in each flower type and how floral characters can enhance the avoidance of ‘pollen discounting’ and ‘self-pollination’ in two gynodioecious species, *Teucrium capitatum* and *Origanum syriacum*.
- **Methods** The relationship between stigma receptivity and pollen viability was studied in two gynodioecious protandrous species of Lamiaceae, in addition to measuring some floral morphological characters over the life span of the flowers.
- **Key Results** Three plant types in each species were found: plants bearing hermaphrodite (or male fertile) flowers (MF), female (or male sterile) flowers (MS) and intermediate flowers (INT). Plant types differed in flower size, with MS types being shorter than the other two types. There was no difference in style length among plant types in *T. capitatum*. Stigma receptivity decayed with floral age and was negative and significantly correlated with pollen viability in the two species, and positive and significantly correlated with style length in *O. syriacum* but only in MS flowers of *T. capitatum*.
- **Conclusions** Reduction in size of floral characters is associated with male sterility, except style length in *T. capitatum*. MF flowers have two successive reproductive impediments: self-pollination and pollen–stigma interference. In both species, self-pollination is avoided by dichogamy (negative correlation between stigma receptivity and pollen viability), and pollen–stigma interference shows two different patterns: (1) style elongation in *O. syriacum* is characterized by a significant length increase, final MF dimensions are greater than those of MS dimensions, and style length is positively and significantly correlated with stigma receptivity; and (2) style movement in *T. capitatum* is characterized by a non-significant increase in style length, final MF floral dimensions are similar to those of MS dimensions, and there is no correlation between style length and stigma receptivity.

Key words: Dichogamy, gynodioecy, Lamiaceae, *Origanum syriacum*, pollen discounting, pollen–stigma interference, pollen viability, protandry, stigma receptivity, style movement, style elongation, *Teucrium capitatum*.

INTRODUCTION

Several breeding systems in plants may favour outcrossing, either by reducing self-fertilization (Stout, 1928; Charlesworth, 1981) or by avoiding pollen–pistil interference (Bawa and Beach, 1981; ‘stigma clogging’ of Lloyd and Webb, 1986; Webb and Lloyd, 1986; Bertin and Newman, 1993). These mechanisms include: gynodioecy – the presence of plants with hermaphrodite flowers and plants with functionally female flowers or individuals with varying degrees of stamen degeneration (Darwin, 1896; Vaarama and Jääskeläinen, 1967; Dulberger, 1984); dichogamy (Lloyd and Webb, 1986) – the temporal separation of male and female functions, either through dehiscence of anthers before the stigma is receptive (protandry) or, vice versa, the stigma being receptive before the dehiscence of anthers (protogyny); and herkogamy (Webb and Lloyd, 1986) – the spatial separation of male and female functions. More strictly, Lloyd and Webb (1986) suggested that the separation of pollen and stigmas acts in general to reduce self-interference and often also reduces self-fertilization. They further suggested that mechanisms preventing

self-fertilization primarily increase maternal fitness, whereas mechanisms avoiding self-interference primarily promote paternal fitness.

Hermaphrodite flowers of gynodioecious species have been considered as pollen donors and, as with all hermaphrodite flowers, have a risk of inbreeding depression (Charlesworth and Charlesworth, 1987) and pollen–pistil interferences, which increase the loss of parental fitness by reducing the flower’s ability to export pollen (‘pollen discounting’ of Holsinger *et al.*, 1984). Flowers reduce those two risks in different ways: the first by means of dichogamy (Lloyd and Webb, 1986) and the second through herkogamy (Webb and Lloyd, 1986; Fetscher, 2001), i.e. by delaying the growth of or moving the female function, which is observed in protandrous species (Lloyd and Webb, 1986).

The presence of gynodioecious species seems to be particularly common in the Lamiaceae (Darwin, 1896; Baker, 1948; García and Muñoz, 1988). Baker (1948) reported that 27 of 76 studied gynodioecious species (approx. 36 %) belonged to the Lamiaceae, but more recent estimates are higher. For example, Owens and Uberta-Jiménez (1992) found that 57 % of species in the Lamiaceae are gynodioecious. Richards (1986) considered the presence of

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gynodioecy in such families as the Lamiaceae to be a well-established breeding system, i.e. the gynodioecy is stable and not just an intermediate step in the evolution of dioecy.

Teucrium capitatum and *Origanum syriacum* have been reported by Roiz and Dulberger (1989) and Ietswaart (1980), respectively, as species presenting two flower types: hermaphrodite or male fertile (MF) and female or male sterile (MS). Gynodioecy is sometimes more complicated than it seems, because of the presence of flowers with different degrees of male sterility. Kesseli and Jain (1984) found intermediate plants in *Limnanthes douglasii* that possessed varying anther size. García and Muñoz (1988) reported two levels of anther degeneration in *Teucrium fruticosum*: anthers that produced no pollen at all and those that produced non-viable pollen. In *Geranium maculatum*, Ågren and Wilson (1991) also observed plants that presented one or more anthers of reduced size, which they classified as intermediate plants (partially male sterile). Uberta-Jiménez and Hidalgo-Fernández (1992) found three principal types of flowers in *Rosmarinus officinalis* [hermaphrodite or male fertile (MF), female or male sterile without pollen (MS) and a set of intermediate forms with non-viable pollen (INT)]. Hidalgo *et al.* (1999) described two events occurring in the MS and INT flowers related to microsporogenesis in total and partial male sterile plants. MS flowers are characterized by the appearance of necrotic areas in the anther tissues before meiosis takes place and in INT flowers by the vacuolization of tapetum in later stages of microsporogenesis than in the anthers of MS flowers.

The aim of the present study was to investigate the relationship between male and female phases across the life span of gynodioecious species. Navarro (1997) treated this aspect briefly in *Salvia verbenaca*. We investigated this relationship in two Lamiaceae present in north-east Israel: *Teucrium capitatum*, distribution of which ranges across the Mediterranean and Irano-Turanian areas; and *Origanum syriacum*, found across the eastern Mediterranean region (Feinbrun-Dothan, 1986; Dudai *et al.*, 1989). The following questions were studied. (1) What is the possible role of the INT plants in this gynodioecy system? (2) What is the relationship between stigma receptivity and pollen viability and between stigma receptivity and style length across the life span of each of the flower types? (3) In MF flowers, how are ‘pollen discounting’ and self-pollination avoided? (4) What is the male–female phase relationship?

MATERIALS AND METHODS

Study population and species characteristics

The populations of *T. capitatum* L. (= *T. polium* L.) and *O. syriacum* L. (= *Majorana syriaca* (L.) Rafin) studied were located in a disturbed area on Mount Carmel (northern Israel). The typical vegetation here is a dwarf shrub community (phrygana) of *Sarcopoterium spinosum*.

T. capitatum is a small shrub presenting nectariferous, white, zygomorphous and unilabiate flowers. It has been described as a gynodioecious species with two plant types, one presenting hermaphrodite, protandrous flowers and the other female flowers. Flowers are principally

visited by *Apis mellifera*, *Lasioglossum* sp. and *Systropha* sp. (Roiz and Dulberger, 1989).

Origanum syriacum is a small shrub presenting nectariferous (Beker *et al.*, 1990), white-pink, zygomorphous flowers. *O. syriacum* has been described as a gynodioecious species (Ietswaart, 1980).

Floral characters

The following floral characters were measured in both species: (1) floral size, as the length from the base of the flower to the top of the flower (to the tip of the lower lip in *Teucrium*); (2) filament length, and (3) style length (from the base of the style to the top of the stigma).

Four flowers were collected per floral stage (0-h flowers = flowers just opening, i.e. floral anthesis; and 3-, 24-, 48- and 72-h-old flowers = 3, 24, 48 and 72 h after anthesis) from 7–8 plants belonging to each plant type in 1998. Floral characters as above were measured in each flower.

Pollen viability and stigma receptivity

To assess pollen viability, via the MTT method, which stains viable pollen but not dead or aborted pollen (Rodríguez-Riaño and Dafni, 2000), pollen from the four flowers was mixed and five random samples of at least 100 pollen grains per sample were counted. All samples were taken at 0800 h and brought after 10 min to the laboratory for examination, except the sample taken 3–4 h after the flower had opened. Stigma receptivity was measured as peroxidase activity using Peroxtestmo KO paper (Dafni and Maués, 1998). Pollen viability in flowers from MS plants (which had no pollen at all) was counted as total abortion. Stigma receptivity was scored as 0 when there was no stain, 1 when only the stigma tip was stained blue, 2 when half the superior part was stained and 3 when the entire stigma was stained.

Statistical analysis

To analyse the effect of age on the size increment of the different floral characters a univariate repeated-measures analysis of variance was used. To analyse differences in final size of plant flower characters, among plant types, a nested ANOVA was used with plants nested within plant types (Sokal and Rohlf, 1981).

RESULTS

Plant types

Three plant types can be distinguished in the studied population for both species (Tables 1 and 2), i.e. those with female or male sterile flowers (MS) that produced no pollen at all, those with intermediate flowers (INT) with a very high percentage of pollen abortion at anthesis (*T. capitatum*, mean = 84.3 %, range = 59–99 %; *O. syriacum*, mean = 64.6 %, range = 22–100 %) and those with hermaphrodite or male fertile flowers (MF) with a very low percentage

TABLE 1. *Floral characteristics (mean $\pm$ s.e.) in the three different flower types of *Teucrium capitatum**

Floral age	<i>n</i>	Flower length (mm)	Filament length (mm)		Style length (mm)	Aborted pollen (%)
			Long stamens (mm)	Short stamens (mm)		
Male sterile plants (MS)						
0 h old	7	7.15 ± 0.15	2.32 ± 0.09	2.31 ± 0.08	5.89 ± 0.21	no pollen
3 h old	7	—	2.10 ± 0.14	2.05 ± 0.13	6.32 ± 0.10	no pollen
24 h old	7	7.32 ± 0.14	2.32 ± 0.13	2.29 ± 0.12	6.46 ± 0.07	no pollen
48 h old	7	—	—	—	6.58 ± 0.12	no pollen
Final size (<i>n</i>)		7.23 ± 0.10 (14)	2.29 ± 0.07 (21)	2.25 ± 0.07 (21)	6.36 ± 0.07 (28)	
Intermediate plants (INT)						
0 h old	8	8.30 ± 0.08	3.65 ± 0.10	3.35 ± 0.13	6.16 ± 0.12	84.28 ± 2.88
3 h old	8	—	3.76 ± 0.18	3.37 ± 0.10	6.28 ± 0.07	92.58 ± 1.15
24 h old	8	8.16 ± 0.16	3.78 ± 0.20	3.43 ± 0.20	6.42 ± 0.11	92.88 ± 1.09
48 h old	8	—	—	—	6.54 ± 0.14	not found
Final size (<i>n</i>)		8.23 ± 0.09 (16)	3.73 ± 0.09 (24)	3.38 ± 0.08 (24)	6.35 ± 0.06 (32)	
Male fertile plants (MF)						
0 h old	7	8.35 ± 0.12	5.33 ± 0.13	4.34 ± 0.15	6.13 ± 0.09	25.89 ± 4.67
3 h old	7	—	5.27 ± 0.16	4.31 ± 0.12	6.14 ± 0.13	34.51 ± 4.08
24 h old	7	8.38 ± 0.09	5.10 ± 0.07	4.07 ± 0.07	6.35 ± 0.09	37.26 ± 6.95
48 h old	7	—	—	—	6.47 ± 0.09	72.03 ± 3.47
Final size (<i>n</i>)		8.38 ± 0.07 (14)	5.25 ± 0.07 (21)	4.26 ± 0.07 (21)	6.27 ± 0.06 (28)	
Nested ANOVA						
Plant type		<i>F</i> = 67.19***	<i>F</i> = 441.69***	<i>F</i> = 221.80***	<i>F</i> = 0.82 ^{ns}	
Plant (plant type)		<i>F</i> = 1.73 ^{ns}	<i>F</i> = 2.08*	<i>F</i> = 1.82 ^{ns}	<i>F</i> = 0.69 ^{ns}	

MS, female flower without pollen (male sterile); INT, intermediate flower with high degree of aborted pollen; MF, hermaphrodite flower (male fertile). The 'final size' row indicates the arithmetic mean for each column (flower length, filament length and style length). A nested ANOVA was performed using the final size of each floral character.

0 h old, just opening flowers (floral anthesis); 3, 24, 48, 72 h old, 3-, 24-, 48- and 72-h-old flowers after anthesis.

Final size: the last two data values were used for each floral character.

n, Plant number.

ns, not significant; *significant at $P < 0.05$; ***significant at $P < 0.001$.

No pollen, flowers have no pollen at all.

Not found, pollen was not found on anthers.

of pollen abortion at anthesis (*T. capitatum*, mean = 25.9 %, range = 6–45 %; *O. syriacum*, mean = 7.7 %, range = 1–20 %).

Floral characters

Flower size. In *T. capitatum* there was no significant increase in flower size over the lifetime of the flower in any of the plant types, but in *O. syriacum* there was an increase in flower size over the lifetime of the flower ($F = 9.76$, $P < 0.001$). This increase was significant after the first 24 h of life of the flower in the three plant types (Table 2). Final flower size was significantly different between plant types in both species, although there were no significant differences between MF and INT plants in *T. capitatum* (Tables 1 and 2).

Filament length. As with flower size, in *T. capitatum* there was no significant increase in stamen filament length over the life of the flower in any of the plant types. In *O. syriacum* an increase in filament length was observed ($F = 15.11$, $P < 0.001$ for long stamens; $F = 12.13$, $P < 0.001$ for short stamens); these increases were produced during the first 24 h of life of the flower in the MF plants but not in the other two types. Final stamen filament length showed significant differences between plant types

in both species, with lengths being greatest for flowers of the MF plants and smallest for those of the MS plants (Tables 1 and 2).

Anthers. In *T. capitatum*, MF anthers were completely exerted from the corolla and reddish-brown, whereas those from MS flowers were included in the corolla tube and brown. INT anthers varied from being exerted to included, but were similar in colour to the anthers of MS flowers. In *O. syriacum*, MF anthers were completely exerted from the corolla and pink, MS anthers were sub-exserted from the corolla on a short filament and brown in colour, and INT anthers varied both in situation and in colour from exerted to included and from pink to brown.

Style length. In both species, there was a constant increase in style length with flower age (Tables 1 and 2), but this increase was only significant in *O. syriacum* ($F = 68.82$, $P < 0.001$), in which the passage from the male to the female phase was indicated by a significant elongation of the style after the first day (24-h-old flowers, Table 1) and also in the next 24 h in MF flowers. In *O. syriacum*, the increase in style length over the life of the flower was significantly greater in MF plants (mean $\pm$ s.e. 3.37 ± 0.27 mm) than in INT plants (1.46 ± 0.24 mm) and MS plants (1.82 ± 0.30 mm). In *T. capitatum*, however, this increase was not significantly

TABLE 2. Floral characteristics (mean $\pm$ s.e.) in the three different flower types of *Origanum syriacum*

Floral age	<i>n</i>	Flower length (mm)	Filament length (mm)		Style length (mm)	Aborted pollen (%)
			Long stamens (mm)	Short stamens (mm)		
Male-sterile plants (MS)						
0 h old	7	3.98 ± 0.18	0.61 ± 0.08	0.20 ± 0.06	3.12 ± 0.19 ^a	no pollen
3 h old	7	—	—	—	3.25 ± 0.05 ^a	no pollen
24 h old	7	4.27 ± 0.13	0.62 ± 0.14	0.34 ± 0.14	4.24 ± 0.34 ^b	no pollen
48 h old	7	4.44 ± 0.16	0.71 ± 0.07	0.29 ± 0.07	4.84 ± 0.22 ^b	no pollen
Final size	14	4.36 ± 0.10	0.66 ± 0.08	0.32 ± 0.07	4.54 ± 0.20	
Intermediate plants (INT)						
0 h old	7	4.65 ± 0.09	1.39 ± 0.15	1.09 ± 0.15	3.58 ± 0.13 ^a	64.63 ± 11.49
3 h old	7	—	—	—	4.20 ± 0.05 ^a	64.20 ± 11.54
24 h old	7	4.88 ± 0.16	1.88 ± 0.40	1.43 ± 0.31	4.94 ± 0.21 ^b	88.57 ± 4.06
48 h old	7	4.95 ± 0.04	1.82 ± 0.35	1.43 ± 0.29	5.04 ± 0.24 ^b	not found
Final size	14	4.91 ± 0.08	1.85 ± 0.26	1.43 ± 0.20	4.99 ± 0.15	
Male-fertile plants (MF)						
0 h old	7	4.45 ± 0.12 ^a	2.03 ± 0.11 ^a	1.22 ± 0.07 ^a	2.53 ± 0.14 ^a	7.71 ± 1.58
3 h old	7	—	—	—	2.95 ± 0.10 ^a	9.34 ± 1.87
24 h old	7	5.15 ± 0.18 ^b	2.77 ± 0.10 ^b	2.02 ± 0.15 ^b	4.32 ± 0.23 ^b	17.09 ± 2.99
48 h old	7	5.22 ± 0.19 ^b	2.83 ± 0.06 ^b	1.96 ± 0.16 ^b	5.64 ± 0.20 ^c	32.89 ± 2.73
72 h old	7				5.90 ± 0.20 ^c	not found
Final size	14	5.18 ± 0.13	2.80 ± 0.06	1.99 ± 0.11	5.77 ± 0.14	
Nested ANOVA						
Plant type		<i>F</i> = 52.74***	<i>F</i> = 486.27***	<i>F</i> = 150.71***	<i>F</i> = 13.99***	
Plant (plant type)		<i>F</i> = 5.62***	<i>F</i> = 21.47***	<i>F</i> = 7.60***	<i>F</i> = 1.25 ^{ns}	

MS, female flower without pollen (male sterile); INT, intermediate flower with high degree of aborted pollen; MF, hermaphrodite flower (male fertile). The 'final size' row indicates the arithmetic mean for each column (flower length, filament length and style length). A nested ANOVA was performed using the final size of each floral character.

0 h old, just opening flowers (floral anthesis); 3, 24, 48, 72 h old, 3-, 24-, 48- and 72-h-old flowers after anthesis.

Final size: the last two data values were used for each floral character.

n, Plant number.

ns, not significant; ***significant at $P < 0.001$.

no pollen, flowers have no pollen at all.

not found, pollen was not found on anthers.

different between plants (MF = 0.34 ± 0.13 mm, INT = 0.26 ± 0.17 mm, MS = 0.49 ± 0.31 mm; Table 1). In *O. syriacum*, final style size was significantly different between the three flower types (Table 2).

Stigma receptivity and pollen viability

Stigma receptivity increased constantly with flower age after anthesis (0-h flowers) in each plant type and in both species (Fig. 1). Peak receptivity occurred in the last 2 d of the flower's life, except in MS plants of *T. capitatum*, which were especially receptive 3 h after anthesis. The degree of receptivity was nil or practically nil in MF plants during the first 24 h of life of the flower, whereas in flowers from INT plants the female phase began gradually 3 h after floral anthesis.

In both species, the temporal evolution of the degree of stigma receptivity increased over the lifetime of the flower, following a logarithmic curve in the MS plants [*T. capitatum*, $r^2 = 0.71$, $P < 0.001$ (Fig. 1A); *O. syriacum*, $r^2 = 0.94$, $P < 0.001$ (Fig. 1D)], with a rapid increase in the first day to almost total receptivity, and a dampening of this increase in the second day. The curve was even steeper in the MS plants of *T. capitatum*,

in which nearly 90 % of total receptivity was reached at 3 h after anthesis (Fig. 1A). In INT flowers, the development of the degree of stigma receptivity was logarithmic with a slight tendency to a linear model in *Teucrium* ($r^2 = 0.91$, $P < 0.001$, Fig. 1B) and almost linear in *Origanum* ($r^2 = 0.95$, $P < 0.001$, Fig. 1E). MF flowers, however, followed an exponential curve in *T. capitatum* ($r^2 = 0.88$, $P < 0.001$, Fig. 1C) and a cubic model in *O. syriacum* ($r^2 = 0.99$, $P < 0.001$, Fig. 1F), with only a small or nil increase in receptivity observed in the first stages of the flower (24-h-old flowers), followed by a continuous increase in the degree of receptivity (48-h-old flowers), again damping out at the end of this increase.

Pollen viability declined steadily with flower age in both species, following a cubic model except for MF flowers in *O. syriacum*, which followed an exponential model (Fig. 1B, C, E, F). This decline was greater in the first 24 h of flower life for the INT plants than that for the MF plants. MS flowers had no pollen at all.

The increase in style length with flower age was positive and significantly correlated with stigma receptivity development in each flower type of *O. syriacum*, but was significant only in MS flowers of *T. capitatum* (Table 3). At the same time, stigma receptivity development over the life

FIG. 1. Style length (mm), degree of stigma receptivity and pollen viability (mean $\pm$ s.e.) over the life span of three flower types in *Teucrium capitatum* (A–C) and *Origanum syriacum* (D–F). Stigma receptivity was scored according to the degree of stigma staining (0, no stain; 1, stain only at the tip; 2, stain upper half; 3, total stain). MS, female flower without pollen (male sterile); INT, intermediate flower with high degree of aborted pollen; MF, hermaphrodite flower (male fertile).

span of the flower was negative and significantly correlated with pollen viability (Table 3), except in MS flowers, which have no pollen at all. Style length and pollen viability were only negative and significantly correlated in MF flowers of *O. syriacum*.

Protandry

In *T. capitatum*, MF flowers are protandrous and the style is initially curved inwards away from the area of pollen presentation, avoiding contact with the anthers.

The stigmatic branches are unequal, practically closed, and during this period the flower is totally male and sheds pollen (Fig. 2A). After 24 h, the style begins to move into position, becoming erect and starting to spread its stigmatic lobes, coinciding with wilting of the stamens (Fig. 2B). MS flowers show neither protandry nor an initially curved style, but they are totally predisposed from the beginning to receive pollen from another plant. INT flowers are protandrous, but their male phase does not last very long (usually less than 24 h). The inward curvature of the style in INT flowers is less pronounced than

TABLE 3. Correlation coefficients among style length, stigma receptivity and pollen viability in *Teucrium capitatum* and *Origanum syriacum*

	Stigmatic receptivity	Style length
<i>Teucrium capitatum</i>		
MS plants		
Style length	$r = 0.60^{***}$	
INT plants		
Style length	$r = 0.30$ ns	
Pollen viability	$r = -0.71^{***}$	$r = -0.36$ ns
MF plants		
Style length	$r = 0.36$ ns	
Pollen viability	$r = -0.71^{***}$	$r = -0.10$ ns
<i>Origanum syriacum</i>		
MS plants		
Style length	$r = 0.67^{***}$	
INT plants		
Style length	$r = 0.68^{***}$	
Pollen viability	$r = -0.60^{***}$	$r = -0.37$ ns
MF plants		
Style length	$r = 0.85^{***}$	
Pollen viability	$r = -0.79^{***}$	$r = -0.74^{***}$

*** $P < 0.001$; ns, not significant ($P > 0.05$).

in MF flowers, and some INT flowers have a style that is almost totally erect (flowers with high levels of anther abortion).

In *O. syriacum*, flowers from the different plant types are protandrous, except those from MS plants (no protandry), and the style is erect but situated below the level of the anthers while the stigma branches are equal and closed.

During this period, the flower is totally male and sheds pollen (Fig. 2C). While reaching female sexual maturity, the style increases in size and the stigma branches separate. These two processes take place continuously during the flower’s lifetime and coincide with wilting of the stamens, moving outwards towards the exterior of the flower (Fig. 2D).

Both species presented asynchronous dichogamy, i.e. there was no synchrony between the male and female phases of flowers of a given plant (definition of Lloyd and Webb, 1986).

DISCUSSION

Plant types

In the definition of gynodioecy, the presence of plants with different degrees of stamen abortion (INT plants) should be taken into account (Vaarama and Jääskeläinen, 1967; Dulberger, 1984). In the two species studied herein, this third type of plant is obvious because, although MS plants present no pollen at all, the flowers from INT plants do possess pollen, even though the percentage of aborted pollen is high or even total, and they show a distinct development of stigma receptivity over the lifetime of the flower. Although these differences are less marked when compared with MF plants, they are nevertheless sufficient for the plants to be considered a different type. Thus, the duration of the male phase is shorter in flowers from INT plants (less than 24 h) than in flowers from MF plants (24 h in *Teucrium* and 48 h in *Origanum*), and the proportion of aborted pollen in these periods in flowers from

FIG. 2. Floral diagram of MF flowers of *Teucrium capitatum* (A, B; side-view) and *Origanum syriacum* (C, D; side-view). ♂ = Male phase and ♀ = female phase.

INT plants is almost four and six times greater, respectively, in *Teucrium* and *Origanum* than in flowers from MF plants.

The presence of INT plants in the studied populations of both species could be due to the degeneration of one of the stages of microsporogenesis, which occurs at later stages in the INT plants than in the MS plants. Hidalgo *et al.* (1999) suggested this for the different degrees of pollen abortion in INT and MS plants for *Rosmarinus officinalis*, where it is due to a failure of one of the stages in the development of microsporogenesis: in the MS plants the failure occurs before meiosis takes place and later during the vacuolate stage in INT plants. The presence of INT flowers possibly indicates that male sterility is a continuous process and it would be considered as a previous step towards total male sterility and not as temporal gynodioecy as in *Rosmarinus officinalis* (Ubera-Jiménez & Hidalgo-Fernández, 1992).

Floral characters

The reduction of floral characteristics, such as the flower size, style length and calyx size, is associated with male sterility (e.g. Darwin, 1896; García and Muñoz, 1988; Roiz and Dulberger, 1989; Ågren and Willson, 1991; Gibson and Diggle, 1997; Puterbaugh *et al.*, 1997; Eckhart, 1999; Hong and Moon, 2003). Similarly to what Hong and Moon (2003) observed in *Lycopus maackianus*, here a reduction of floral, staminal filaments and style size associated with male sterility was seen in *O. syriacum*, i.e. the smaller the flower, style and staminal filaments, the greater the degree of pollen abortion, with the smallest flowers presenting no pollen (MS flowers). *T. capitatum* revealed a different pattern: floral size and staminal filaments depended on male sterility, as in *O. syriacum*, but style length was not different, as Kesseli and Jain (1984) found in *Limnanthes douglasii*, i.e. petals, sepals and stamens were significantly greater in male-fertile flowers than in male-sterile flowers, but showed no differences in style length. By contrast, Delph and Lively (1992) found no difference in floral size between hermaphroditic and female flowers in *Hebe stricta*. Owens and Ubera-Jiménez (1992) found that the flower size of females was larger than that of hermaphrodites in *Lycopus europaeus*, while Delph (1996) reported the opposite results. Female flowers have longer styles than hermaphrodites in *Plantago maritima* (Dinnéztz, 1997) and *Eritrichum aretioides* (Puterbaugh *et al.*, 1997).

Protandry: pollen viability and stigma receptivity

In protandrous self-compatible species, a natural behaviour to reinforce each of the two stages – male and female phases – predicts a steady increase in stigma receptivity and decrease in pollen viability over the lifetime of the flower. Together with the reduction in the number of pollen grains per flower in later stages of flower development, this probably guards more effectively against possible self-pollination. Yet Roiz and Dulberger (1989) did not reject the possibility of spontaneous self-pollination on the second day of anthesis in the flowers from MF plants in *T. capitatum*. Such behaviour is not typical, however;

Navarro (1997) pointed out in *Salvia verbenaca* that the relationship between flower age and pollen germinability was the opposite of what one would have expected, with the time of greatest pollen germinability coinciding with the moment of maximum stigma receptivity (*S. verbenaca* is in part cleistogamous, so the result is not unexpected). This could be an adaptation to allow self-fertilization when cross-pollination fails. The results from Navarro (1997) and the present study support the hypothesis that the occurrence or non-occurrence of selfing in protandrous species is more dependent on pollen germinability patterns than on temporal separation of the stamen dehiscence and stylar elongation phases.

MF flowers of protandrous species have two successive problems: self-pollination and pollen–stigma interference. While pollen is presented, the stigma is not receptive (not exposed, stigma branches closed), so it cannot receive pollen in an effective way. At this moment, the female phase could obstruct pollen export from the anthers, so it must be avoided. *Teucrium* and *Origanum* MF flowers solve both these problems in two different ways.

To avoid self-pollination, both species follow the same model, presence of dichogamy, which is characterized by a negative correlation between stigma receptivity and pollen viability over the life span of the flower, although the presence in both species of asynchronous dichogamy (definition of Lloyd and Webb, 1986) cannot prevent pollination of the flower by pollen from another flower on the same plant (geitonogamy of Proctor *et al.*, 1996) but they follow two different models in order to avoid pollen–stigma interference.

The first model, ‘style movement’ (Lloyd & Webb, 1986), is present in *T. capitatum* and is characterized by the absence of any kind of correlation between style length and stigma receptivity over the life span of the flower. In this model, the style is always separated from the anthers to avoid pollen discounting so that, to achieve the correct position to receive pollen at the female phase, only a style movement of almost 180° is required and not a significant increase in length. This feature is associated with nototribic pollination in the subfamily Ajugoidae in which the upper lip is absent and allows free style movement (van der Pijl, 1972). Pollen always contacts the same part of the insect’s body, so it is advantageous for plants to keep the style in a more or less constant position to optimize the position of the stigma to receive pollen. In probability, in *T. capitatum*, the final style length is a characteristic that depends on the type of pollination, rather than being associated with male sterility.

The second model, ‘style elongation’ (Lloyd & Webb, 1986), is present in *O. syriacum* and is characterized by a negative correlation between style length and stigma receptivity over the life span of the flower. In this model the style is in a correct position to receive the pollen, but at a level below that of the anthers, so that to be able to attain a correct position to receive pollen from the body of an insect there has to be a significant elongation of the style but without movement. Only in this way does the style have a chance to make contact with the ventral part of the insect’s body (sternotribic pollination) without interference

from other parts of the flower (e.g. the corolla) when it lands on the upper part of the corolla. This would mean that in order to avoid such interference, the style needs to surpass in length those flower characters likely to interfere with a correct reception of pollen by the stigma and thus depends on the size of these other flower characters (e.g. corolla and anthers). In other words, because larger flower characters demand longer styles, style length may be associated with male sterility.

By contrast, pollen–stigma interference (pollen discounting) of homogamous plants is avoided by herkogamy (separation of stigma receptivity and pollen release within a flower in space; Webb and Lloyd, 1986). That has been shown to provide a good model in *Mimulus aurantiacus* (Fetscher, 2001).

Gynodioecy can be grouped into two groups, stable and unstable (Ross, 1978; Bawa, 1980). In the former, male sterility is usually controlled cytoplasmically or nuclear-cytoplasmically (Lewis, 1941); this stable gynodioecy can be considered as a well-established breeding system, and not an intermediate step in the evolution of dioecy, in such families as the Lamiaceae, Asteraceae and Dipsacaceae (Richards, 1986). In the latter, the control is nuclear; in this case, the gynodioecy can represent an intermediate condition towards the evolution of dioecy. However, male sterility is not always under genetic control; flowers that are produced abnormally early or late in a hermaphrodite flowering season are frequently male sterile, but this condition is not inherited (Richards, 1986). Atlan *et al.* (1992) pointed out that in the self-compatible gynodioecious *Thymus vulgaris*, inbreeding depression yields an accelerating gain curve for female function and hence selecting for male sterility. The data presented herein show a relatively high frequency of female plants, at a ratio of about 1:0.5:1 (MF:INT:MS) in both species and thus agree with this view. Roiz and Dulberger (1989) also found that hermaphrodite and female plants occurred in a 1:1 ratio. They attributed the high frequency of females mainly to the superiority of out-crossed progeny rather than to higher fecundity. This suggests cytoplasmatic rather than nuclear inheritance. The presence of stable gynodioecy can be considered as a well-established breeding system, as was suggested by Richards (1986) for most species of the Lamiaceae, rather than a first stage in the evolution from hermaphroditism to dioecy, via subdioecy (Ross, 1980). Stable gynodioecy has been established in other species of Lamiaceae (Thompson *et al.*, 1998; Manicacci *et al.*, 1998), which also show high female frequencies and cytoplasmatic inheritance.

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