

Self-incompatibility, floral parameters, and pollen characterization in the narrow endemic and threatened species *Artemisia granatensis* (Asteraceae)

by

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Abstract

Peñas, J., Lorite, J., Alba-Sánchez, F. & Taisma, M.A. 2011. Self-incompatibility, floral parameters, and pollen characterization in the narrow endemic and threatened species *Artemisia granatensis* (Asteraceae). *Anales Jard. Bot. Madrid* 68(1): 97-105.

Artemisia granatensis Boiss. is a paradigmatic species for plant conservation in Spain and Europe. It is a critically endangered (CR) endemic species growing above 2500 m in the Sierra Nevada (southern Spain). Natural populations have been considerably devastated in the past due to intensive human exploitation for folk medicine. The sparse available data concerning the reproductive biology of this species under natural conditions indicate a low reproductive success. To provide additional information on the reproductive biology of *A. granatensis*, and consequently information useful for the management and conservation of this species, we studied the breeding system through pollen-tube growth. In addition, some floral and pollen traits were recorded. No differences were found between populations in terms of the morphological traits of flowers and inflorescences. *A. granatensis* is an anemophilous species, and the data indicate that pollen transfer may be limited between isolated populations, and so contributing to an extremely low fruit-set. Results show *A. granatensis* is self-incompatible, probably with a sporophytic self-incompatibility system, and with no evidence of partial self-incompatibility. Reproductive traits, related to pollen morphology and settling speed may explain the low rate of recruitment in the small populations separated by geographical barriers.

Keywords: reproductive biology, pollen-tube growth, endemic and threatened species, conservation.

Resumen

Peñas, J., Lorite, J., Alba-Sánchez, F. & Taisma, M.A. 2011. Autoincompatibilidad, parámetros florales y caracterización de polen en la especie endémica y amenazada *Artemisia granatensis* (Asteraceae). *Anales Jard. Bot. Madrid* 68(1): 97-105 (en inglés).

Artemisia granatensis Boiss. es una especie paradigmática en la conservación de flora a nivel español y europeo. Es una especie catalogada como En Peligro Crítico (CR) endémica de Sierra Nevada (sur de España), donde habita por encima de los 2500 m. Las poblaciones naturales han sido casi exterminadas en el pasado debido a una recolección masiva de la especie, utilizada en medicina popular. Los escasos datos disponibles acerca de su biología reproductiva en condiciones naturales indican que existe un bajo éxito reproductivo. Con el objetivo de proporcionar información adicional acerca de la biología reproductiva de *A. granatensis*, útil para la conservación y el manejo de la especie, evaluamos el sistema de compatibilidad a través del crecimiento del tubo polínico. Además se registraron datos sobre algunos rasgos florales y polínicos de la especie. No se encontraron diferencias entre poblaciones en términos de rasgos morfológicos de flores e inflorescencias. *A. granatensis* es una especie anemófila para la cual los datos obtenidos sobre capacidad de dispersión sugieren que la transferencia de polen podría ser difícil entre poblaciones aisladas o muy distanciadas, pudiendo ser un factor más a tener en cuenta entre las causas que provocan un limitado éxito reproductivo y una paupérrima producción de semillas. Los resultados muestran que *A. granatensis* tiene autoincompatibilidad esporofítica sin evidencias de autoincompatibilidad parcial. Los rasgos reproductivos relacionados con la morfología y la velocidad de sedimentación del polen pueden explicar la baja tasa de reclutamiento de poblaciones pequeñas, a veces separadas por barreras geográficas.

Palabras clave: biología reproductiva, crecimiento del tubo polínico, especie endémica y amenazada, conservación.

Introduction

The southern area of the Iberian Peninsula has long been recognized as a centre of plant diversity and endemism (Molero, 1994; Domínguez & al., 1996; Peñas & al., 2005), and a “phytogeographical hotspot”; that is, significant reservoirs of unique genetic diversity favourable to the evolutionary processes of Mediterranean plant species (Médail & Diadema, 2009). The largest number of endemic plant species, and indeed one of the largest in Europe, is found in Sierra Nevada (Blanca & al., 1998). This mountain harbours the narrow endemic *Artemisia granatensis* Boiss. (Asteraceae), a paradigmatic species for the plant conservation in Spain and Europe (Council Directive 92/43/EEC; Fay, 1992), since it was included in the first Spanish catalogue of threatened species (BOE, 1990). Today, this species is considered critically endangered (CR) in the latest national (Moreno, 2008) and regional (Cabezudo & al., 2005) list of threatened species, with the main threats being overgrazing and collection for folk medicine. Estimates of population sizes suggest that about 3000 individuals of the species survive (Blanca & al., 1998), distributed in 12 populations (Blanca, 2002).

Habitat fragmentation leading to small isolated populations may be the most apparent cause for reproductive failure and species loss (Koul & Bhatnagar, 2007). Up to the present, the efforts to recover natural populations of *A. granatensis* have not been based on a knowledge of the reproductive biology of natural populations. Furthermore, the difficult access to the small and distant populations of *A. granatensis* has constrained reproductive studies *in situ*. However, field studies on reproductive traits, compatibility systems, and pollination mechanisms are necessary to define conservation strategies for the species. Reproductive-biology studies, thus, should be an integral feature of any conservation project (Weller, 1994; Weekly & Race, 2001; Koul & Bhatnagar, 2007).

Self-incompatibility, a genetic barrier to prevent inbreeding that is broadly distributed among angiosperms, could be a main constraint against reproductive success in *A. granatensis* because in self-incompatible species there may be a loss of genetic diversity among individuals in fragmented and scattered populations that dooms such isolated populations to extinction (Weller, 1994). Since *A. granatensis*, belonging to Asteraceae, a family with self-incompatibility established in around 40 genera (Charlesworth, 1985), and with some reports on partial self-incompatibility (Ortiz & al., 2006), could have a self-incompatibility system which has not previously been tested.

Some studies have related self-incompatibility systems to floral and inflorescence size (Gibbs & al., 1975; Ortiz & al., 2006). For example, in the genus *Hypochaeris* L. partial self-compatible heads are larger than self-incompatible heads (Ortiz & al., 2006). Thus, the evaluation of a self-incompatibility system must include the flower and inflorescence morphology in order to gain a full understanding of the reproductive potential.

Artemisia is known to be an anemophilous genus (O'Brien, 1980; Watson & al., 2002), and ecological characteristics of in *A. granatensis* populations could hamper the transfer of pollen between distant populations due to geographical barriers. Currently we have no data about the potential *A. granatensis* pollen movement in the atmosphere. To help fill this gap, theoretical data on the settling speed and residence time in air are presented. This information was obtained based on some morphological pollen features. Data about some physical properties of the *A. granatensis* pollen are important building blocks in a model of its pollen movement and, as such, later will be helpful in establishing the main factors influencing the dispersion degree of pollen between *A. granatensis* populations. Consequently, the settling speed of this pollen is fundamental for determining the distance that this particle can be transported in the atmosphere as well as its probability of being deposited on the plants or on the ground (Aylor & al., 2005).

The aim of the study is provide information on the reproductive biology of *A. granatensis*, in relation to pollen features and breeding system, useful for the management and conservation of this species. For this propose, the present study *i*) characterizes the basic pollen morphological and functional parameters and compares floral morphology within and between populations, and *ii*) evaluates the operation presence of a self-incompatibility system by means of hand pollinations and pollen-tube growth.

Material and methods

Study area and selected sites

Sierra Nevada, a mountain range of some 2,100 km² located in SE Spain (37°N, 3°W), has a complex orography, bedrock, and soil composition (e.g. areas with dolomitic soils), and reaches a height of 3482 m. This massif marks the southernmost limit of the influence of the Quaternary glaciations in Europe, when this mountain range was covered with glaciers in areas above around 2,500 m while large areas remained free of permanent ice (Gómez & al., 2001). All these features have contributed to make the Sierra Nevada a

refuge for many plant species during glacial ages (Blanca & al., 1998), thus harbouring isolated populations that have evolved under particular conditions (e.g. soil type or isolated summit areas), and which have encouraged speciation and a high level of endemism (Blondel & Aronson, 1999; Peñas & al., 2005; Thompson, 2005). In fact, the area above 2,000 m contains about 100 endemic or rare taxa. Many of these species are threatened by different factors (Blanca & al., 1998; Bañares & al., 2003), with 38 taxa included on the regional protection list (Blanca & al., 1999). Today most of this entire area lies within National and/or Natural parks. Climatic conditions are typically alpine (with a Mediterranean character), with mean temperatures below 0 °C during winter months and snow cover that can persist up to 8 months in the highest places (occasionally up to 10 months in small, protected areas). See Gómez (2002) for a detailed description of climatic conditions in Sierra Nevada range.

Two populations of *A. granatensis* were selected in the summit area of the Sierra Nevada. The first (AG1 hereafter), at 2790 m on the northern slope, included 45 mature individuals, and the second (AG2 hereafter), at 3045 m on the southern slope, had 55 mature individuals. In both cases there are no other patches or isolated individuals in the surroundings (at least 300 m around). The populations were c. 6 km apart. Because of the difficult access to the populations, the low number of individuals per population, and the use of a semi-extractive sampling design, we limited the sampling effort to the minimum necessary to allow the statistical analysis of the data.

Studied species

Artemisia granatensis Boiss. belongs to the large family of *Asteraceae*. *Artemisia* is the largest genus in the tribe *Anthemideae* and one of the largest in the family, with over 500 species (Martin & al., 2001). Taxonomically, *A. granatensis* is closely related to other alpine species such as *A. splendens* Willd. and *A. umbelliformis* Lam. (Watson & al., 2002), and it occasionally forms a hybrid, *A. × fragosoana* Font Quer (Blanca, 2002) with the latter. *A. granatensis* is a long-lived perennial herb, caespitose and white-sericeous, with stems 5-12 cm long, erect, simple or scarcely branched. Basal leaves are numerous, petiolate, divided with segments of flabellate outline. Flowers are arranged in terminal discoid capitula of 5-8 mm in diameter, with 1-5 capitula per stem. The capitula have external flowers female and internal ones hermaphrodite, being a gynomonocious species. Flowers are actinomorphic, tubulose, dark purple in

colour, with a papillose stigma. Flowering ranges from July to August and fruit ripening (small achene) occur in September. Although information concerning the breeding system of the species is scant, *A. granatensis* is known to be an anemophilous species (e.g. Blanca & al., 1999).

A. granatensis appears in perennial high-mountain pastures on mica-schists, from 2500 m to the highest peaks (above 3400 m). Traditionally, the main threat has been the harvest of complete individuals for medicinal purposes; also ungulates (wild and domestic ones) browse a large percentage of the reproductive stems, despite the production of sesquiterpenes that make the foliage bitter (Watson & al., 2002). The result of these pressures is a major decrease in seed set (decreasing 20-90 % of the total seed set, depending on the population; author's unpublished data).

Inflorescence and flower morphological data

A total of 18 mature inflorescences (heads/capitula) were randomly collected from each population (AG1 and AG2). The heads were dissected under a bifocal magnifying glass and the number of female and hermaphrodite flowers per capitulum was counted. A subsample of 25 mature hermaphrodite flowers were randomly taken from these 18 inflorescences, and stamens, ovary and style length were measured using a digital calliper (± 0.001). Data were analysed by means of one-way ANOVA.

Pollen morphological analysis and settling speed

Samples of 30 anthers (15 per population) from 30 individuals were randomly selected and collected before anthesis and acetolysed using the method of Erdtman (1960) as modified by Hideux (1972), and then mounted in glycer-gelatin for light microscopy. The pollen terminology used is based on Punt & al. (2007). For scanning electron microscopy (SEM), non-acetolysed grains were dehydrated in an alcohol series, pipetted onto a SEM stub in a few drops of 100% alcohol, and allowed to dry. Samples were coated with gold/palladium and examined using a SEM-microscopy. In parallel, the principal features of individual pollen grains (emphasizing diameters i.e. polar axis and equatorial diameter) were measured using a light microscope at a magnification of 400 \times in order to determinate their characteristic dimensions and shapes. Then, the diameters measures (i.e. the major and minor diameters P and E) were used to calculate the volume-equivalent sphere of the pollen grain given by ${}^3PE^2$. Taking into account the latter measure both the "theoretical settling speed" (Fuchs, 1964;

Leith, 1987), as well as, the “theoretical residence time” of *A. granatensis* pollen (see Chatigny & al. (1979) for further information about residence times estimation) assuming dry deposition were calculate.

The settling speed (V_t) of a pollen grain falling in still air at a constant temperature and pressure is described by Fuchs (1964). Taking into account the volume-equivalent diameter (d) of the pollen grains, the V_t of *Artemisia* pollen was calculated based on Stokes’ Law for a sphere (in the absence of electrostatic forces). This equation calculates the settling speed as a function of particle size, particle density, acceleration due to gravity, and the density and viscosity of the air.

Stokes’ Law Equations: solving for settling speed or terminal velocity

$$V_t = \frac{gd^2(\rho_p - \rho_m)}{18\mu}$$

Inputs: acceleration of gravity (g); particle diameter (d); density of particle (ρ_p); density of medium (ρ_m); viscosity of medium (μ).

Pollen-tube growth and compatibility system

Pollinations were achieved by rubbing a dissection needle against the anthers of pollen-bearing flowers and then against the stigmas of outer female flowers of the same head (self-crosses, SC) or the female flowers in the head of a different individual in the same population (intra-population outcrosses, IPC) or against those of a different head in the other population (AG1 and AG2) i.e. inter-population outcrosses (InPC). All hand-pollinated heads were bagged with cellophane bags. Some naturally pollinated heads (no hand-pollinated, and no bagged) were collected to measure natural tube growth (no hand-pollinated NP).

Prior to hand pollination stylar arms were observed with a magnifying glass to ensure that the arms were fully expanded (mature) and had no pollen on them.

We collected five capitula per population and placed individually in cellophane bags in order to use as donors in the inter-population outcrosses (InPC), the time passed between collection and hand-pollination was two to three hours, roughly.

Afterwards all hand-pollinated (SC, IPC, InPC) flowers were collected (24h-48 h after) and fixed in 70% ethanol. After rinsing in distilled water, isolated gynoecia were softened and cleared in 8 mol/l NaOH for 48-72 h. Softened gynoecia were placed in distilled water for at least 1 hour before staining with a 0.1% solution of aniline blue in 0.1 mol/L K₃PO₄ for 12 h (as described in Martin, 1959). Each gynoecium was examined under UV light (range 360-390 nm for selective excitation of DAPI fluorescence) to observe tube growth.

Pollen-tube growth was observed of selfs and crosses at the stigma, and along the style. Pollinations were classified as SI or SC based on whether pollen tubes reached the base of the style or not. Pollen-tube growth was compared using the non-parametric X² test.

Results

Floral morphology (Table 1)

Artemisia granatensis capitula had around 80 flowers each. Heads from plants in AG1 and AG2 populations showed no differences in hermaphrodite and female flower number. There were no significant differences in total flowers/head, hermaphrodite flowers/heads, female flowers/head and natural fruit-set/head between populations, although the natural fruit:flower ratio was higher in AG2. We found no differences between morphological traits of reproductive structures between AG1 and AG2 (Table 2) with the exception of mature stamen length, which was higher for AG2 flowers (Table 2).

Table 1. Mean (SD) of inflorescence traits gathered from *Artemisia granatensis* populations (AG1 and AG2) ($n = 18$ inflorescences).

	AG1	AG2	F-ratio	P
Total flowers/ head	74.0 (19.1)	83.1 (30.3)	F (1, 35) = 0.877	0.356 n.s.
Hermaphrodite flowers/ head	70.7 (18.1)	74.2 (30.5)	F (1, 35) = 0.701	0.408 n.s.
Female flowers/ head	5.4 (3.7)	6.8 (3.7)	F (1, 35) = 0.573	0.454 n.s.
Fruits/ head	0.4 (2.2)	2.1 (1.0)	F (1, 35) = 4.108	0.051 n.s.
Fruit:flower ratio	0	2.29%		

Mean (SD) values for floral traits and fruit set of n.s.: not significant.

Table 2. Mean (SD) values of female and male floral traits of *Artemisia granatensis* flowers at AG1 and AG2 populations ($n = 25$).

	AG1	AG2	Anova Results F	P
Ovary length (mm)	0.88 (0.12)	0.87 (0.09)	F (1,405) = 0.330	0.569 n.s.
Style length (mm)	1.50 (0.32)	1.59 (0.26)	F (1,405) = 1.091	0.302 n.s.
Style arms length (mm)	0.74 (0.12)	0.77 (0.12)	F (1,405) = 0.277	0.601 n.s.
Stamen length (mm)	2.12 (0.16)	2.35 (0.23)	F (1,405) = 5.447	0.035 *

Mean (SD) values for floral traits of * = significant, n.s.: no significant.

Pollen morphology and settling speed

A. granatensis pollen has the typical anemophilous syndrome, based on morphological features and settling speed. It is isopolar, with radial symmetry. In polar view it is circular-lobate (Fig. 1a), and in equatorial view it is circular-elliptical (Fig. 1b). It is spheroidal or prolate-spheroidal, with a P:E ratio of 1.0, small to medium in size, the length of the polar axis (P) being 18-20 (19.2 ± 0.8) μm and the equatorial diameter (E) being 17-22 (19.3 ± 1.3) μm . The pollen grain is trizonocolporate, fossaperturate. The ectoaperture is a colpus 12-16 (13.7 ± 1.6) μm long; the endoaperture is a circular or lalongate porous 1.5-3 (1.9 ± 0.5) μm long. The surface relief of the pollen grain is microechinate, ornamentation of spines shorter than 1 μm , (c. 0.7 μm), showing delicate verrucate surface sculpturing, broader than high and less than 1 μm in diameter (around 0.24 μm).

The resulting volume-equivalent diameter of *A. granatensis* pollen is c. 19.3 μm . Known this measure and under the assumption of a dry deposition as well as a density of 1 g/cm^3 , the theoretical settling speed or terminal velocity (V_t) was calculated at around 1.18 cm/s (or 42.48 m/h); taking into account the above parameter, the resulting theoretical residence time in the atmosphere was 0.6 days (c. 14 h).

Pollen-tube growth and compatibility system

Self-incompatibility was determined based on pollen-tube growth inhibition. The inhibition of incompatible pollen occurred at the stigma surface, where grains either failed to germinate, or the emerging pollen tube was usually inhibited before penetrating the stigma surface. Compatible pollen grains produced pollen tubes growing through the style. Viability tests showing full fluorochromatic reaction demonstrated that *A. granatensis* pollen collected from populations AG1 and AG2 was viable.

Self crosses (SC) produced failed to germinate, or did not penetrate in the stigma both for AG1 and

AG2 plants (Fig. 2), signifying a rejection of self pollination and sporophytic self-incompatibility. The X^2 test showed that SC produced a significantly lower

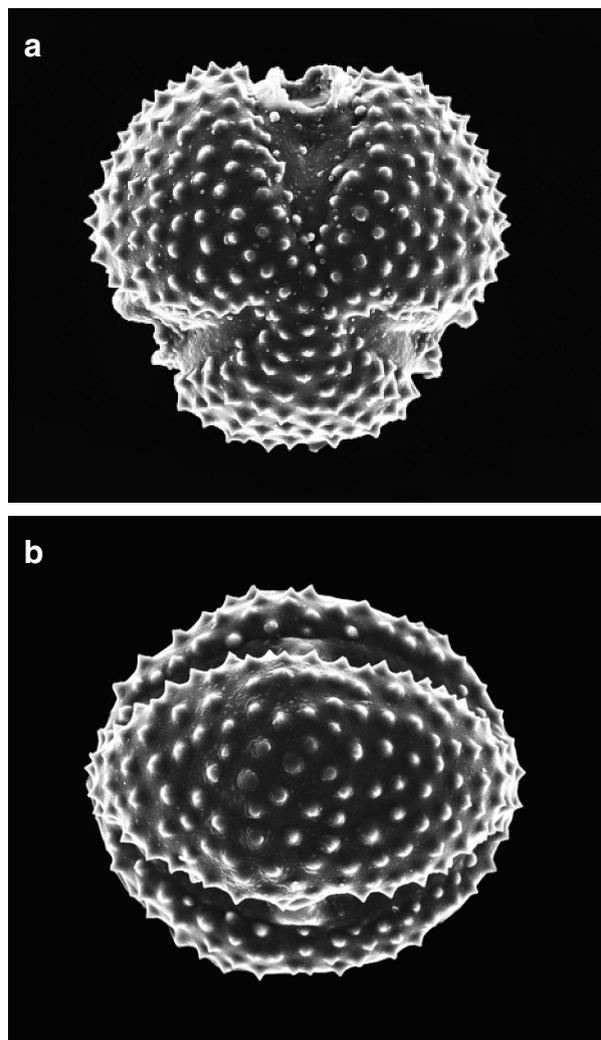


Fig. 1. View of *Artemisia granatensis* pollen grain (SEM microscopy). **a**, whole grain: polar view showing three apertures; **b**, whole grain: meridional view showing two apertures and details of spines.

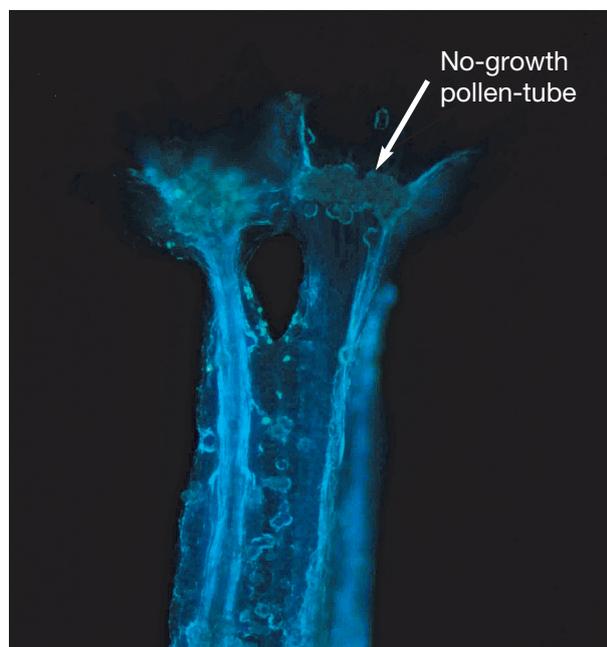


Fig. 2. Inhibition of pollen tube at the stigmas of *Artemisia granatensis* for a SC cross in AG2.

number of gynoecia with pollen tubes than did IPC in both AG1 and AG2 (Table 3).

The number of gynoecia with pollen tubes was higher for IPC than for SC in both AG1 and AG2. (Table 3; Fig. 3). The X^2 test for the comparison of NP and IPC in AG1 and AG2 showed no significant differences with respect to the number of gynoecia with pollen tubes (Table 3). InPC showed a high number of gynoecia with pollen tubes (Table 3; Fig. 4) and also a higher number of gynoecia with pollen tubes than NP in both AG1 and AG2 (Table 3).

Discussion

Artemisia granatensis, as with many *Asteraceae*, has a gynomonocious sexual system, in which female and bisexual flowers occur in the same inflorescence (capitulum). The isolated *A. granatensis* populations studied showed no significant differences between morphological traits. Flower number per head and the ratio of hermaphrodite to female flowers showed no differences between AG1 and AG2. Additionally, stamen, ovary, and style sizes were comparable. Previous reports in some *Asteraceae* showed that flower-size differences could be related to the loss of self-incompatibility in small populations (Ortiz & al., 2006), and this trend has also been found for another self-incompatible species in fragmented habitats (Taisma & Varela, 2005). Our results indicate that AG1 and AG2 plants have no morphological differences and, therefore, these traits could not be related to differential self-incompatibility expression.

Pollen-tube growth after controlled hand pollination in natural populations of *A. granatensis* showed that the species has a sporophytic self-incompatibility system with no evidence of increased self-incompatibility or partial-self incompatibility as has been found in small, isolated and peripheral populations (Fausto & al., 2001; Vallejo-Marin & Uyenoyama, 2004; Taisma & Varela, 2005). These results agree with findings for other members of *Asteraceae* and suggest that, although population size is small and isolation is high, there is no breakdown of the self-incompatibility system. These data support the idea that the main constraint on reproductive success in *A. granatensis* under natural conditions (Fig. 5) is the reduced number of compatible mates due to failure in wind pollination.

Table 3. Results of pollination treatments in *Artemisia granatensis* individuals in populations AG1 and AG2.

Cross Type		n	Number of gynoecia with growing tubes	Number of gynoecia without growing tubes	X^2 critical value (1gl; $p < 0.01 = 6.63$)
AG1	SC	72	0	72	SC vs. IPC = 77.88 *
	IPC	6	6	0	
	InPC	66	56	10	SC vs. InPC = 11.25 *
	NP	29	8	21	InPC vs. NP = 2.03 n.s.
AG2	SC	122	0	122	SC vs. IPC = 20.69 *
	IPC	104	26	78	
	InPC	48	16	32	SC vs. InPC = 33.49 *
	NP	48	7	41	InPC vs. NP = 4.62 n.s.

SC = self crosses, IPC = intra-population outcrosses, InPC = inter-population outcrosses, NP = no hand-pollinated, natural tube growth; n.s.: not significant.

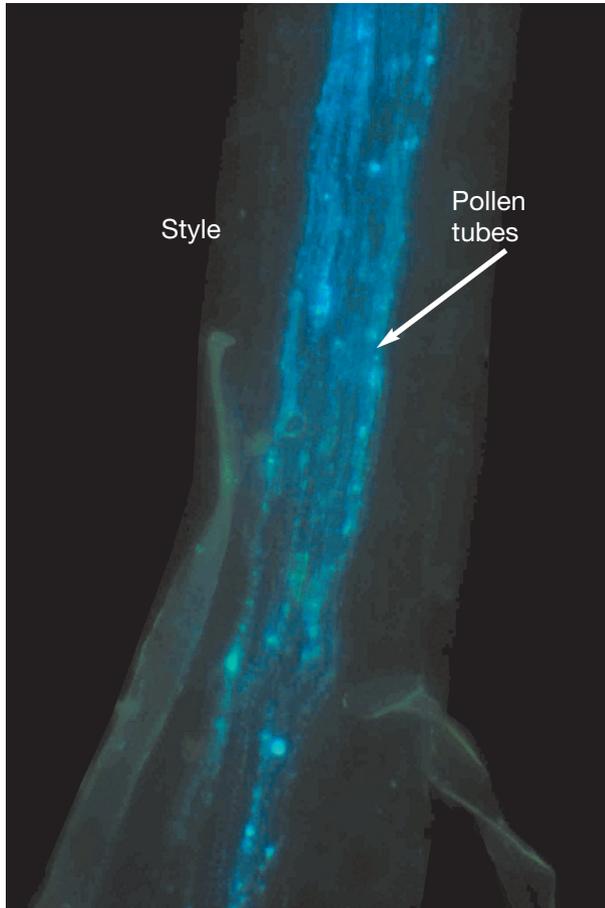


Fig. 3. Growing pollen tubes at the style after a IPC cross in AG1.

Fragmentation of wind-pollinated populations seriously reduces pollen availability, limiting reproduction (Knapp & al., 2001). Davis & al. (2004) found 9-fold more pollen on stigmas of high-density *Spartina alterniflora* plants than on those occurring at low density; they also found that the consequences of loss of appreciable numbers of seed caused by pollen limitation persists for decades.

The low natural fruit set of *A. granatensis* (Hernández-Bermejo & al., 2003) and the low number of gynoecia with viable pollen tubes after NP (natural tube growth) agree with the expected reduced seed-set in small populations of self-incompatible, wind-pollinated species (Widen, 1993, Lienert & Fisher, 2003; Davis & al., 2004). A critical event for *A. granatensis* reproduction could be pollen deposition, because this species is self-incompatible in very small isolated populations, and thus compatible pollen flow could be a critically limiting condition for fruit set. In this sense, further studies addressing pollen limitation in relation with population size and isolation are needed.

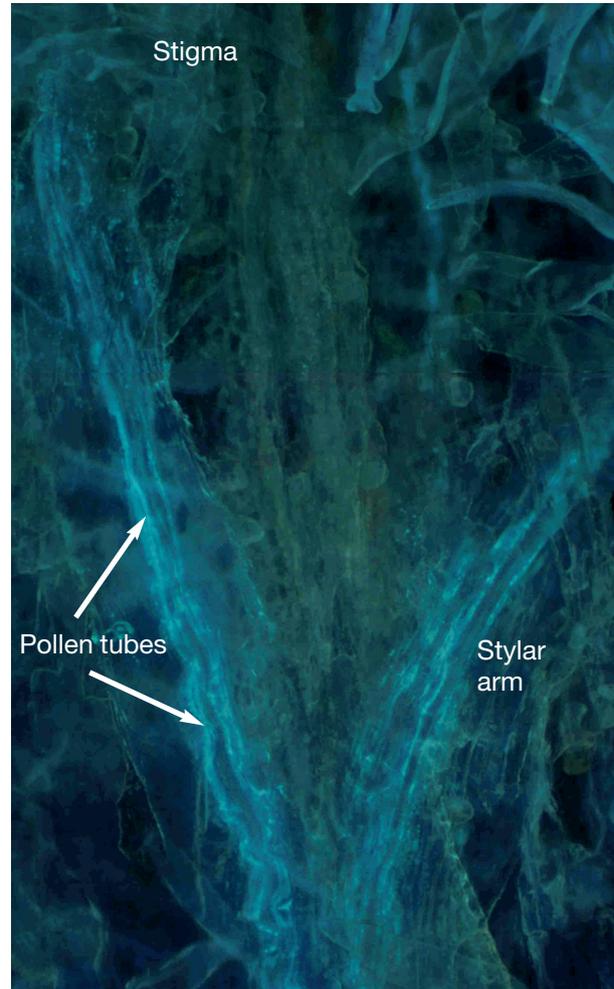


Fig. 4. Growing pollen tubes at the stigma, stylar arms and style after a InPC cross in AG2.

Transport and dispersal of pollen grains by the moving atmosphere as well as their residence time as airborne particles are strongly linked to physical atmospheric characteristics at their time of flight (Comtois & al., 2000). The settling speed and residence time estimated for *A. granatensis* pollen can vary greatly in nature (Sierra Nevada) owing to turbulence and atmospheric humidity, which can alter the density of biological particles (Aylor, 2002); including topographic barriers against pollen displacement. However, a cornerstone parameter in any future model of pollen transport in the atmosphere is calculating the gravitational settling speed in still air, V_t , of individual pollen grains, because V_t largely determines both the distance of travel and the efficiency of deposition on target organs (Aylor & al., 2005). According to Kohler & al. (2007) this theoretical information is an essential parameter for reliably modelling the atmospheric dispersal of pollen *in situ*. Despite this, very little is

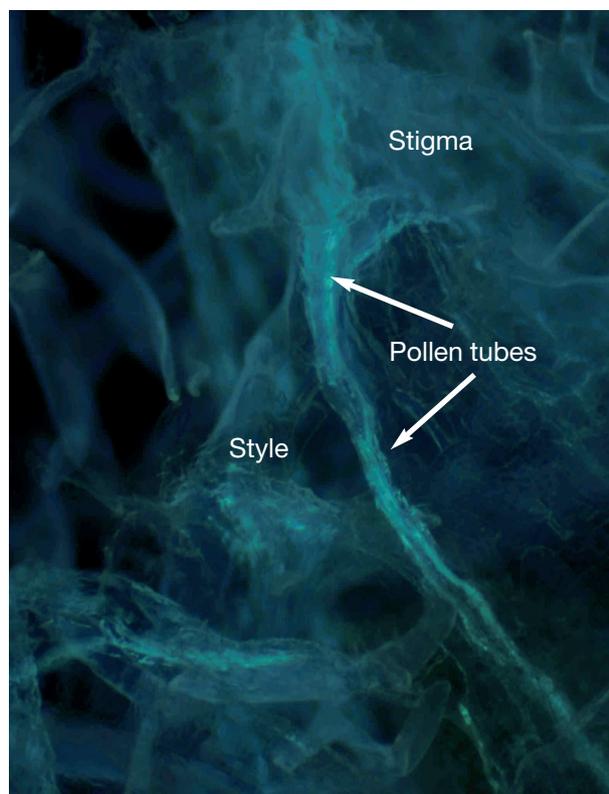


Fig. 5. Growing pollen tubes at the stigma and style level after NP in AG2.

known about *A. granatensis* pollen mobility in the atmosphere. As far as we know, this study is the first to determine physical characteristics of *A. granatensis* pollen, which can contribute to defining the main parameters involved in reproductive biology in this species. The model equations for settling speed presented here offers a means for evaluating dispersal potential for a range of environmental conditions.

Our results suggest the potential distance that *A. granatensis* pollen can be transported during the estimated residence time (14 h) was 600 m. According to Mandrioli (1998) small or medium particles, such as *A. granatensis* pollen, have a relatively long or medium residence time in the atmosphere, supporting the hypothesis of intra- and inter-populational pollination success. Nevertheless, natural pollen-tube growth suggests a pollen-deposition limitation, probably related to the viability and longevity of pollen grain as well as pollen dispersion capacity, both intimately linked to the likelihood of reproductive success (Mandrioli, 1998). These facts suggest that the reproductive success depends on the time spent by the viable pollen grain to reach the nearest population and the inter-population range. Obviously, further details about pollen flow within and between populations is

required for acquiring a thorough knowledge the reproductive biology and pollination ecology in the case of *A. granatensis*.

In any case, it seems obvious that *A. granatensis* reproductive efficiency has been critically affected by the devastation of natural population due to human use. Current population size and plant densities may be a serious limitation to guarantee enough pollen from compatible mates. Additionally, present populations are isolated by major geographic barriers that could also seriously limit compatible pollen flow between populations. Programmes for the recovery of *A. granatensis* may need to include reintroduction of compatible mates in order to enhance pollen flow and fruit-set efficiency by means of connecting isolated patches.

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