

# Pollen: ovule ratio and its relationship with other reproductive traits in some *Passiflora* species (Passifloraceae)

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## Abstract

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The pollen/ovule (P/O) ratio has been used as predictor of the reproductive system of angiosperms (lowest P/O values correspond to obligate autogamous species while the highest correlate with obligate xenogamous species) but it does not keep for all the taxa. The relation of P/O index with pollen and stigma size, flower diameter, pollen vitality, stigmatic area/pollen-bearing area of the pollinators ratio (SA/PBA), taxonomy and breeding system was analyzed in four *Passiflora* species with different degree of compatibility, pollination system and taxonomic placement. P/O of the self-compatible species (with shorter anthesis, scarce pollinator visits or minor longevity) was lower than P/O of the self-incompatible ones. The P/O values of the outcrossers could be related with the highly efficient pollination performed by the frequently visiting *Xylocopa* species; these bees transport *Passiflora* pollen in a usually monospecific mass on the thorax, resembling grouped pollen dispersal. All the taxa exhibited pollen with unimodal size and high vitality, except for *P. misera*, in which pollen size was bimodal, with similar quantities of grains in both classes, the large being more vital than the small ones. Correlations of P/O were positive with flower size, negative with stigma area/pollen-bearing area of the pollinator, null with stigma area and not significant with pollen size. The P/O ratio did not reflect taxonomic affinities; this index and the breeding system were more related with pollinator type, anthesis, flower size and SA/PBA than with pollen grain or stigma size.

**Keywords:** pollen size, pollen vitality, breeding systems, flower size, stigma area, pollen-bearing area, pollinators, anthesis, plant longevity, sexual system.

## INTRODUCTION

The widely cited paper by Cruden (1977) about the proportion of pollen/ovules (P/O) of the angiosperms as indicator of their reproductive system and the ecological successional stages they occupy (P/O value increases with degree of xenogamy and successional stage) stimulated much subsequent research. Various authors have used the P/O ratio directly as predictor of breeding systems (Lindsey, 1982; Ortega Olivencia & al., 1997; López & al., 1999); others have analyzed if the P/O ratio coincides or not with the predictions of that foundational work (Preston, 1986; Plitman and Levin, 1990; Spira, 1980; Wyatt, 1984; Roitman and Medán, 1994; Affre and Thompson, 1998; Weller & al., 1998; Galloni & al., 2007; Hokche and Ramírez, 2008). Even Cruden and McClain (1996) tested the P/O ratio in additional species.

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## Resumen

Amela García, M.T., Miguez, M.B. & Gottsberger, G. 2014. El índice polen/óvulos y su relación con otros rasgos reproductivos en varias especies de *Passiflora* (Passifloraceae). *Anales Jard. Bot. Madrid* 71(2): e009.

La utilidad del índice polen/óvulos (P/O) para predecir el sistema reproductivo de las Angiospermas (los valores más bajos corresponden a especies autógamas obligadas mientras que los valores más altos a especies xenógamas obligadas) no siempre ocurre. Analizamos la relación del P/O respecto del tamaño del polen y estigma, diámetro floral, viabilidad polínica, proporción área estigmática/área de acarreo del polen por los polinizadores, la taxonomía y el sistema reproductivo en cuatro especies de *Passiflora* con grado de compatibilidad, polinizadores y posición taxonómica distintos. El P/O de las especies auto-compatibles (con antesis más corta, polinizadores infrecuentes o menos longevidad) fue menor que el de las auto-incompatibles. Esto estaría relacionado con la polinización altamente eficiente que realizan asiduamente *Xylocopa* spp., transportando polen de *Passiflora* en masa, usualmente mono-específica sobre el tórax, semejando dispersión polínica agrupada. Todos los taxones exhibieron polen de tamaño unimodal y alta vitalidad, excepto *P. misera*, en que fue bimodal, con cantidades similares de granos en ambas clases, los grandes más vitales que los pequeños. La correlación del P/O fue positiva con el tamaño floral, negativa con la proporción área del estigma/área de acarreo del polen por el polinizador, nula con el área del estigma y no significativa con el tamaño del polen. El P/O no reflejó afinidades taxonómicas; este índice y el sistema reproductivo estuvieron más relacionados con el tipo de polinizador, la antesis, el tamaño floral y la razón área del estigma/área de acarreo del polen por el polinizador que con el tamaño del polen o del estigma.

**Palabras clave:** tamaño del polen, vitalidad del polen, sistemas reproductivos, tamaño de la flor, área estigmática, área de acarreo del polen, polinizadores, antesis, longevidad de la planta, sistema sexual.

P/O adjustments have been probed at various levels, i.e., between populations (Wyatt, 1984) or races (Schoen, 1977) and within genera (Spira, 1980; Ortega Olivencia & al., 1997; Affre and Thompson, 1998), tribes (Small, 1988; López & al., 1999; Galloni & al., 2007) and families (Preston, 1986; Plitman and Levin, 1990; Weller & al., 1998; Wyatt & al., 2000; Choteau & al., 2006; Hokche and Ramírez, 2008; Devoto and Medán, 2008).

In most cases, P/O ratio falls within the ranges calculated by Cruden (1977) or keeping the tendency (P/O of autogamous species lower than P/O of xenogamous species) (Schoen, 1977; Spira, 1980; Wyatt, 1984; Preston, 1986; Graumann and Gottsberger, 1988; Ortega Olivencia and Devesa Alcaez, 1993; Weller & al., 1998; Christ & al., 2001; Lehnebach and Riveros, 2003; Medán, 2003), but some exhibit higher (Choteau & al., 2006; Devoto and Medán, 2008; Hokche and Ramírez, 2008) or lower (Wild & al.,

2003; Galloni & al., 2007) values than expected. Rather than breeding system, in several taxa P/O ratio reflects better pollination mechanism (Galloni & al., 2007) or pollination efficiency (Choteau & al., 2006). P/O ratio was significantly associated with pollination mechanism in species with unknown breeding system (López & al., 1999) and in 21 xenogamous legumes (Etcheverry & al., 2012). Lower P/O ratios usually coincide with precise or highly specialized pollen transfer mechanisms (Small, 1988; Jürgens & al., 2002; Choteau & al., 2006). This has been exemplified in the Mimosoideae (Cruden, 1977). The extreme is represented in Asclepiadoidea (Cruden, 1977; Wyatt & al., 2000). Lack of adjustment to expected values of P/O were also discussed in relation to pollination ecology (Affre and Thompson, 1998).

Cruden (2000) admitted that this index might be confusing, especially for xenogamous species with low P/O ratio, many of which have large pollen grains and/or large stigmas, based on the relation between P/O ratio, pollen size and the ratio of stigma area to the pollen-bearing area of the pollinators (SA/PBA) (Cruden and Miller-Ward, 1981). The stigma area is expected to be large enough to receive sufficient pollen grains to fertilize a large number of ovules (Cruden and Miller-Ward, 1981); the SA/PBA was used as a measure of the likelihood of a pollen grain reaching a stigma, and it was hypothesized that P/O would be inversely related to SA/PBA (Cruden and Miller-Ward, 1981).

P/O ratio, breeding system and pollen vitality, which implies the proportion of grains capable of fertilization, have been investigated concomitantly in few studies (Lindsey, 1982; Hoc and Amela García, 1998, 1999; López & al., 1999; Lehnebach and Riveros, 2003; Wild & al., 2003), but pollen vitality has not been related to P/O or breeding system.

P/O ratio has further been explored in relation to sexual system (Lindsey, 1982; Jürgens & al., 2002; Choteau & al., 2006), pollination system (Plitman and Levin, 1990; Götzenberger & al., 2008), life form (Cruden, 1977; Small, 1988; Jürgens & al., 2002), seed mass (Götzenberger & al., 2006) and floral size (Etcheverry & al., 2012), among other parameters (see Ortega Olivencia & al., 1997).

P/O ratio has not been examined in *Passiflora* L. yet. Preliminary records in species with known breeding system (Amela García, 1999) suggest that this index is lower than expected by the predictions of Cruden (1977). The aim of this work was to explore the relationship between P/O ratio, pollen and stigma size, flower size, pollen vitality, pollen-bearing area and pollen transfer efficiency in *Passiflora* species with different degree of self-compatibility and from different infrageneric categories, to know how these floral traits interrelate and corroborate if the P/O ratio predicts the breeding system or reflects taxonomic affinities in *Passiflora*.

## MATERIAL AND METHODS

### *Plant material*

Samples of the following species were obtained at the following respective sites in Argentina: *P. caerulea* (Merlo, Buenos Aires province), *P. foetida* (Charata, Chaco province), *P. suberosa* (San José, Misiones province), *P. misera* (Iguazú National Park, Misiones province). The first two taxa are included in subgenus *Passiflora* while the last two belong to subgenus *Decaloba* (Feuillet & MacDougal, 2004).

Buds ready to open were collected and fixed in ethanol 70° for pollen and ovule counts, pollen and stigma size measurements, whereas other buds were dried between paper sheets for pollen vitality tests.

### *Pollen and ovule counts*

For pollen grain number estimation, anthers were opened under a dissecting microscope and all the pollen was transferred to plastic tubes containing 5 ml of 0.9% NaCl solution. Pollen grains were counted with a cell counter and analyzer system CASY (Schärfe System, Reutlingen, Germany). The number obtained was multiplied by the number of anthers of the bud. The ovules were counted dissecting the ovary under a stereoscopic microscope. The P/O index (ratio between pollen grains and ovules of a bud) defined by Cruden (1977) was calculated, the values obtained were compared with the ranges given by that author and with the breeding system, which had been determined with controlled pollinations (Amela García, 1999).

### *Pollen size and vitality*

The pollen grain size was measured with the automatic cell counter and analyzer system mentioned above. This device gives the diameter of the figure according to the position it is, so it is somewhat like an average between the equatorial and polar axis in these subspheroidal grains (Amela García & al., 2002). Pollen vitality was estimated by optical contrast-fluorescence microscopy (Greissl, 1989) and by staining the main pollen constituents, lipids in all of them (Amela García, 1999), with Sudan IV (Kerns and Inouye, 1993).

### *Stigma area and pollen-bearing area of the pollinators*

*Passiflora* species have capitate stigmas, whose most resembling geometric form is an ellipse, so the longer and the shorter axis were measured with an ocular micrometer of a dissecting microscope, converted to mm and then the ellipse area was calculated. The deposition site of the pollen of *Passiflora* species on their pollinators is the thorax dorsum, which can be resembled to a rectangle, so the length and the width were measured and then the rectangle area was calculated. Dimensions were taken at naked eye with a ruler made of graph paper, on specimens deposited at the Museo Argentino de Ciencias Naturales "B. Rivadavia". Pollinators were chosen based on previous works (Amela García, 1999; Amela García and Hoc, 1997, 1998a, 2001). Specimens of the pollinators of *P. suberosa* could not be measured as they were not available for us. Afterwards, the stigma area/pollen bearing area was calculated for each species.

### *Flower size*

The major diameter (distance between the apexes of opposite sepals) was measured on fresh material with graph paper at naked eye in these actinomorphic flowers.

### *Statistical analysis*

The data were subjected to ANOVA and correlation analysis using the statistical program InfoStat 9.0 (2009).

## RESULTS

### *P/O ratio and breeding system*

P/O ratios differed significantly between the self-incompatible and the self-compatible species:  $F=50.66$ ,  $p<0.0001$ ,  $n=89$  (Table 1); P/O of self-incompatible species were higher (3 folds) than those of the self-compatible ones.

### *Pollen grain size and vitality*

The pollen grain size was rather constant within each species, considering the little standard deviation (Table 2) and differed significantly between some species ( $F=308.11$ ,  $p<0.0001$ ,  $n=94$ ) (Table 2); *P. misera* exhibited greater variation, in this species a tendency to a bimodal distribution was detected (Fig. 1). Pollen vitality was high in all species with both tested staining methods (Table 3); in *P. misera*, vitality was notoriously higher in the bigger grains (Table 3).

### *P/O, pollen grain size, flower size, stigma area and SA/PBA*

The correlation between P/O and pollen grain size was not significant (Fig. 2A).

Flower size (Table 2) varied significantly between some species ( $F=109.97$ ,  $p<0.0001$ ,  $n=47$ ) and was positively correlated with P/O (Fig. 2B).

The stigmatic area differed significantly between some species ( $F=27.918$ ,  $p=0$ ,  $n=62$ ; Table 4). The P/O index was not correlated with stigma area (Fig. 2C) but it was inversely and strongly ( $-167.40$ ) correlated with the SA/PBA ( $r=0.418$ ,  $p=0.0002$ ,  $n=73$ ) (Fig. 3).

## DISCUSSION

### *P/O, breeding system, pollinators, anthesis and plant longevity*

The lower P/O ratios of *P. foetida* and *P. suberosa* as compared to the ones of *P. caerulea* and *P. misera* are in accordance with the self-compatibility of the former and the self-incompatibility of the latter (Amela García, 1999), and the tendency coincides with the one discovered by Cruden (1977). Nevertheless, P/O values were lower than expected by the predictions of Cruden (1977) for all the species, irrespective of their breeding system; however, P/O of self-compatible species resulted more closely to Cruden's values, but the ones of xenogamous species were substantially lower than those proposed by him. Based on the ranges given for xenogamous taxa, Cruden and Miller-Ward (1981) argued that species with very efficient pollen transfer mechanisms had lower P/O ratios than expected as compared to species with equivalent breeding systems but less efficient pollen flow. The specialization between bees and flowers results in a more efficient pollination with less pollen loss (Pijl, 1954). Probably the low P/O ratio in *P. caerulea* and *P. misera* is related with the efficient pollen transfer performed by *Xylocopa* species (Amela García and Hoc, 1997 and Amela García unpublished. data, respectively), becoming unnecessary a major pollen production. Their reproductive system could be assimilated to the one of "sweepstakes" (Cruden, 1977), as, although pollen is not clustered by abundant pollenkit or viscin threads nor grouped in pollinia, the transport in mass on the pollinators thorax, comprising the majority or even the whole pollen load in this body part, would be comparable with those agglutinations. Every time a pollinator visits a flower it takes a considerable amount of grains

**Table 1.** Breeding system (determined by experimental pollinations), pollen and ovule number, P/O ratio and estimated breeding system by P/O ratio of four *Passiflora* species

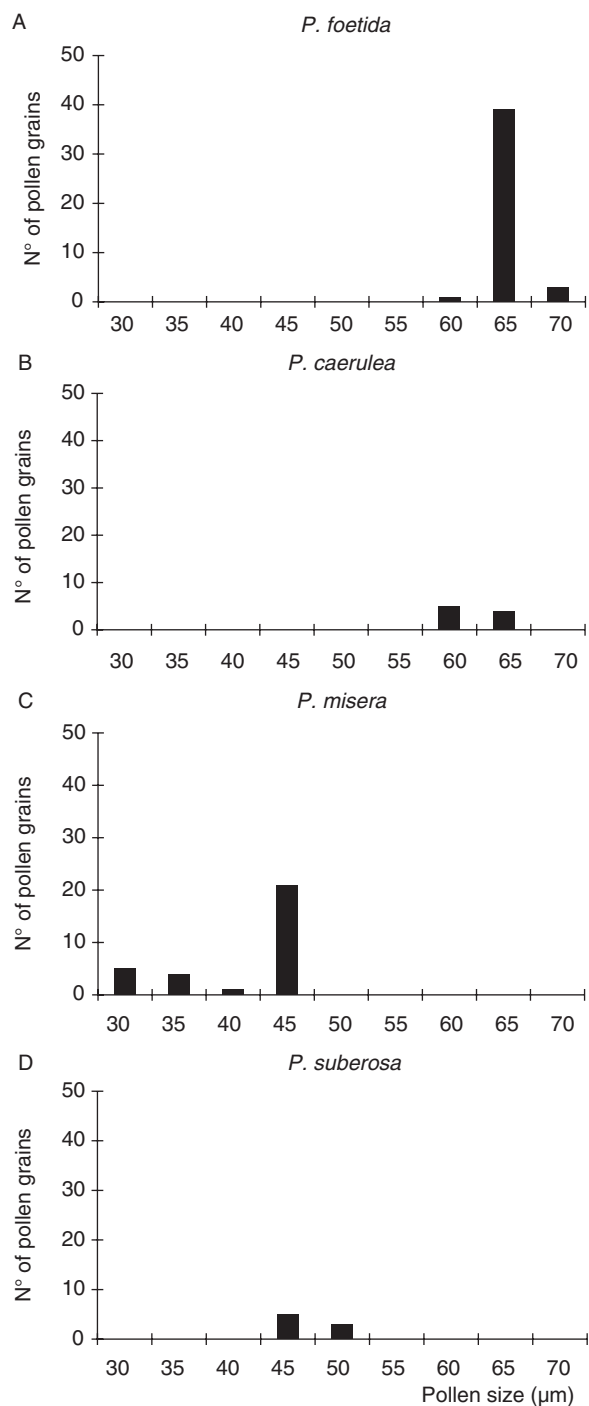
Species	<i>P. foetida</i>	<i>P. caerulea</i>	<i>P. misera</i>	<i>P. suberosa</i>
Breeding system	psc	si	si	hsc
Pollen	6,667±1,241 26 (14)	164,216±43,537 26 (10)	23,873±6,145 25 (3)	6,319±4,342 14 (3)
Ovules	31±4 26 (14)	252±45 25 (10)	41±8 24 (3)	24±2 14 (3)
P/O	216.62 <sup>a</sup> ±40.39 26 (14)	656.67 <sup>b</sup> ±141.73 25 (10)	601.44 <sup>b</sup> ±209.74 24 (3)	262.26 <sup>a</sup> ±183.59 14 (3)
Estimated breeding system	fa	fa-fx	fx	fa

Numbers within each cell represent: average±standard deviation and n=number of flowers (plants); si=self-incompatible; psc=partially self-compatible; hsc=highly self-compatible; fa=facultative autogamous; fx=facultative xenogamous. Values with different letters differed significantly at  $p<0.0001$ .

**Table 2.** Pollen grain size and flower size of four *Passiflora* species

Species	<i>P. foetida</i>	<i>P. caerulea</i>	<i>P. misera</i>	<i>P. suberosa</i>
Pollen diameter (µm)	63.39 <sup>a</sup> ±1.46 44 (13)	59.56 <sup>a</sup> ±1.26 10 (2)	38.28 <sup>b</sup> ±6.02 32 (1)	43.45 <sup>c</sup> ±1.79 8 (1)
Flower size (mm)	41.44 <sup>a</sup> ±3.05 9 (9)	40.73 <sup>a</sup> ±8.68 11 (11)	27.4 <sup>b</sup> ±3.5 15 (5)	9 <sup>c</sup> ±0.85 12 (12)

Values with different letters differ significantly at  $p<0.01$ . Numbers within each cell represent the average±standard deviation and n=number of anthers or flowers (number of plants).

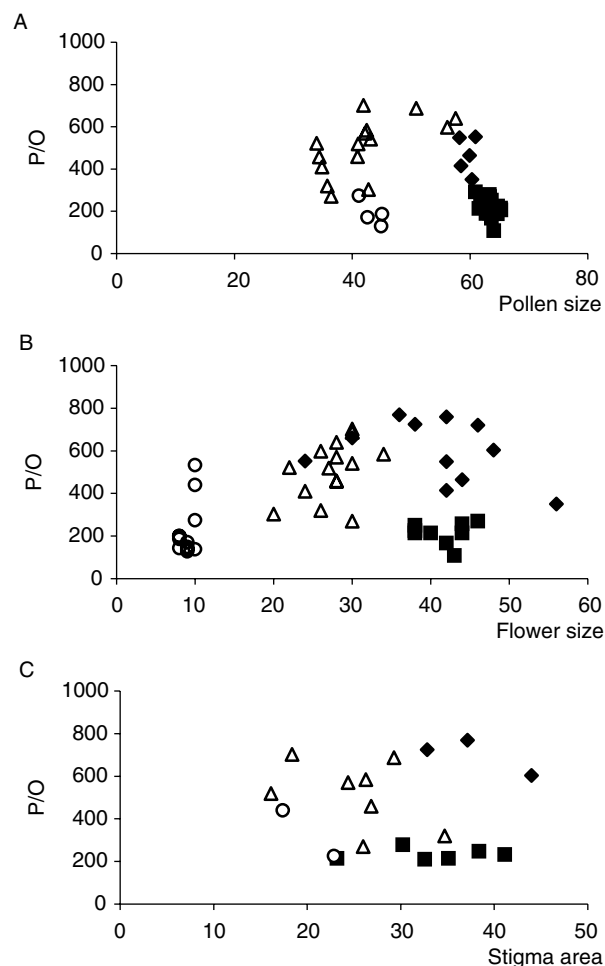


**Fig. 1.** Frequency distribution of pollen grains in pollen size classes of four *Passiflora* species.

**Table 3.** Pollen vitality of four *Passiflora* species with two different techniques: fluorescence and main constituents

Species	<i>P. foetida</i>	<i>P. caerulea</i>	<i>P. misera</i>	<i>P. misera</i>
fluorescence % (n)	90 (100)	86 (89)	l: 100 (164) s: 4 (160)	82 (206)
constituents % (n)	98 (363)	96 (307)	-	96 (644)

n=number of pollen grains tested; l=large grains; s=small grains.



**Fig. 2.** Relationship between pollen/ovule ratio (P/O) and other floral traits of four *Passiflora* species. ■=*P. foetida*, ◆=*P. caerulea*, △=*P. misera*, ○=*P. suberosa*.

from the anthers (usually getting most of the notothorax covered with pollen), and leaves a similar quantity of pollen through its wide contact with the stigmas when they are deflected. Cruden and Miller-Ward (1981) found that P/O was negatively correlated with the pollen-bearing area of the pollinators and with pollen grain size. An additional coincidence with these authors' observations is that most of the xenogamous species with the lower P/O ratio have larger pollen grains: *P. caerulea* (this work) and *P. mooreana* (Amela García and Hoc, 1998b).

Still, P/O ratios of self-compatible species were a bit lower than expectations of Cruden (1977). An inverse relation between P/O ratio and pollinator reliability was anticipated by Cruden and Miller-Ward (1981) but low P/O ratio of a self-compatible orchid also coincided with low pollinator visitation rate (Lehnebach and Riveros, 2003). Pollinator constancy of *P. foetida* is high (Amela García and Hoc, 1998a). In contrast, scarce frequency of pollinators to *P. suberosa* might be compensated by its longer anthesis and its higher degree of self-compatibility (Amela García, 2008) as compared to *P. foetida* (Amela García and Hoc, 1997). In all the *Passiflora* species studied so far, stigma receptivity lasts the whole anthesis (Amela García, 1999), so anthesis



**Table 4.** Stigma area of four *Passiflora* species, thorax area of their pollinators and stigma area/pollen-bearing area (SA/PBA)

Species	stigma area	pollinator	thorax area	SA/PBA
<i>P. caerulea</i>	46.15 <sup>b</sup> ±12.56 n=24	<i>Xylocopa frontalis</i>	101.9±18.57 n=10	0.43±0.12
		<i>Xylocopa artifex</i>	43.7±7.41 n=10	1.03±0.35
		<i>Xylocopa nigrocincta</i>	87.8±11.14 n=10	0.49±0.08
		<i>Xylocopa augusti</i>	84.6±13.35 n=10	0.53±0.17
<i>P. foetida</i>	32.45 <sup>a</sup> ±7.48 n=10	<i>Ptiloglossa tarsata</i> (male)	116.4±9.15 n=10	0.58±0.20
		<i>Ptiloglossa tarsata</i> (female)	43.1±11.25 n=10	0.77±0.18
		<i>Pseudaugochloropsis graminea</i>	12.5±2.12 n=10	2.69±0.99
<i>P. misera</i>	26.26 <sup>a</sup> ±5.86 n=10	<i>Ptiloglossa</i> sp.	42±0 n=2	0.64±0
		<i>Xylocopa artifex</i>	43.7±7.41 n=10	0.62±0.21
<i>P. suberosa</i>	21.28 <sup>a</sup> ±5.14 n=18	-----	-----	-----

Numbers within each cell represent averages±standard deviation and n=number of measured specimens. Dimensions are in mm. Values with different letters differ significantly at  $p<0.01$ .

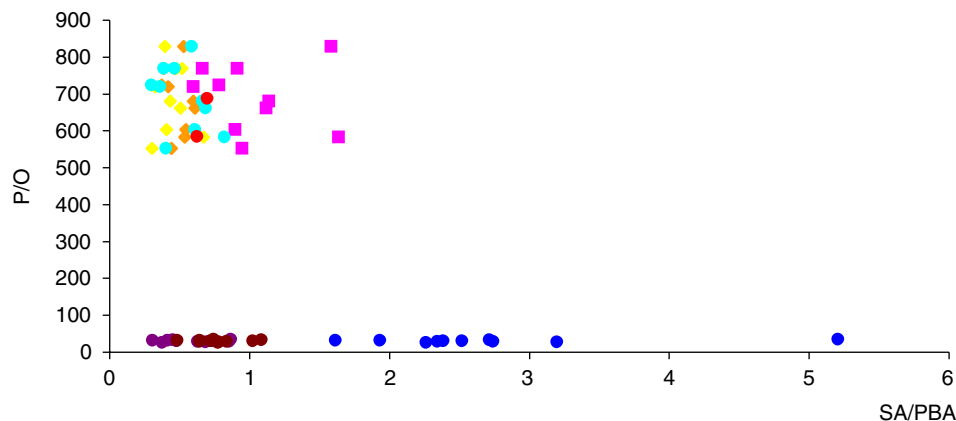
duration is a direct indicator of receptivity in them. A positive relation between P/O ratio, degree of outcrossing and flower life span occurred between different populations analyzed by Wyatt (1984) and species analyzed by Choteau & al (2006). A tendency in these traits may be seen in the *Passiflora* species analyzed: anthesis duration of *P. foetida*<*P. suberosa* similar to *P. misera*<*P. caerulea*.

Among the vines studied, there is also a trend between degree of self-compatibility, P/O and longevity. The self-compatible species, with lower P/O ratio, are annuals (the whole plant or the aerial stems) or biannuals (except from *P. suberosa*, that lives longer) whilst the self-incompatible ones, with higher P/O ratio, render successfully more years. This tendency, discovered by Cruden (1977), was further confirmed in various taxa (Small, 1988; Plitman and Levin,

1990; Galloni & al., 2000; Jürgens & al., 2002; Choteau & al., 2006) with isolated exceptions (López & al., 1999).

### *P/O, pollen grain size, pollen vitality and breeding system*

Although the pollen grains of the examined *Passiflora* species were large (*P. foetida* and *P. caerulea*) or medium-sized (*P. misera* and *P. suberosa*), considering the categories established by Erdtman (1952), no significant correlation was found between P/O and pollen grain size, in contrast to predictions by Cruden & Miller-Ward (1981). This relation varies among taxa: positive but weak for Asclepiadaceae (Wyatt, 1984), null for Genisteae (López & al., 1999) and negative for other taxa (Cruden and Miller-Ward, 1981; Wyatt, 1984; Cruden, 2000). Götzenberger & al. (2007)



**Fig. 3.** Relationship between pollen/ovule (P/O) and stigma area/pollen-bearing area of the pollinators (SA/PBA) of four *Passiflora* species. Symbols: yellow=*Xylocopa frontalis*, pink=*Xylocopa artifex*, orange=*Xylocopa nigrocincta*, light blue=*Xylocopa augusti*, violet=*Ptiloglossa tarsata* (male), brown=*Ptiloglossa tarsata* (female), red=*Ptiloglossa* sp., blue=*Pseudaugochloropsis graminea*.

concluded from 311 angiosperms that P/O depends on pollen grain number but not size. In *Passiflora*, pollen grain size is related with taxonomic position (Amela García & al., 2002) rather than with breeding system.

As in *P. misera*, more than one pollen grain size classes were encountered in the same anther of several Caryophylloideae but, unlike *Passiflora*, number of grains in each size class was dissimilar (Jürgens & al., 2012). A bimodal pollen size distribution with the larger grains viable and the smaller ones aborted was also detected in a conifer (Aizen and Rovere, 1995). In the species with two pollen size classes, in which the smaller ones are not vital, the useful P/O ratio would be minor than the estimated one, nearly the half in the examined *Passiflora* species. So pollen vitality, i.e., proportion of pollen grains capable of germination, should be taken in account in pollen or P/O counts.

All the *Passiflora* species studied exhibited high pollen vitality, regardless of their breeding system. Relatively high degrees of pollen vitality have been detected for self-incompatible species (Wild & al., 2003; Hoc and Amela García, 1998) as well as self-compatible ones (Hoc and Amela García, 1999; Lehnebach and Riveros, 2003). Low levels of pollen vitality occur in species with different degrees of inbreeding and similar pollen longevities were found in two out of four *Cyclamen* species with the most opposite levels of inbreeding (Affre and Thompson, 1998). So pollen vitality seems to have no relation with breeding system.

In any case, the high pollen vitality suggests that the majority of the grains would be able to fertilize the conspecific ovules, and, as the transfer is directional (by means of constant visitors), a great part of the grains would reach their destiny. Besides, in *P. caerulea*, vitality is not affected by rain (Amela García and Hoc, 1997), as in well protected anthers (Hoc and Amela García, 1999) but not in very exposed ones (Hoc & al., 1995). In addition, the high vitality benefits the pollinators that feed their larvae with pollen, most *Passiflora* spp. pollinators carried *Passiflora* pollen in their hind legs (Amela García, 1999), being profitable the major part of the pollen load. In the species with large vital grains and small non vital ones, there would be a tendency to diminish the energetic cost of the pollen loss that the hymenopterans use for nesting, offering a certain non vital quantity; but that kind of grains also can reach the stigmas, as they are in the same anthers as the big ones, so this strategy would be inefficient.

### ***P/O, flower size, stigma area and SA/PBA***

The positive correlation between flower size with P/O was also found in 14 *Vicia* spp. (Ortega-Olivencia & al., 1997) but not in 21 papilionoid legumes (Etcheverry & al., 2012). P/O ratio was not correlated with stigma area in the *Passiflora* species analysed, perhaps because stigma area was not so different between them. On the contrary, P/O ratio was inversely related to the stigmatic area in two species of Stylidaceae and eight species from three genera of Proteaceae (Cruden, 1997). In the last ones, taxa with higher P/O produced larger number of smaller pollen grains and had smaller stigmas (Cruden, 1997). The co-occurrence of large stigmas with many ovules was corroborated in several species (Cruden and Miller-Ward, 1981).

In spite of the different degree of autogamy of the studied species and the minor than expected P/O ratio in the self-incompatible and with more efficient pollination ones, P/O index was inversely and strongly related with the SA/PBA, as in the 19 xenogamous angiosperms studied by Cruden and Miller-Ward (1981). It is interesting to note that the pollinators that do not always pollinate, due to their behavior on the flowers, i.e., *X. artifex* in *P. caerulea* and *Pseudaugochloropsis graminea* in *P. foetida* (Amela García and Hoc, 1997 and 1998a, respectively), have smaller pollen-bearing areas and, in consequence, a major SA/PBA resulted, i.e., a lower probability of pollination.

### ***P/O and sexual system***

Hermaphroditic species exhibit lower P/O ratios and larger pollen grains than sexually dimorphic congeners (Weller & al., 1998); lower P/O ratios have been computed for more than a hundred hermaphroditic taxa as compared to those with other sexual systems (see Cruden, 2000, Fig. 4). Having this in mind, the hermaphroditic flowers of *Passiflora* would be expected to have low P/O ratios; however, other factor needs to be considered: a certain proportion of the flowers of at least 13 *Passiflora* spp. do not bend the styles during anthesis, thus the stigmas do not get in contact with the pollen (either of the same flower or carried by a pollinator), turning the sexual system functionally andromonoecious (Amela García and Hoc, 2011 and cites therein). Considering this sexual system, the P/O ratio would be greater, as pollen grain number varies as a function of the percentage of male flowers (Cruden, 2000), either they are morphologically or functionally staminate. P/O from andromonoecious species are greater than from homoecious ones (Cruden, 2000).

### ***P/O and taxonomy***

The P/O index was lower than expected in *P. caerulea* and *P. misera* whereas it approached the expected values for *P. foetida* and *P. suberosa*, so the P/O index did not reflect taxonomic affinities in the species analysed. Wyatt & al (2000) concluded that pollen grain and ovule number did not reflect taxonomy but ecogeographical pressures in 38 species from 4 tribes of Asclepiadoidea. Lack of consistency between phylogenetic relationships and P/O was also detected by Mione and Anderson (1979) cited in Cruden (2000) and Affre and Thompson (1998), who found P/O more related with ecology. Instead, a tendency to xenogamy or autogamy within various taxa at different levels (genus or tribe) of Polemoniaceae (in which P/O ratio is a good predictor of the breeding system) was perceived by Plitmann and Levin (1990). P/O variations were associated to genus but not to tribe in 21 papilionoid Legumes (Etcheverry & al., 2012). Jürgens & al (2002) suggested that taxonomy is indirectly related with P/O as a consequence of the combination of features typical of each taxonomic group. P/O, like other traits related to pollination, are open to selection; as random evolution also takes part in phylogeny, P/O could not necessarily reflect taxonomic affinities, as suggested by Affre and Thompson (1998) and Galloni & al. (2007).

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