

Carolina Torres

## Pollen size evolution: correlation between pollen volume and pistil length in *Asteraceae*

Received: 4 November 1999 / Revision accepted: 14 February 2000

**Abstract** Based on the assumptions that pollen tube length is predetermined by provisions in the pollen and that it is a function of pistil length, I hypothesise that species with longer pistils will have larger pollen grains than species with shorter pistils, and that pistil length and pollen size will be positively correlated in a linear manner. To test this hypothesis, the relationship between pollen grain volume and pistil length was compared in 43 *Asteraceae* species from Argentina. A positive linear correlation was found between pollen volume and pistil length. This correlation remained significant even after potential effects of phylogenetic relatedness were removed. The maintenance of this correlation suggests that in *Asteraceae* the association between pistil length and pollen volume may reflect a functional rather than a phylogenetic relationship. In addition, the pistil length: pollen volume ratio (PPR) was analysed in relation to the phylogenetic position of the species. High values of PPR would imply a reduction of the male gametophyte in relation to the minimal volume that a pollen grain must have to grow and fertilise an ovule. Thus, the general pattern of pollen volume reduction in relation to pistil length previously found among many angiosperm families will be also present within a family, i.e., PPR values of derived *Asteraceae* would be higher than those of basal species. Results indicated that reduction of pollen volume in derived *Asteraceae* was three times greater than the concomitant shortening of pistil length. Consequently, PPR increased with the phylogenetic position of the taxa. This work supports the correlation between pistil and pollen characters previously found for other plant families and confirms the influence of post-pollination processes on pollen size evolution.

**Key words** Pollen evolution · Pollen diameter · Pollen tube growth · Common ancestry effects · Post-pollination processes

### Introduction

Among angiosperm species, pollen volume varies by almost five orders of magnitude (Wodehouse 1935; Muller 1979), implying diverse opportunities for pollen size evolution. On the other hand, within species pollen diameter commonly has a coefficient of variation less than 5% (Vonhof and Harder 1995), suggesting that particular reproductive conditions favour a specific pollen size. This contrasting pattern of pollen size variation among and within species implies strong selection promoting pollen performance. According to this interpretation, an adaptive explanation of pollen size variation in angiosperms would help in understanding how pollen size affects plant siring success.

Although pollen size is presumably the result of a multitude of physiological, morphological, ecological, and historical constraints on a plant species, we can distinguish three broad reproductive processes that would be related to the evolution of pollen size: (1) resource allocation to male function (trade-off between pollen grain size and number), (2) pollination (pollen removal, transport and deposition, pollinator type, etc.), and (3) post-pollination processes (pollen germination and tube growth, fertilization, and pistil characteristics). Trade-off between pollen size and number is a consequence of subdivision of limited resources and should constrain the evolution of pollen size. However, pollen grain size varies considerably less than the number of pollen grains produced per flower (Vonhof and Harder 1995), suggesting that natural selection optimises resource investment per offspring and not the offspring number (Smith and Fretwell 1974; Lloyd 1987). Thus, pollen size would not be involved in an energy trade-off for greater pollen production. Although the correlation between pollen size and mode of pollination (i.e., biotic or abiotic) is well

C. Torres (✉)  
Instituto Multidisciplinario de Biología Vegetal,  
Universidad Nacional de Córdoba-CONICET,  
Casilla de Correo 495, 5000, Córdoba, Argentina  
E-mail: ctorres@imbiv.unc.edu.ar,  
Fax: +54-351-4332104

known (Wodehouse 1935; Bolick 1990; Ackerman 1995), no other correlations have been detailed with the influences of pollination events on pollen size (Harder 1998). In contrast to these two reproductive processes, there are numerous works in which relationships between pollen size and post-pollination events are evident. In particular, the correlation between pollen size and pistil characteristics has been reported in many plant groups (Covas and Schnack 1945; Rosen 1973; Lee 1978; Plitmann and Levin 1983; Cruden and Lyon 1985; Williams and Rouse 1990; Kirk 1993; Vonhof and Harder 1995; Harder 1998).

Given that pollen grains must send tubes through the pistil on their way to the ovule, we may ask whether differences in pollen size are accompanied by differences in some attribute of the pistil, such as pistil length. In general, the growth of the pollen tube is conditioned by the components of the pollen grain and by physiochemical processes that involve stigma and style. The pollen may contain carbohydrates, lipids, enzymes, membranes, and amino acids, all of which are essential for growth; however, many of them are not synthesised during pollen tube growth. Some nutrients, such as polysaccharides and amino acids, can be absorbed from the style whereas some other materials (especially mRNA) cannot be obtained this way and thus function as limiting factors (Tupy et al. 1986). The amount of stored energy may correspond to pollen grain volume or size. If so, larger pollen grains are most likely to contain enough stored energy to support tube growth down longer styles, whereas smaller grains would be sufficient for pollen tube growth through shorter styles. Therefore, based upon the assumptions that pollen tube length is predetermined mainly by provisions in the pollen and is a function of style length, I hypothesise that species with long styles will have larger pollen size than species with short styles, and that style length and pollen size will be positively correlated in a linear manner.

Within a taxonomic framework, Covas and Schnack (1945) calculated pistil length: pollen volume ratio (PPR) for 240 species of 68 angiosperm families, considering the relationship between PPR and the phylogenetic position of the taxa. These authors suggest that high values of PPR imply a reduction of the male gametophyte in relation to the minimal volume that a pollen grain must have to grow and fertilise an ovule. Covas and Schnack (1945) found that pollen volume reduction was greater in derived angiosperm families and, in consequence, it would be reasonable to suppose that the same pattern could also be present within families. Based upon these antecedents, I hypothesise that the general pattern of pollen volume reduction in relation to pistil length found among many angiosperm families will be also present within *Asteraceae*, so PPR values of derived species would be higher than those of basal species. *Asteraceae* is one of the most derived plant families and, despite its manifest success on almost all terrestrial ecosystems, relatively little investigation of its sexual reproduction has been done (Lane 1996). Although some studies

that analyse the functional significance of angiosperm pollen considered some *Asteraceae* species (Wodehouse 1935; Covas and Schnack 1945; Lee 1978; Blackmore 1982; Bolick 1978; 1990; Kirk 1993; Torres 1998), no comparative investigation of pollen size and pistil length within the family has been made.

## Materials and methods

Measurements of pollen and pistil characters were made on flowers of 43 species of *Asteraceae*. Voucher specimens are deposited in the herbarium of the National University of Córdoba. Samples were collected from living plants in natural populations from Argentina, Córdoba and Neuquén Provinces, Departamento Santa María, Los Aromos. Capitula were preserved in 70% ethanol. Lengths of equatorial and polar axes were measured in ten pollen grains obtained from anthers of two individuals for each species, with an ocular micrometer at  $\times 400$  in a Zeiss Axiolab light microscope. Prior to obtaining the measurements, the grains were stained with basic fuchsin. Pollen projections larger than  $0.5 \mu\text{m}$  (such as spinules) were not considered. Pollen grain volume was calculated as  $\pi PE^2/6$  where  $P$ =polar axis diameter and  $E$ =equatorial axis diameter.

Pistil length was considered an approximation to the maximum distance that a pollen tube must grow to fertilise an ovule. This distance corresponds to the length between stigmata branch extremities and the base of the ovary, since the sole ovule of an *Asteraceae* gynoecium has basal placentation. To estimate mean pistil length for each species, the pistil of one flower from each of five individuals was measured with a digital caliper (resolution=0.01 mm) and the assistance of a Zeiss Stemi SV 6 magnifying glass. Measurements were made taking care that flowers were in female phase (stigmas fully elongated). In species with different morphological flower types in the capitulum, pistil length was calculated as the mean of values obtained for each flower type (five flowers of each type were measured). The pistil length:pollen volume ratio (PPR) for each species was calculated using the mean pistil length ( $\mu\text{m}$ ) and the mean pollen grain volume ( $\mu\text{m}^3$ ).

Data underwent correlation (Pearson coefficient) and linear regression analyses. Tests for normality (both graphic and Kolmogorov-Smirnov) and homogeneity of variances (Levene) were performed on all the variables (Sokal and Rohlf 1995). Pollen volume, pistil length and PPR were log-transformed prior to analysis to meet the assumptions of parametric tests. The statistical program package SPSS (1992) was used. Means of original data and standard deviations are given.

According to Silvertown and Dodd (1997), it is necessary to consider the phylogeny and the lack of independence that common ancestry may confer on samples. That is to say, if two species share similar traits due to common descent, then treating them as independent data points when testing for trait correlations is the equivalent of pseudoreplication (Rees 1995). The taxonomic unit considered in the first pollen volume-pistil length correlation analysis was species. Thus, in order to remove the potential effects of common ancestry, an additional correlation analysis was made considering tribes as the taxonomic unit. The phylogenetic diagram proposed by Bremer (1994) was used to arrange tribes in an evolutionary sense. Although there is a large amount of work on phylogenetic reconstruction of *Asteraceae*, there are still problems in general and basal resolution of many tribes. In this way, although data pertain to 11 tribes, only seven unrelated groups of species could be determined: (1) subfam. Barnadesioideae,  $N=1$ ; (2) tribe Mutisieae,  $N=6$ ; (3) tribe Cardueae,  $N=2$ ; (4) tribes Lactuceae and Vernonieae,  $N=5$ ; (5) tribe Inuleae,  $N=1$ ; (6) tribes Astereae and Senecioneae,  $N=6$ ; and (7) tribes Helenieae, Heliantheae, and Eupatorieae,  $N=22$ . Groups of tribes with four or fewer taxa sampled are treated only in the analysis of the family as a whole. Analyses (ANOVA and  $t$ -test) were performed to evaluate differences in mean pollen volume and pistil length between basal

and derived species, at both the tribe and the subfamily levels. The Bonferroni test (Sokal and Rohlf 1995) was used for multiple, a posteriori comparisons.

## Results

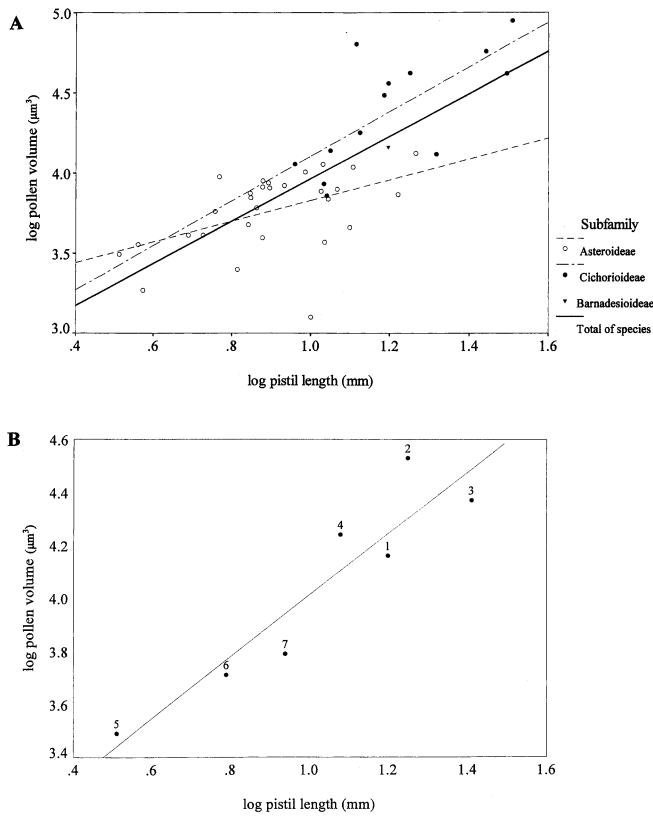
There was manifest variation in pollen grain volume and pistil length among *Asteraceae* species (Table 1). A positive linear correlation was found between pollen grain volume and pistil length ( $r=0.76$ ,  $P=0.0001$ , Fig. 1A). Pistil length ( $l$ ) explained significant proportions of the variation in  $v$ , the pollen grain volume ( $\log v=2.64+1.32$

$\log l$ ;  $F_{[1,41]}=55.85$ ,  $P<0.00001$ ,  $r^2=0.58$ ), i.e., pollen grain volume is proportional to the pistil length, and subsequently to the distance a pollen tube has to grow to reach the ovule. The additional analysis made in order to assess the effects of phylogenetic relatedness on this correlation (see Materials and methods) also showed high positive correlation between pollen volume and pistil length ( $r=0.78$ ,  $P=0.037$ , Fig. 1B), and the linear fitting of this relation was significant ( $v=-999.11+1426.01 l$ ;  $F_{[1,41]}=7.91$ ,  $P<0.038$ ,  $r^2=0.61$ ), i.e., the effect of pistil length on pollen size remained significant after controlling for phylogenetic relatedness.

**Table 1** Pollen grain size and pistil length for 43 *Asteraceae* species. Values show means $\pm$ SD. PPR=relation between pistil length and pollen volume. Species were arranged according to the phylo-

genetic tree proposed by Bremer (1994), following an ascending evolutionary position

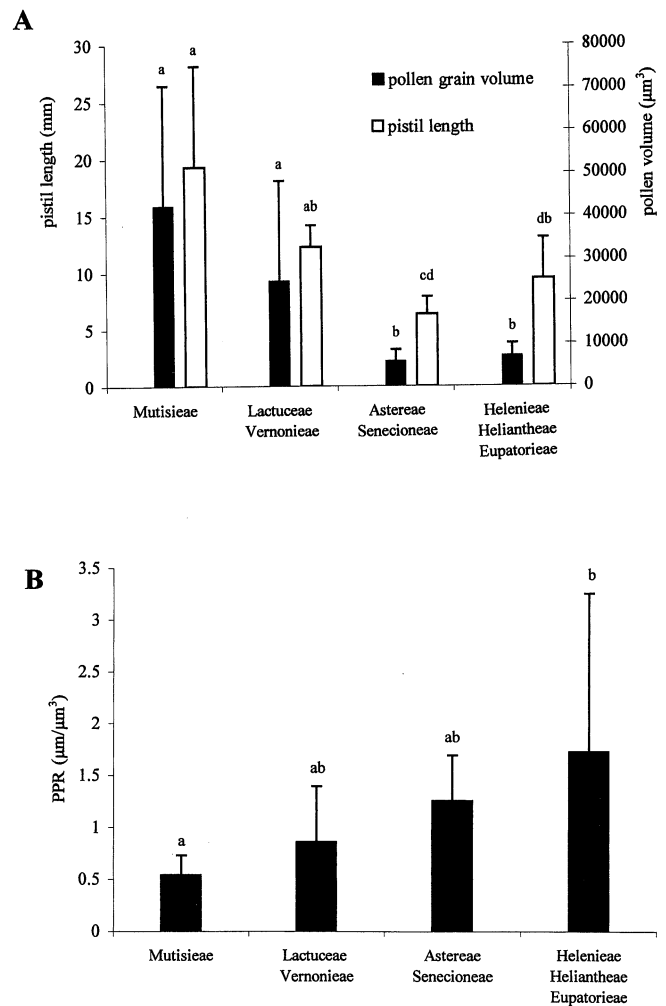
Subfamily	Tribe	Species	Pollen diameter ( $\mu\text{m}$ )	Pollen volume ( $\mu\text{m}^3$ )	Pistil length (mm)	PPR ( $\mu\text{m}/\mu\text{m}^3$ )	
Barnadesioideae		<i>Chiquiraga erinacea</i>	30.25 $\pm$ 1.94	14,672 $\pm$ 3949	15.71 $\pm$ 0.75	1.07	
Cichorioideae	Mutisieae	<i>Chaptalia nutans</i>	33.37 $\pm$ 2.41	19,708 $\pm$ 5425	13.31 $\pm$ 3.20	0.67	
		<i>Hyalis argentea</i>	44.81 $\pm$ 2.65	47,407 $\pm$ 9105	17.76 $\pm$ 3.18	0.37	
		<i>Mutisia decurrens</i>	57.49 $\pm$ 2.84	100,217 $\pm$ 20,868	32.33 $\pm$ 0.60	0.32	
		<i>M. spinosa</i>	49.93 $\pm$ 1.58	65,534 $\pm$ 11,859	27.66 $\pm$ 2.94	0.42	
		<i>Trichocline reptans</i>	42.75 $\pm$ 3.42	41,344 $\pm$ 10,365	15.69 $\pm$ 1.05	0.38	
		<i>Trixis divaricata</i> var. <i>discolor</i>	29.25 $\pm$ 1.42	13,182 $\pm$ 2491	9.12 $\pm$ 1.32	0.69	
	Cardueae	<i>Carduus thoermeri</i>	43.00 $\pm$ 2.65	42,104 $\pm$ 10,898	31.32 $\pm$ 2.07	0.74	
		<i>Centaurea solstitialis</i>	29.25 $\pm$ 2.30	13,346 $\pm$ 4380	20.73 $\pm$ 0.32	1.55	
	Lactuceae	<i>Hypochoeris radicata</i>	24.00 $\pm$ 2.19	7419 $\pm$ 2810	10.98 $\pm$ 1.21	1.48	
		<i>Sonchus oleraceus</i>	29.75 $\pm$ 1.15	13,848 $\pm$ 2262	11.20 $\pm$ 1.52	0.81	
		<i>Taraxacum officinale</i>	25.37 $\pm$ 0.84	8578 $\pm$ 1201	10.78 $\pm$ 0.67	1.26	
	Vernonieae	<i>Vernonia mollissima</i>	49.50 $\pm$ 1.58	63,700 $\pm$ 8603	13.02 $\pm$ 1.25	0.20	
		<i>V. nudiflora</i>	38.75 $\pm$ 2.12	30,739 $\pm$ 7078	15.33 $\pm$ 1.45	0.50	
	Asteroideae	Inuleae	<i>Achyrocline tomentosa</i>	18.12 $\pm$ 1.35	3167 $\pm$ 986	3.25 $\pm$ 0.16	1.03
		Astereae	<i>Baccharis articulata</i>	15.25 $\pm$ 0.99	1880 $\pm$ 512	3.75 $\pm$ 0.49	1.99
<i>B. rufescens</i>			20.87 $\pm$ 1.18	4805 $\pm$ 1143	6.94 $\pm$ 0.30	1.44	
<i>Grindelia anethifolia</i>			23.75 $\pm$ 4.12	7648 $\pm$ 5214	7.04 $\pm$ 0.87	0.92	
<i>G. discoidea</i>			25.00 $\pm$ 1.18	8236 $\pm$ 1639	7.54 $\pm$ 0.40	0.91	
<i>Solidago chilensis</i>			19.87 $\pm$ 0.71	4123 $\pm$ 623	5.33 $\pm$ 0.43	1.29	
<i>Senecio pampeanus</i>			25.75 $\pm$ 1.47	9027 $\pm$ 2168	7.55 $\pm$ 1.01	0.84	
Helenieae		<i>Gaillardia megapotamica</i>	25.50 $\pm$ 1.05	8726 $\pm$ 1518	7.80 $\pm$ 0.06	0.89	
		<i>Helenium argentinum</i>	26.25 $\pm$ 1.32	9543 $\pm$ 2022	5.85 $\pm$ 0.27	0.61	
		<i>Porophyllum ruderale</i>	29.37 $\pm$ 1.98	13,440 $\pm$ 3808	18.40 $\pm$ 0.38	1.37	
		<i>Schkuhria pinnata</i>	22.25 $\pm$ 2.11	5923 $\pm$ 2327	5.71 $\pm$ 0.51	0.96	
		<i>Tagetes minuta</i>	27.87 $\pm$ 2.13	11,533 $\pm$ 3682	10.73 $\pm$ 0.70	0.93	
Heliantheae		<i>Acanthospermum hispidum</i>	19.00 $\pm$ 1.15	3631 $\pm$ 923	3.64 $\pm$ 0.21	1.01	
		<i>Acmella decumbens</i> var. <i>affinis</i>	19.87 $\pm$ 1.37	4166 $\pm$ 1203	4.89 $\pm$ 0.28	1.17	
		<i>Bidens pilosa</i>	24.87 $\pm$ 1.24	8114 $\pm$ 1705	7.86 $\pm$ 1.01	0.97	
		<i>Cosmos sulphureus</i>	23.62 $\pm$ 1.50	6983 $\pm$ 1861	11.06 $\pm$ 1.34	1.58	
		<i>Flourensia campestris</i>	26.87 $\pm$ 2.06	10,337 $\pm$ 3310	9.67 $\pm$ 1.24	0.93	
		<i>Verbesina encelioides</i>	22.62 $\pm$ 1.24	6115 $\pm$ 1411	7.28 $\pm$ 0.70	1.19	
		<i>Wedelia glauca</i>	24.25 $\pm$ 1.34	7535 $\pm$ 1752	7.01 $\pm$ 0.73	0.93	
		<i>Zexmenia buphtalmiflora</i>	24.50 $\pm$ 1.05	7742 $\pm$ 1401	10.62 $\pm$ 1.52	1.37	
		<i>Zinnia peruviana</i>	24.75 $\pm$ 1.64	8021 $\pm$ 2204	11.65 $\pm$ 1.25	1.45	
		Eupatorieae	<i>Eupatorium argentinum</i>	16.87 $\pm$ 1.06	2544 $\pm$ 671	6.51 $\pm$ 0.42	2.56
<i>E. arnotianum</i>			19.62 $\pm$ 0.84	3976 $\pm$ 719	10.01 $\pm$ 0.23	2.52	
<i>E. clematideum</i>			25.18 $\pm$ 3.57	8863 $\pm$ 5062	8.56 $\pm$ 0.30	0.96	
<i>E. inulaefolium</i>			19.62 $\pm$ 0.60	3966 $\pm$ 513	7.54 $\pm$ 0.63	1.90	
<i>E. hookerianum</i>			27.50 $\pm$ 3.38	11,383 $\pm$ 5707	12.80 $\pm$ 1.05	1.12	
<i>E. subhastatum</i>			19.20 $\pm$ 2.52	3897 $\pm$ 2075	10.84 $\pm$ 0.75	2.78	
<i>Mikania urticifolia</i>	24.12 $\pm$ 1.87		7480 $\pm$ 2421	16.63 $\pm$ 1.15	2.22		
<i>Stevia satuireifolia</i>	20.62 $\pm$ 1.98		4717 $\pm$ 1876	12.56 $\pm$ 0.66	2.66		



**Fig. 1** Relationships between pollen grain volume and pistil length in *Asteraceae*. **A** Correlation analyses considering all species ( $N=43$ ) and subfamily groups (Subfam. Cichorioideae,  $N=13$ ; Subfam. Asteroidae;  $N=29$ ). **B** Correlation analysis considering groups of unrelated taxa (Bremer 1994) in order to control for possible phylogenetic relatedness effects. Groups of unrelated tribes were numbered according to ascending phylogenetic position (Bremer 1994). 1=subfam. Barnadesioideae ( $N=1$ ); 2=tribe Mutisieae ( $N=6$ ); 3=tribe Cardueae ( $N=2$ ); 4=tribes Lactuceae and Vernoniae ( $N=5$ ); 5=tribe Inuleae ( $N=1$ ); 6=tribes Astereae and Senecioneae ( $N=6$ ); and 7=tribes Helenieae, Heliantheae, and Eupatorieae ( $N=22$ );  $N$ =Species sample size

Additional analyses examined pollen volume and pistil length considering the phylogenetic position of species. First, I searched for differences between subfamilies Cichorioideae and Asteroidae, basal and derived, respectively (Bremer 1994). Cichorioideae had larger mean values than Asteroidae for the two variables ( $33,217 \pm 25,067$  and  $6589 \pm 3008 \mu\text{m}^3$  for pollen volume,  $17.63 \pm 8.01$  and  $8.59 \pm 3.59$  mm for pistil length, respectively, Fig. 1A). These differences were significant ( $t=5.83$ ,  $P<0.0001$  for pollen volume, and  $t=5.09$ ,  $P<0.0001$  for pistil length). The same trend was observed at the tribe level. Significant differences were found among the tribes; basal showed higher values for pollen volume and pistil length than derived tribes ( $F_{[3, 35]}=14.48$ ,  $P<0.00001$ ;  $F_{[3, 35]}=9.38$ ,  $P<0.0001$  respectively, Fig. 2A).

The relationship between pistil length and pollen volume (PPR) was also related to species phylogeny. Although both variables decreased (Fig. 2 A), PPR increased with the phylogenetic position of species (Fig. 2



**Fig. 2** Pollen grain volume and pistil length in relation to phylogeny in *Asteraceae*. **A** Pollen grain volume and pistil length, **B** pistil length:pollen volume ratio (PPR). Groups of unrelated tribes were arranged according to the phylogenetic tree proposed by Bremer (1994), following an ascending evolutionary position (left to right, basal to derived). Bars show mean  $\pm$  SD. Letters (a, b, c, d) on the bars indicate results of the post hoc test; significant differences between means are marked with different letters

B). Significant differences were found between basal and derived tribes ( $F_{[3, 35]}=6.86$ ,  $P<0.0009$ ). The same trend was observed at the subfamily level, where PPR was significantly higher in Asteroidae (derived) than in Cichorioideae ( $1.61 \pm 1.36$  and  $0.76 \pm 0.44$ , respectively;  $t=-3.88$ ,  $P<0.001$ ). The pattern of variation of PPR values can be explained by considering the behaviour of each variable separately. Although both variables decrease with the phylogenetic position, pistil length and pollen volume of derived species are one-half and one-fifth, respectively, those of basal species. In other words, reduction of pollen volume in derived species is greater than the decrease in pistil length, making evident a male gametophyte volume reduction relative to the distance it must grow to reach an ovule.

## Discussion

These results are consistent with the hypotheses proposed, since there was a strong correlation between pistil length and pollen size. The trend found for *Asteraceae* was previously reported for many angiosperm families (Schnack and Covas 1945; Rosen 1973; Lee 1978; Plitmann and Levin 1983; Williams and Rouse 1990; Dulberger 1992; Kirk 1993; Vonhof and Harder 1995; Harder 1998). Furthermore, the strong influence of pistil length on pollen size remained significant even after potential effects of phylogenetic relatedness were removed. The maintenance of this correlation suggests that in *Asteraceae* the association between pistil length and pollen volume may reflect a functional rather than a phyletic relationship.

The correlation between pollen size and pistil length would be a manifestation of an integrated compensatory evolution of the gametophytic and sporophytic generations, and of the androecium and gynoecium. Changes in one character of the fertilization system would imply obligate changes in other characters in order to maintain the functional integrity of the system. In particular, response to selection for pistil length increase probably would be minimal without a simultaneous pollen size increase (which would be accompanied by the increase of those provisions which function as limiting factors). Otherwise, many pollen tubes would fail to reach the ovules. On the other hand, the evolution of shorter pistils would be accompanied by less selection pressure on pollen size, since pollen tubes would be able to reach the ovule without major changes. Thus, a system modification is most likely to come about in response to selection for pistil length than for pollen size changes. In addition, considering that the amount of pollen produced per flower in many families is independent of pollen volume (Vonhof and Harder 1995), pollen size seems to be an unlikely prime target for natural selection.

Although most studies that involve related species showed a strong correlation between pollen grain size and style or pistil length, significant correlations have not been reported in some studies based on a miscellaneous array of species from different families (e.g. Cruden and Miller-Ward 1981, Cruden and Lyon 1985). These contradictory results could be because (1) the influence of phylogenetic relatedness (family) was not considered, so the ability to detect a significant correlation may have been compromised (Silvertown and Dodd 1997), and (2) pollen tubes can receive unlimited resources from the stigma and/or the style. This nutritional support given by the pistil to pollen tube growth has been previously recorded for some species (e.g. Amici 1830; Labarca and Loewus 1973; González et al. 1996) and appears to be associated with the change from the autotrophic to the heterotrophic pollen tube phase on entering the style (Herrero and Dickinson 1980; Mulcahy and Mulcahy 1983). In a number of species, specialised pistil cells are rich in starch which disappears after pollination and secretions are produced. Some authors have

found that these secretions supply nutrition and provide a pathway toward the ovules for the pollen tubes (Tilton et al. 1984 and references therein). Nevertheless, these secretions could be involved in other processes, in addition to pollen tube nutrition and guidance, that could effectively explain the emptying of pistil cells after pollen tube passage. Therefore, it has been suggested that through interaction of pollen tubes and pistil reserves mate choice can occur in plants (Marshall and Folsom 1991), and that particular pollen genotypes could be selected by the pistil (Hormaza and Herrero 1994).

The specializations between pollen size and pistil length are sometimes so precise that certain types of cross pollination are impossible, so they can play a part as isolating mechanisms (Heslop-Harrison 1981). The most compelling evidence comes from comparisons of pollen tube length in legitimate and illegitimate pollinations with a particular pollen size in tristylous species of *Pontederia* (Anderson and Barrett 1986; Scribailo and Barrett 1991). The fact that small and medium pollen grains produce pollen tubes of the same length in both legitimate and illegitimate pollinations suggests that incompatibility in certain pollen-pistil combinations may be simply a function of pollen storage reserves. Thus, in each morph general failure to set seed on illegitimate pollination appears to result from a mismatch between pollen size and pistil length.

The differences in pistil length/pollen volume ratio (PPR) between basal and derived *Asteraceae* are in accordance with the pattern previously reported by Covas and Schnack (1945) among many angiosperm families. PPR increases with the rise in the phylogenetic position of the species since pollen volume reduction in derived *Asteraceae* was three times greater than the shortening of pistil length. Although there is a natural tendency to suppose that pollen size might be similar in species with close phylogenetic positions, taxa evidently affiliated (e.g., species pertaining to a same family, tribe, or genera) may present pollen grains with very different sizes (e.g. Plitmann and Levin 1983; Cruden and Lyon 1985, this work). So, pollen grain volume must not be used as an indicator for phylogenetic positions of taxa. Nevertheless, if pollen volume is considered in relation to the distance that a pollen tube must grow to fertilise the ovule, the PPR values of species with close phylogenetic positions tend to be similar. Thus, a more effective way to estimate the phylogenetic position of a species is to consider its PPR value. Until now, PPR has been measured for only a few angiosperm families (Covas and Schnack 1945) and the importance of this relationship remains still undetermined. Particularly in *Asteraceae*, since there are still many problems in phylogenetic reconstruction of this family (Bremer 1994), PPR might usefully be evaluated as a trait for the resolution of doubtful phylogenetic positions of taxa.

To conclude, and despite the attractiveness of the stated hypotheses, little experimental work has been done; the inferences reported up to now are mainly speculative. This paper represents a new contribution that supports a

strong correlation between pistil and pollen features and confirms the influence of post-pollination processes in pollen size evolution. Future studies on the functional significance of pollen volume should be directed at identifying how pollen size affects post-pollination processes.

**Acknowledgements** I thank Leonardo Galetto for valuable discussions on early drafts of this manuscript, Cecilia Eynard and Gabriel Bernardello for useful comments, and Luis Ariza Espinar for his assistance in species identification. The study was supported by Consejo de Investigaciones Científicas y Técnicas de la Provincia de Córdoba (CONICOR), Consejo Nacional de Investigaciones Científicas y Técnicas, Agencia Nacional de Promoción Científica y Tecnológica, and Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba. Thanks are due to CONICOR for the fellowship to the author.

## References

- Ackerman JD (1995) Convergence of filiform pollen morphologies in seagrasses: functional mechanisms. *Evol Ecol* 9:139–153
- Amici JB (1830) Note sur le mode d'action du pollen sur le stigmate: extrait d'une lettre de M. Amici à M. Mirbel. *Ann Sci Nat* 21:329–332
- Anderson JM, Barrett SCH (1986) Pollen tube growth in tristylous *Pontederia cordata* (Pontederiaceae). *Can J Bot* 64:2602–2607
- Blackmore S (1982) A functional interpretation of *Lactuceae* (Compositae) pollen. *Plant Syst Evol* 141:153–168
- Bolick MR (1978) Taxonomic, evolutionary, and functional considerations of *Compositae* pollen ultrastructure and sculpture. *Plant Syst Evol* 130:209–218
- Bolick MR (1990) The pollen surface in wind-pollination with emphasis on the *Compositae*. *Plant Syst Evol [Suppl]* 5:39–51
- Bremer K (1994) *Asteraceae: cladistics and classification*. Timber Press, Oregon
- Covas G, Schnack B (1945) El valor taxonómico de la relación longitud del pistilo:volumen del grano de polen. *Darwiniana* 7:80–90
- Cruden RW, Miller-Ward S (1981) Pollen-ovule ratio, pollen size, and the ratio of stigmatic area to the pollen-bearing area of the pollinator: an hypothesis. *Evolution* 35:964–974
- Cruden RW, Lyon DL (1985) Correlations among stigma depth, style length, and pollen grain size: do they reflect function or phylogeny? *Bot Gaz* 146:143–149
- Dulberger R (1992) Floral polymorphisms and their functional significance in the heterostylous syndrome. In: Barrett SCH (ed) *Evolution and function of heterostyly*. Springer, Berlin Heidelberg New York
- González MV, Coque M, Herrero M (1996) Pollen-pistil interaction in kiwifruit (*Actinidia deliciosa*; Actinidiaceae). *Am J Bot* 83:148–154
- Harder LD (1998) Pollen-size comparisons among animal-pollinated angiosperms with different pollination characteristics. *Biol J Linn Soc* 64:513–525
- Herrero M, Dickinson HG (1980) Pollen tube growth following compatible and incompatible intraspecific pollinations in *Pentunia hybrida*. *Planta* 148:217–221
- Heslop-Harrison Y (1981) Stigma characteristics and angiosperms taxonomy. *Nord J Bot* 1:401–420
- Hormaza JJ, Herrero M (1994) Gametophytic competition and selection. In: Williams EG, Knox RB, Clarke AE (eds) *Genetic control of self-incompatibility and reproductive development in flowering plants*. Kluwer, The Netherlands
- Kirk WDJ (1993) Interspecific size and number variation in pollen grains and seeds. *Biol J Linn Soc* 49:239–248
- Labarca C, Loewus F (1973) The nutritional role of pistil exudate in pollen tube wall formation in *Lilium longiflorum*. *Plant Physiol* 52:87–92
- Lane MA (1996) Pollination biology of Compositae. In: Caligari PDS, Hind DJN (eds) *Compositae: biology and utilization*. Proceedings of the International Compositae Conference, Kew, 1994, vol. 2. Royal Botanic Gardens, Kew
- Lee S (1978) A factor analysis of the functional significance of angiosperm pollen. *Syst Bot* 3:1–19
- Lloyd DG (1987) Selection of offspring size at independence and other size-versus-number strategies. *Am Nat* 129:800–817
- Marshall DL, Folsom MW (1991) Mate choice in plants: an anatomical to population perspective. *Annu Rev Ecol Syst* 22:37–63
- Mulcahy GB, Mulcahy DL (1983) A comparison of pollen tube growth in bi- and trinucleate pollen. In: Mulcahy DL, Ottaviano E (eds) *Pollen: biology and implications for plant breeding*, Elsevier Biomedical, New York, NY
- Muller J (1979) Form and function in angiosperm pollen. *Ann Missouri Bot Gard* 66:593–632
- Plitmann U, Levin DA (1983) Pollen-pistil relationships in the *Polemoniaceae*. *Evolution* 37:957–967
- Rees M (1995) EC-PC comparative analyses? *J Ecol* 83:891–892
- Rosen WG (1973) Pistil-pollen interactions in *Lilium*. In: Heslop-Harrison J (ed) *Pollen: development and physiology*. Butterworths, London
- Schnack B, Covas G (1945) Hibridación interespecífica en *Glandularia* (Verbenáceas). *Darwiniana* 7:71–79
- Scribailo RW, Barrett SCH (1991) Pollen-pistil interactions in tristylous *Pontederia sagittata* (Pontederiaceae). II. Patterns of pollen tube growth. *Am J Bot* 78:1662–1682
- Silvertown J, Dodd M (1997) Comparing plants and connecting traits. In: Silvertown J, Franco M, Harper JL (eds) *Plant life histories*. The Royal Society, University Press, Cambridge
- Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. *Am Nat* 108:499–506
- Sokal RR, Rohlf FJ (1995) *Biometry*. Freeman, San Francisco
- SPSS (1992) *SPSS for Windows: base system user's guide*, release 5.0. SPSS, Chicago
- Tilton VR, Wilcox LW, Palmer RG, Albertsen MC (1984) Stigma, style and obturator of soybean, *Glycine max* (L.) Merr. (*Leguminosae*) and their function in the reproductive process. *Am J Bot* 71:676–686
- Torres C (1998) Estructura del capítulo y caracteres florales en relación a la polinización en dos especies de *Vernonia* (*Asteraceae*). *Kurtziana* 26:65–82
- Tupy J, Suss J, Rihova L (1986) RNA synthesis and ribosome status in pollen tube growth of *Nicotiana tabacum* L.: effects of external pH. *J Plant Physiol* 123:467–476
- Vonhof MJ, Harder LD (1995) Size-number trade-offs and pollen production by papilionaceous legumes. *Am J Bot* 82:230–238
- Williams EG, Rouse JL (1990) Relationships of pollen size, pistil length and pollen tube growth in *Rhododendron* and their influence on hybridization. *Sex Plant Reprod* 3:7–17
- Wodehouse RP (1935) *Pollen grains*. McGraw-Hill, New York