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Reproductive biology and hybridization of the species of *Asphodelus* sect. *Verinea* (Pomel) Baker (Asphodelaceae)

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Abstract

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Reproductive biology and hybridization of the tetraploid *A. fistulosus* L. and the diploids *A. tenuifolius* Cav. and *A. ayardii* Jahand. & Maire are studied. In the three species flowering occurs in spring, and a high number of flowers open per plant a day. In *A. fistulosus* and *A. tenuifolius* the stigma rest between the anthers, while in *A. ayardii* the style is 3–4 mm longer than stamens, which may favours outcross pollination. Stigmas may receive pollen along the day, but pollen grains germinate only early in the evening. Pollen to ovule ratio, nectar secretion and breeding system indicates that there is a high degree of self-compatibility in the three species. However, the presence of pollen vectors is absolutely necessary for seed production in *A. ayardii* which behaves as an allogamous plant. *A. tenuifolius*, and *A. fistulosus* behave as autogamous plants, with high level of fructification in controlled conditions of pollination. Suggestions about the relationships among the three species are given on account of the reproductive features. Artificial hybridization was carried out between *A. tenuifolius* and *A. ayardii*, but in none of the crosses any mature fruit was obtained.

Key words: *Asphodelus*, Asphodelaceae, breeding systems, self-incompatibility, pollen, nectar, hybridization.

Introduction

Asphodelus sect. *Verinea* (Pomel) Maire includes a tetraploid annual to triennial species (2n=56), *A. fistulosus* L., and two diploid species (2n=28), one annual, *A. tenuifolius* Cav., and another perennial, *A. ayardii* Jahand. & Maire (= *A. cirerae* Sennen).

Recent detailed morphological, karyological, palynological and electrophoretic studies on the group have been published Ruíz-Rejón et al. (1990) and Díaz-Lifante (1991). However, the exact relationships between these three species have not been established yet, although some suggestions have been made. As Stace (1989) stated, a detailed knowledge of the breeding system is essential for a complete taxonomic understanding

of any group. In this context, a study of the breeding system and hybridization capacity of *A. fistulosus, A. tenuifolius* and *A. ayardii* has been undertaken; the results are presented in this paper.

Stebbins (1957), Solbrig (1976) and Cruden (1977) pointed out the adaptative advantages of allogamy and autogamy. Allogamous plants show a higher adaptative capacity to environmental changes, on account of a higher heterozigosity and a broader genetic flexibility. But autogamy can be adaptative in variable and extreme environmental conditions, as well as in disturbed habitats, and consequently, this is the most frequent breeding system in colonizing plants (Baker 1974).

Morphological floral traits and floral development are in accordance with the breeding system of the plants concerned (Ornduff 1969). In this sense Primack (1985) pointed out that floral longevity and the number of flowers simultaneously opened per plant, are very important factors to be considered in reproduction, because they define the length of floral exposition to pollinators and the possibility of cross pollination. There are many reports on the existence of this correlation, as that of *Arenaria uniflora* (Wyatt 1984), *Trycirtis* (Takahashi 1987), and *Erysimum* (Nieto 1991). A decrease in flower size or inflorescence has been related to an evolution towards autogamy in *Leavenworthia* (Rollins 1963), and in many Compositae (Gibbs et al. 1975, Mejías 1992). The pollen to ovule ratio has been considered by Cruden (1977) as a good indicative parameter of the breeding system. In addition, quantities and patterns of nectar production are important factors in relation to the activity of the pollinators (Cruden et al. 1983).

There are several publications related to the reproductive biology of genus *Asphodelus*. Observations on floral biology were reported by Loew and Kirschner (1911) and Obeso and Villalba (1991) in *A. albus* Miller, and by Kugler (1977), Pantis and Stamou (1991) and Schuster et al. (1993) in *A. ramosus* L. (sub *A. aestivus* Brot.). A new contribution to the knowledge of the reproductive biology of the genus is provided in the present study on species of *Asphodelus* sect. *Verinea*.

Material and methods

Plants from 32 natural populations were used. The plants were transplanted to pots and grown to flowering in the experimental garden of the Departamento de Biología Vegetal y Ecología, Universidad de Sevilla. Some observations were carried out in the wild. The origin of the populations and voucher specimens, kept in the herbarium of this Department (SEV), are listed in Table 1.

Flowering process

Observations on the floral phenology were made in the field, and the number of flowers opened per day and inflorescence were counted. The life-span and the opening pattern of flowers were observed, as well as the time of dehiscence of anthers and pollen exposition. Forty flowers, one or two per plant, were observed for this purpose and several records were made every thirty minutes from the opening of flower to midday.

To determine the moment when the stigma becomes receptive, hand-pollinations were carried out in *A. fistulosus* and *A. ayardii*. Flowers of inflorescences, kept in containers with water since their collection, were used the following day. For *A. ayardii* and *A. fistulosus*, several styles from flowers pollinated at midday were collected at three different times in the afternoon-evening (A1–A3 in Table 3). For *A. ayardii*, all the styles were collected at the end of the evening, but the flowers were pollinated at three different times of the day (B1–B3 in Table 3). In the controls for both sets of experiments, stigmas were not hand-pollinated. The styles were fixed in formalin-acetic-ethanol (F.A.A., Sass 1940). To observe the pollen grains attached to the stigmas, the styles were squashed

Tab. 1. Origin of populations studied.

Population	Location	Experiments *
Asphodelus ayardii Jahand. & Maire		
AYA-1	Spain, Almería, between Gádor and Benahadux (SEV 133010)	FB, N
AYA-2	Spain, Almería, Vélez Rubio, cross-road to Taberno (SEV 127518)	FB, P:O
AYA-3	Spain, Almería, Vera, Los Gallardos (SEV 129707)	P:O, N, BS, H
AYA-4	Spain, Málaga, Cala del Moral, arroyoTotalán (SEV 133011)	P:O, N
AYA-5	Spain, Murcia, Almudena, Casas de D. Gonzalo (SEV 129706)	BS, H
AYA-6	Spain, Murcia, Jumilla, Finca La Esperanza (SEV 127524)	FB, BS
AYA-7	Spain, Murcia, Totana (SEV 127507)	FB, BS, H
AYA-8	Spain, Murcia, Ulea (SEV 129716)	BS, H
AYA-9	Spain, Toledo, Mora de Toledo (SEV 127512)	P:O
AYA-10	Morocco, Nador, Mont Arroui (SEV 127520)	P:O
AYA-11	Morocco, Nador, Selouane (SEV 133012)	N
A. fistulosus L.		
FIS-1	Spain, Albacete, Hellín (SEV 128136)	P:O, N
FIS-2	Spain, Almería, Almócita (SEV 133018)	FB, P:O
FIS-3	Spain, Huelva, road Sevilla-Huelva (SEV 127351)	BS
FIS-4	Spain, Málaga, Riogordo	N
FIS-5	Spain, Murcia, Jumilla	P:O, N, BS
FIS-6	Spain, Murcia, between Jumilla and Venta del Olivo (SEV 129711)	BS, H
FIS-7	Spain, Murcia, FFCC Blanca (SEV 127386)	BS, H
FIS-8	Spain, Murcia, Totana	FB
FIS-9	Spain, Sevilla, Alcalá de Guadaira (SEV 128134)	P:O, N
FIS-10	Spain, Sevilla, Sanlúcar la Mayor (SEV 129704)	FB, P:O, N, BS, H
A. tenuifolius Cav.		
TEN-1	Spain, Almería, Benahadux	N
TEN-2	Spain, Almería, between Gádor and Benahadux (SEV 129703)	FB, N
TEN-3	Spain, Almería, between H. Overa and P. Lumbreras (SEV 129709)	FB, P, N, BS, H
TEN-4	Spain, Almería, Urcal (SEV 127503)	BS
TEN-5	Spain, Almería, between Venta de Yesos and Tabernas (SEV 127492)	H
TEN-6	Spain, Murcia, Totana (SEV 127487)	BS
TEN-7	Spain, Murcia, between Totana and Lorca, "La Hoya" (SEV 127479)	H
TEN-8	Spain, Granada, Almuñécar, Arroyo de la Miel (SEV 127493)	P:O
TEN-9	Spain, Málaga, Cala del Moral, arroyo Totalán (SEV 133022)	P:O
TEN-10	Spain, Murcia, Puerto Lumbreras (SEV 129708)	FB, P:O, N, BS, H
TEN-11	Morocco, Nador (SEV 127501)	P:O

* Experiments carried out in each population. (FB, floral biology; P:O, pollen-ovule ratio; N, nectar; BS, breeding systems; H, hybridization).

on a slide, in a solution of lactic acid with some drops of cotton-blue, and gently heated at 60 °C. The number of pollen grains attached to the stigma in the phase of the germination was counted. In *A. fistulosus* stigmatic receptivity was also tested following Galen et al. (1985). This experiment is based on the peroxidase activity present in the receptive stigmas.

Pollen to ovule ratio (P:O)

To estimate the total number of pollen grains produced per flower, flower buds from plants grown in the wild and fixed in F.A.A. were used. The P:O ratio was determine for five populations of each species. In *Asphodelus*, which always has six ovules and six stamens per flower, the P:O ratio is simply the number of pollen grains present in an anther.

The number of pollen grains per anther was lower than 1000. The anthers were dissected in the two thecas, the content of one theca was spread on a drop of water in a stripped slide and all the grains were counted under the microscope. The number of pollen grains in the thecas of outer and inner stamens was recorded separately. The P:O ratio for each population was calculated as the addition of the mean number of pollen grains of two thecas, from a same flower, one from the inner and another from the outer whorl.

Nectar production

Nectar produced by flower was studied in inflorescences from natural populations. They were cut and put in containers with water. Measurements were taken one day after they were collected, on flowers starting to wither, when the nectar accumulation is maximum. Volume of nectar was measured by using micropipettes of 1.5 µl, which were applied to the opening of the septal nectaries, to collect nectar which accumulated as a viscose drop. Sugar concentration was measured as sucrose equivalent in % w/w with a temperature-compensated hand-refractometer K-Fuji 91463 (0–32%). Sugar amount was calculated in mg as the product of volume (in µl) and concentration (in w/w).

Breeding system

Tests of open pollination, controlled pollination and hand-self pollination were performed. Plants collected in the field, and cultivated in the experimental garden were used. To test the possibility of natural self-pollination, plants were caged, to prevent the access of insects. To test incompatibility, flowers of caged plants were hand-self pollinated by rubbing the stigma with several anthers of the same flower. Fruit and seed set in open pollination was estimated in flowers freely exposed to pollinators in the field.

Reproduction efficiency was evaluated in all cases as the percentage fruit set (Fr/Fl ratio), and also as the percentage seed set (S/O ratio); the product of both parameters (Fr/Fl × S/O) indicates the total percentage of ovules maturing into seeds per plant (pre-emergent reproductive success (PERS), Wiens et al. 1987), an alternative approach to determine relative reproductive success or fecundity.

Hybridization

Flowers of caged plants were emasculated early in the morning, before anthesis. Pollination was carried out at midday, by rubbing the stigma with anthers of several plants of the donor species. Control crossings were made, for which flowers were cross-pollinated with pollen from the same population. Two sets of crossings were carried out. In 1989 the populations crossed were TEN-5, TEN-7, FIS-7, AYA-7 and AYA-8; in 1990, TEN-3, TEN-10, FIS-6, FIS-10, AYA-3 and AYA-5.

Results

Flowering process

Flowering in the three species occurs in spring. *A. tenuifolius* flowers between January and March, with all plants flowering in each population. *A. ayardii* flowers between

Tab. 2. Total number of flowers produced per inflorescence in *Asphodelus tenuifolius* (TEN), *A. fistulosus* (FIS) and *A. ayardii* (AYA). (*n*: number of inflorescences).

Population	Range	Mean	*n*
TEN-2	34–594	330.8	10
TEN-3	73–610	289.4	4
TEN-10	311–632	421.4	8
FIS-2	145–253	208.5	4
FIS-10	124–604	254.3	9
AYA-1	98–212	186.4	10
AYA-2	116–224	168.3	6

February and May. In *A. fistulosus* the height of flowering occurs between January and March, though some plants can flower in December, or after March, even as late as August or September. In *A. ayardii* and *A. fistulosus* the flowering period can extend during a long period due to a sequential production of scapes in a same rhizome.

A plant produces several scapes and several branches per inflorescence. There are 1 to 3 (sometimes 4) flowers opened at the same time on each branch. This results in a high number of flowers opened per plant and day, and a very high number of flowers produced per plant during the flowering period. This implies a great reproductive effort in these species.

The total number of flowers produced per inflorescence has been evaluated in several populations. Results are shown in Table 2. *A. tenuifolius* produces the higher number of flowers per inflorescence, followed by *A. fistulosus* and *A. ayardii.* The results are only tentative, and a higher number of counts should be carried out.

Floral phenology and morphologic floral traits

A. ayardii presents the largest flowers in the group, with perianth segments between 13 and 16.5 mm long. In *A. fistulosus* they are between 8 and 12.5 mm. In *A. tenuifolius* the smallest size is found, between 3 and 7.5 mm. The colour and morphology of flowers is similar in the three species: white slightly stained with pink, with a mean longitudinal brown nerve, though in *A. fistulosus* and *A. tenuifolius* the pink shade of the flowers is somewhat deeper. Pollen is clearly orange in *A. fistulosus* and A. *tenuifolius*, and yellowish-orange in *A. ayardii.*

There is a difference of 1–3 mm in length between the outer and inner stamens, which are the longest. In the anthesis they are in a zygomorphic disposition, with the anthers of outer stamens below those of the inner stamens. In *A. fistulosus* and *A. tenuifolius* the style is approximately as long as the stamens, and the stigma rests between the anthers. This allows self-pollen deposition, by any small vibration of the inflorescence, or when flowers close. In *A. ayardii* the style is always 3–4 mm longer than stamens, which avoids spontaneous self-pollination.

In the three species the opening of flower is simultaneous and synchronized in all the flowers of the same plant. The whole process has been followed in a wild population of *A. fistulosus* from Sevilla (Spain). The previous night the pedicel changes from erect to patent position, and at the same time the tips of the perianth segments begin to separate. At about 5 am the pedicel moves to a patent position and the flowers start to open. Over 7–8 daylight hours, flowers are completely opened.

Dehiscence of anthers takes place in the three species early in the morning. The outer anthers open 15–30 minutes later than the inner. The exact moment of the opening depends on the environmental humidity, and it can be delayed up to noon on rainy days. In normal conditions anthers open between 9:15 and 9:30 am. Observations made in a population of *A. ayardii* from Jumilla (Murcia, Spain) showed that, in a day with a normal activity of pollinators, no pollen was left in the anthers after 4 pm. The closing of the flowers and withering of anthers takes place between 4 and 5 pm in *A. tenuifolius*, between 4 and 7 pm in *A. fistulosus*, and between 6 and 8 pm in *A. ayardii*.

Stigma receptivity

In Table 3 the results are shown obtained from the hand-pollinations carried out in *A. ayardii* and *A. fistulosus*. They were done to determine the beginning of stigma receptivity and pollen germination. The stigma retains the pollen independently of the time at which pollination occurs. In consequence, adhesion of pollen to the stigma can occur at any moment from flower opening. Nevertheless, pollen grains germinate only in the styles collected after 5.30 pm in *A. fistulosus*, and after 7 pm in *A. ayardii*, coincident with the beginning of the flower closing. In *A. fistulosus*, the stigmas of unpollinated flowers used as control had germinating pollen when they were collected, which indicates the existence of spontaneous self-pollination. This did not happen in *A. ayardii*.

Test of stigmatic receptivity corroborated this result; stigmas were not yet receptive at 4 pm but were so by 6.45 pm.

Tab. 3. Stigma receptivity. The number of pollen grains per stigma and the time of pollen germination is given for pollinations made at different hours in plants of *Asphodelus ayardii* and *A. fistulosus* from Totana (Murcia). The number of stigmas (No. stigmas) with 0,1–50 or more than 50 pollen grains, and the number of them belonging to each phase of germination (Ph. germination) is indicated. (A1–A3, stigmas pollinated at about midday and collected at different times in the evening; B1–B3, stigmas pollinated at different times of the day and collected at the end of the evening; C: control; I: no germination; II: pollen tubes begin to protrude; III: pollen tubes grow through the stigma. C: control).

Taxa	Test	No. styles	Pollination [h]	Collection [h]	No. stigmas			Ph. germination		
					0	1–50	>50	I	II	III
A. fistulosus	A1	11	14:00	17:30	0	4	7	5	0	0
	A2	10	14:00	19:00	0	0	10	0	0	10
	A3	11	14:00	21:00	0	0	11	0	0	11
	C	4	–	19:00	0	0	2	0	2	0
A. ayardii	A1	16	11:30	15:30	0	13	3	16	0	0
	A2	15	11:30	19:00	0	9	6	15	0	0
	A3	16	11:30	22:00	0	1	15	0	4	12
	B1	6	11:30	22:00	0	0	6	1	1	4
	B2	8	13:30	22:00	0	1	7	2	1	5
	B3	8	15:30	22:00	0	0	8	0	0	8
	C	4	–	22:00	4	0	0	4	0	0

Tab. 4. Pollen grains per theca (mean values and standard deviation for inner and outer anthers are given) and P:O ratio in *n* flowers from five populations of *Asphodelus tenuifolius* (TEN), *A. fistulosus* (FIS) and *A. ayardii* (AYA). (LA: Length of anthers in mm).

Population	*n*	Pollen grains per theca		P:O	LA
		Outer	Inner		
TEN-3	5	186.3±27.2	202.0±42.7	388.3	1.6
TEN-8	5	170.4±20.4	187.0±15.4	357.4	1.1
TEN-9	6	169.4±50.5	188.0±44.9	357.4	1.4
TEN-10	5	180.2±45.1	187.2±46.2	367.4	1.3
TEN-11	5	161.2±33.5	167.6±37.8	328.8	1.2
FIS-1	5	254.8±24.9	288.2±16.4	543.0	2.1
FIS-2	5	261.4±28.2	310.6±43.1	572.0	2.0
FIS-5	6	232.7±58.1	258.2±30.2	490.8	1.9
FIS-9	7	197.4±42.8	237.3±22.5	434.7	2.2
FIS-10	5	194.4± 9.12	211.4±17.3	405.8	2.3
AYA-2	5	443.8±66.3	487.2±30.7	931.0	2.2
AYA-3	5	389.0±45.4	413.4±42.4	802.4	2.2
AYA-4	5	434.7±89.7	480.4±81.9	915.1	2.4
AYA-9	5	400.2±69.8	456.8±39.7	857.0	2.2
AYA-10	5	635.2±31.9	691.8±54.8	1327.1	2.7

Pollen to ovule ratio

In Table 4 the mean number of pollen grains found per theca in five populations of each species is shown. In the three species the outer whorl has always less pollen grains than the inner, but the differences are not significant. In *A. tenuifolius* the number of pollen grains per theca reaches the lower values, and also the size of the anthers is the smallest. In *A. ayardii* the anthers are the biggest in the group, and the highest values are reached for number of pollen grains per theca. *A. fistulosus* shows intermediate values between the other two species. The P:O ratio shows a mean value for populations of 359.9±21.4 in *A. tenuifolius*, 489.3±70.1 in *A. fistulosus*, and 966.5±207.9 in *A. ayardii*. According to Cruden (1977), ratios for *A. tenuifolius* and *A. fistulosus* show that they can be classified as autogamous facultative to allogamous facultative; *A. ayardii* shows the values expected in allogamous facultative plants.

Nectar secretion

Nectar secretion is continuous through the day, from opening to withering. Parameters of nectar secretion are given in Table 5. *A. tenuifolius* produces very little nectar, with a mean value for the populations studied of 0.09±0.01 mg. *A. ayardii* and *A. fistulosus* produce more nectar, with a amount of sugar of 0.28±0.09 mg in *A. fistulosus* and 0.32±0.12 mg in *A. ayardii*. The volume and concentration show a larger range of variation, because they are subjected to the environmental conditions.

Tab. 5. Nectar production per flower in *Asphodelus tenuifolius* (TEN), *A. fistulosus* (FIS) and *A. ayardii* (AYA). Amount of sugar (mg sugar/flower), volume (µl nectar/flower) and nectar concentration (% w/w) are indicated as mean value for the N flowers studied for each population.

Population	N	Sugar amount	Volume	Concentration
TEN-1	19	0.11±0.02	0.89±0.18	12.87± 2.48
TEN-2	9	0.08±0.01	0.12±0.01	27.50±10.05
TEN-3	12	0.09±0.04	1.05±0.39	9.47± 3.01
TEN-10	10	0.09±0.03	1.02±0.48	10.48± 4.61
FIS-1	14	0.29±0.06	2.10±0.53	13.75± 1.63
FIS-4	13	0.33±0.99	4.01±1.07	8.16± 0.47
FIS-5	25	0.38±0.09	2.16±0.69	18.38± 5.26
FIS-9	25	0.16±0.06	1.12±0.76	17.75± 5.59
FIS-10	15	0.18±0.05	2.08±0.81	8.88± 1.83
AYA-1	30	0.18±0.08	1.10±0.01	15.69± 3.29
AYA-3	20	0.28±0.08	2.09±0.84	14.16± 3.85
AYA-4	24	0.44±0.13	2.65±0.85	17.05± 2.69
AYA-11	11	0.40±0.16	4.77±2.27	10.56± 2.58

Breeding system

In Table 6 the results obtained in the tests of controlled pollination (caged plants) and open pollination are indicated for each population. The results of each plant are grouped per population. In Table 7 mean values for all the populations of each species are given.

In *A. tenuifolius* and *A. fistulosus* fruit set and seed set reach high values both for caged and open pollination conditions. As both species have stamens and style with the same length, the proximity of the anthers to the stigma render self-deposition of pollen possible. In *A. tenuifolius* these values are similar in both conditions, but in *A. fistulosus* values for open conditions are higher. This suggests a more effective mechanism of self-pollination in *A. tenuifolius* than in *A. fistulosus*, probably by a closer proximity between anthers and stigma. In open conditions *A. ayardii* shows similar values to *A. tenuifolius* and *A. fistulosus* but a very low fruit set was obtained in caged inflorescences, with a mean value for PERS between populations of 0.03 (s.d.=0.02). This indicates that the presence of pollen vectors is absolutely neccessary for seed production. In this species, a distance of 3–4 mm between anthers and stigma is sufficient to avoid self-pollination. The occasional seed set obtained in caged conditions corresponds to terminal flowers in the inflorescence, smaller in size and with stigma and anthers closer than in the rest of the flowers, conditions in which self-pollination may occur.

Hand-selfings were successful in the three species, both for fruit set and seed set, indicating that there is a high degree of self-compatibility. *A. fistulosus* and *A. tenuifolius* show higher values for fruit set than *A. ayardii*, but similar values for seed set were obtained in the three species. The mean values for PERS are 0.65 (s.d.=0.23, range=0.38–0.93) in *A. tenuifolius*, 0.66 (s.d.=0.13, range=0.49–0.80) in *A. fistulosus*, and 0.54 (s.d.=0.21, range=0.28–0.58) in *A. ayardii*.

Hybridization

In Table 8 the results of the two sets of crossing carried out in 1989 and 1990 are shown, and the number of plants and flowers used in the experiments are indicated. In

Tab. 6. Fruit and seed set of *Asphodelus tenuifolius* (TEN), *A. fistulosus* (FIS) and *A. ayardii* (AYA) in open pollination (open), in controlled pollination (caged), and hand-self-pollination (forced) conditions. (*n*: number of plants; No. Fl: number of flowers, No. Fr; number of fruits; No. S: number of seeds; Fr/Fl: percentage of flowers which developed fruits; S/O: percentage of ovules which produced seeds; PERS: Preemergent reproductive success (Fr/Fl × S/O).

Population	Test	*n*	No. Fl	No. Fr	No. S	% Fr/Fl	% S/O	PERS
TEN-3	caged	13	735	673	3358	91.56	83.16	0.76
	open	14	272	255	1374	93.75	89.80	0.84
	forced	7	57	49	242	85.96	82.31	0.71
TEN-4	caged	2	74	56	293	75.68	87.20	0.66
	open	1	27	19	110	70.37	96.49	0.68
	forced	8	83	66	303	79.51	76.52	0.61
TEN-6	caged	2	76	62	323	81.58	86.83	0.71
	open	1	81	68	357	83.95	87.50	0.74
	forced	1	27	17	61	62.96	59.80	0.38
TEN-10	caged	7	296	276	1499	93.24	90.52	0.84
	open	6	173	158	887	91.33	93.57	0.85
	forced	4	33	31	185	93.93	99.46	0.93
FIS-3	caged	7	477	373	1559	78.20	69.66	0.55
	open	3	329	245	1185	74.47	80.60	0.60
	forced	2	15	14	52	93.33	61.90	0.58
FIS-5	caged	4	607	405	1675	66.72	68.93	0.46
	open	4	540	429	2099	79.44	81.55	0.65
	forced	4	219	192	866	87.67	75.17	0.66
FIS-6	caged	5	473	384	1648	81.18	71.53	0.58
	open	5	206	192	987	93.20	85.68	0.80
	forced	1	44	44	212	100	80.30	0.80
FIS-7	caged	3	314	190	735	60.51	64.47	0.39
	open	1	25	22	108	88.00	81.82	0.72
	forced	2	44	29	129	65.91	74.14	0.49
FIS-10	caged	8	550	349	1421	63.45	87.86	0.43
	open	8	245	226	1083	92.24	79.87	0.74
	forced	4	42	38	193	90.48	84.65	0.77
AYA-3	caged	11	1271	97	370	7.63	63.57	0.05
	open	9	213	184	992	86.38	89.86	0.78
	forced	4	36	21	110	58.33	87.30	0.51
AYA-5	caged	9	1411	100	344	7.09	57.33	0.04
	open	2	105	69	335	65.71	80.92	0.53
	forced	4	56	39	195	69.64	83.33	0.58
AYA-6	caged	7	120	5	18	4.77	60.00	0.02
	open	6	718	491	1965	68.38	66.70	0.46
	forced	1	51	46	260	90.20	94.20	0.85
AYA-7	caged	9	782	18	57	2.30	52.78	0.01
	open	5	135	102	514	75.56	83.99	0.63
	forced	6	93	34	155	36.56	75.98	0.28
AYA-8	caged	5	540	17	54	3.15	52.94	0.02
	open	2	40	40	207	100	86.25	0.86
	forced	6	125	70	363	56.00	86.43	0.48

Tab. 7. Mean and standard deviation for fruit-set (Fr/Fl), seed-set (S/O) and coefficient PERS of N populations of *Asphodelus tenuifolius*, *A. fistulosus* and *A. ayardii* for self-, open- and hand-self-pollination.

Taxa	N		Self-pollination	Open-pollination	Hand self-pollination
A. tenuifolius	4	Fr/Fl	85.51 ± 8.33	84.85 ± 10.51	80.59 ± 13.15
		S/O	86.92 ± 3.01	91.84 ± 3.92	79.52 ± 16.30
		PERS	0.74 ± 0.08	0.78 ± 0.08	0.65 ± 0.23
A. fistulosus	5	Fr/Fl	70.01 ± 9.16	85.47 ± 8.21	87.48 ± 12.90
		S/O	68.49 ± 2.61	81.90 ± 2.25	75.23 ± 8.56
		PERS	0.48 ± 0.08	0.7 ± 0.08	0.66 ± 0.13
A. ayardii	5	Fr/Fl	4.87 ± 2.38	79.21 ± 14.11	62.15 ± 12.69
		S/O	57.32 ± 4.64	81.54 ± 8.92	85.45 ± 6.62
		PERS	0.03 ± 0.02	0.65 ± 0.17	0.54 ± 0.21

Tab. 8. Crossing carried out between and within species in two sets of experiments made in 1989 and 1990. Number of plants used (pl), total number of flowers (fl) hybridized and coefficient of pre-emergent reproductive success (see text) (in parentheses) are indicated.

		A. tenuifolius	*A. fistulosus*	*A. ayardii*
A. tenuifolius	1989	8 pl, 37 fl (0.78)	4 pl, 83 fl (0)	13 pl, 178 fl (0)
	1990	21 pl, 62 fl (0.78)	18 pl, 152 fl (0)	14 pl, 138 fl (0)
A. fistulosus	1989	3 pl, 39 fl (0)	2 pl, 17 fl (0.55)	3 pl, 43 fl (0)
	1990	9 pl, 93 fl (0)	11 pl, 40 fl (0.86)	10 pl, 68 fl (0)
A. ayardii	1989	12 pl, 168 fl (0)	4 pl, 81 fl (0)	6 pl, 41 fl (0.75)
	1990	14 pl, 96 fl (0)	10 pl, 104 fl (0)	8 pl, 52 fl (0.67)

spite of the high number of interspecific crosses made, none of them produced any mature fruit. The crosses between plants of the same population, which act as control, have resulted in good fruit set, and the values for the coefficient PERS are as they have been expected from the results of the breeding system indicated above.

Discussion

The above results indicate that *A. tenuifolius* is facultatively autogamous. Self-pollination can occur in any phase of the lifespan of the flower. This species shows the smallest flowers in the section, and a shorter life-span, with a position of anthers and stigma which favours self-pollination. In this species autogamy reaches very high values, but cross-pollination is possible and is likely to occur, given that some nectar secretion occurs and that P:O ratio is high for an autogamous species.

In *A. fistulosus* breeding system can be considered as intermediate between facultative autogamy and a facultative allogamy. Spontaneous self-pollination reach high values, but they are increased by open pollination. The secretion of some nectar and a presumably high P:O ratio suggests that cross-pollination is important. Self-pollination may occur in any phase of the life of the flower.

A. ayardii is an outbreeder. The distance between stigmas and anthers prevents self-pollination, and makes pollinators indispensable for sexual reproduction. However self-compatibility is high. All the characters analysed related to morphology, nectar and pollen production and seed set concur with this finding.

Generally *A. fistulosus* shows, for the analyzed characters, intermediate values between those of *A. ayardii* and *A. tenuifolius*: total number of flowers opened per inflorescence, life-span, size of flowers and anthers, P:O ratio and amount of nectar secreted. With respect to the breeding-system, though *A. fistulosus* is clearly a selfer, as is *A. tenuifolius*, an increase of seed-set results from open pollination. The high number of flowers produced by *A. tenuifolius*, as compared with *A. ayardii*, is remarkable and relates to the reproductive effort of this annual species. The flowering time in *A. tenuifolius* is shorter than in the other two species, since it occurs during a shorter period of favourable environmental conditions. *A. ayardii* and *A. fistulosus* extend the flowering by sequential production of new floral stems in the same plant.

In these three species, *A. tenuifolius* represents the maximum specialization to an arid environment, with an annual life cycle and high seed production, which is guaranteed by self-pollination. This is reflected in the evolution of the flower: flowers and anthers are small, their number of pollen grains are few, and nectar secretion is reduced. *A. fistulosus* behaves as a pioneer and colonizer plant, showing the typical characters of this life history (Stebbins 1957, Baker 1974, Richards 1986, Grant 1989). Thus, plants can flower even in August, and produce fruits under these conditions. Self-pollination is a strategy followed by colonizer species, due to the high number of seeds produced by this mechanism (Bocquet 1968, Lefebvre 1970, Baker 1974, Solbrig 1976, Aeschimann 1983, Mulligan and Findlay 1970), which is advantageous in the colonization of homogeneous habitats. In these plants, a certain degree of cross-pollination allows some genetic variability, which eventually provides ecological plasticity (Stebbins 1957). *A. ayardii*, an perennial and allogamous, represents the less evolved taxa, according to the tendencies indicated by Stebbins (1957, 1974).

Autogamy in the group is derived from allogamy, and evidence of it is the persistence in *A. tenuifolius* and *A. fistulosus* of morphologic flower traits that favour cross-pollination, such as a zygomorphic disposition of stamens, some nectar secretion, a high P:O ratio, and delayed pollen germination, which takes place when the flower closes. This last characteristic represents a longer period of exposition of stigma to pollination, a higher possibility to receive foreign pollen and, in consequence, cross-pollination. Delayed stigma receptivity until sunset has also been found in *Gasteria verrucosa* (Asphodelaceae, Alooideae), in which Willemse and Franssen-Verheijen (1986) reported that germination of pollen starts when the perianth withers, five days after anthesis.

The results of this study are in agreement with the broad tendencies accepted by many authors in relation to breeding systems, including the relationships between the breeding system, biological cycle and habit of the plants. Thus perennial species are frequently allogamous and annuals are autogamous. But at the same time breeding systems and biological cycle are often correlated with ploidy level (Ornduff 1969, Gustafsson 1948, Stebbins 1950, 1957 and 1985, Richards 1986). Annual species are frequently autogamous and diploid, sometimes tetraploid. Perennials are often allogamous, with active vegetative reproduction and high levels of ploidy, and their autopolyploid derivatives tend to be allogamous and perennial, too. In relation to the three species studied, here a hypothesis of an amphiploid origin for *A. fistulosus* (annual-biennial autogamous) from *A. tenuifolius* (annual diploid autogamous), and *A. ayardii* (perennial diploid allogamous) can be suggested.

In spite of the high number of crosses made between *A. ayardii* and *A. tenuifolius*, hybrid fruits have not been produced, though control crosses were positive, as could be expected. Nevertheless, a null result in a hybridization experiment does not prove absolutely that hybridization could not occur or that it had not taken place in the past history of the species. Addressing the question in a molecular study would be desirable to confirm the hybrid hypothesis. This investigation is now in progress.

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