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RESEARCH PAPER

# Pollen:ovule ratio and its relationship with other floral traits in Papilionoideae (Leguminosae): an evaluation with Argentine species

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## ABSTRACT

**The pollen:ovule ratio (P/O) has traditionally been used as a rough estimator of plant breeding systems. It has been shown that plant breeding systems are associated with particular floral traits. In this study, we determined the P/O in 21 Leguminosae species from Argentina and explored relationships between P/O and taxonomic position, flower size, floral rewards, pollen presentation and pollination mechanisms. According to the results, 15 out of the 21 species classified were obligate xenogamous, although some of them have been recorded as facultative xenogamous in previous studies. There was a significant effect of taxonomic position (genus), reward type and pollination mechanism on P/O. Species offering only nectar as a floral reward (which were species with a brush mechanism) had a significantly lower P/O than species offering pollen or pollen and nectar. Species with the brush pollination mechanism had the lowest P/O, while species with valvular and pump mechanism had the highest P/O. However, pollen presentation (primary and secondary) and flower size did not have a significant effect on P/O. Our results demonstrate that P/O variability is determined by taxonomic position and pollination mechanism in this plant group.**

## INTRODUCTION

Cruden (1977) proposed that the pollen:ovule ratio (P/O) is a good estimator of the breeding system of a plant. In his study, Cruden (1977) determined the P/O in 80 different species, concluding that the greater the degree of autogamy, the lower the P/O. Cruden (2000) considered that the P/O indicated the efficiency with which pollination takes place, *i.e.* the likelihood of a pollen grain reaching the stigma to result in maximum seed set. Thus, autogamous plants would have a low P/O because pollination efficiency in these plants is higher than that in outcrossing plants. A number of studies have confirmed the validity of the P/O as an indicator of the plant breeding system (Schoen 1977; Campbell *et al.* 1986; Plitmann & Levin 1990; Mione & Anderson 1992; Jürgens *et al.* 2002; Michalski & Durka 2009). Cruden's hypothesis of pollination efficiency has been criticised because it considers only the individual fitness in terms of pollen limitation and its implications for seed production. For example, Charnov (1982) suggested that local mate competition in hermaphrodite plants and sex allocation of resources should also be considered, because both the male and female sexual functions are equally important for fitness. This model states that plants with a certain degree of autogamy will experience strong inbreeding. Although these hypotheses were proposed independently, they are not mutually exclusive. Thus, either local mate competition or pollination efficiency, or both, may cause a lower relative allocation of resources to the male function in inbreeders compared to closely related outcrossers (reviewed in Delesalle & Mazer 2009).

Numerous factors, both intrinsic and extrinsic, may determine the evolution of pollen and ovule number, and thus the P/O. Among the intrinsic factors are the sexual system of the species (*e.g.* monoecy, dioecy, gynodioecy), the evolutionary history of the species, plant size, rewards offered, flowering time, life form, pollination mechanisms and pollen presentation, among others. On the other hand, among the extrinsic factors are pollen vectors (groups and availability), nutrient availability, herbivory and location of populations within the species' range of distribution (periphery *versus* central), among others (*e.g.* Cruden & Jensen 1979; Lord 1980; Cruden & Miller-Ward 1981; Queller 1984; Wyatt 1984; Cruden & Lyon 1989; Cruden 2000; Busch 2005; Delesalle & Mazer 2009). For instance, the P/O varies among plants depending on the floral rewards offered. Flowers offering nectar and pollen, or only pollen, as a reward tend to have higher P/O ratios than those offering only nectar as a reward for pollination services (Vogel 1978; Dulberger 1981). Species offering only pollen as a reward produce it in great quantities because it is necessary to compensate for the amount of pollen consumed by pollinators. In contrast, species with special pollen-transporting structures (pollinia, viscin threads, polyads) have lower P/Os than species lacking these structures because pollen aggregation enhances pollen transfer efficiency (Cruden 1977; Cruden & Jensen 1979; Schlising *et al.* 1980; Mehrhoff 1983; Koptur 1984; Harder & Johnson 2008).

Concerning the extrinsic factors, it has been demonstrated that pollen vectors affect the P/O (*e.g.* Cruden 2000). For example, wind-pollinated species have a much higher P/O than their insect-pollinated relatives because wind is a less efficient mechanism for pollen dispersal than insects, such that a high number of pollen grains are lost during the process (*e.g.* Melampy & Hayworth 1980; Ackerman 2000; Michalski & Durka 2009; Friedman & Barrett 2011). Howell *et al.* (1993), Yeo (1993) and Cruden (2000) suggested that the P/O from animal-pollinated plants depends on the mechanism of pollen presentation. In plants with primary pollen presentation, pollen is delivered directly from the anthers to the vector's body. On the other hand, in plants with secondary pollen presentation, pollen grains are delivered first on a floral part (*e.g.* the keel petals in Papilionoideae) and then on the body of the vector. Therefore, secondary pollen presentation would imply an accurate delivery of pollen on the vector's body, efficient pollination and a low P/O (Howell *et al.* 1993).

The ecological and evolutionary success of Leguminosae has been related to biotic pollination mechanisms (Arroyo 1981; Schrire 1989). Each one of the three subfamilies within the Leguminosae has achieved a characteristic floral architecture, in which plants within the subfamily Papilionoideae have developed the most complex floral mechanisms.

Plants within the Papilionoideae have keel zygomorphic flowers that are mainly bee-pollinated (Faegri & Van der Pijl 1979; Westerkamp 1997); although bird pollination and bat pollination have also been recorded within the subfamily (Arroyo 1981; Bruneau 1997; Etcheverry & Trucco Alemán 2005; Ortega-Olivencia *et al.* 2005). It has been proposed that in bee-pollinated flowers, each part of the corolla is specialised for a particular role in pollinator attraction and the success of pollination. For example, the vexillum usually helps to attract pollinators; the keel protects androecium and gynoecium and, together with the wings, provides a platform for the insects to land on. The wings also operate as levers that raise or lower the keel (Arroyo 1981; Stirton 1981).

Within the Papilionoideae, primary and secondary pollen presentations have been recorded associated with the four types of basic pollination mechanisms (valvular, pump, explosive and brush). In the valvular type, pollen presentation is primary, whereas in the other three mechanisms it is secondary (Yeo 1993; Leppik 1966; Westerkamp 1997). In the explosive mechanism, commonly only one pollination event occurs and it has evolved independently in several tribes (Small 1988), while in the other three mechanisms, repeated visitation is possible (Westerkamp 1997). The four pollination mechanisms are associated with a particular floral architecture and kinetics (Westerkamp 1997).

It has been suggested that within Papilionoideae, the P/O varies according to the pollination mechanism (Arroyo 1981; Small 1988). For instance, Arroyo (1981) and Small (1988) suggested that plants with the explosive mechanism have a low P/O because a single pollinator visit is needed for efficient transference of pollen. However, there is a lack of studies determining the P/O within the Leguminosae and its relationship with pollination mechanisms. There are only four studies addressing this question (Small 1988; López *et al.* 1999; Rodríguez-Riaño *et al.* 1999; Galloni *et al.* 2007). Small (1988) analysed the P/O associated with pollination mechanisms in the tribe Trifolieae. He found that the P/O ratio for *Medicago,* which has an explosive mechanism, was three to 11 times smaller than that of the other five genera within the tribe, which have other mechanisms of pollen presentation. Small (1988) argued that the low P/O estimated for *Medicago* is a consequence of its highly specialised, irreversible pollination mechanism, which allows only one effective exchange of pollen with pollinators. López *et al.* (1999) determined P/Os in 34 species within the tribe Genisteae. They found that, contrary to expectations, the highest P/Os were recorded in taxa with the specialised explosive mechanism. Rodríguez-Riaño *et al.* (1999) analysed the P/O associated with pollination mechanisms in 168 taxa within nine tribes (Galegeae, Genisteae, Hedysareae, Loteae, Trifolieae, Psoraleae, Robinieae, Thermopsideae, Trifoleae and Vicieae) and reported that the highest P/O ratio was presented by the pump type. The study of Galloni *et al.* (2007) in 32 Mediterranean legume species showed that species with the brush mechanism had the lowest P/O, the intermediate value corresponded to the explosive mechanism, while the highest P/O was associated with the pump and valvular mechanisms. Clearly, further studies are required in order to test the generality of these patterns.

In this study, we determined P/O in 21 Argentine Papilionoideae species and analysed the relationship between the P/O and (i) taxonomic position, (ii) flower size, (iii) floral rewards (pollen, nectar or pollen and nectar), (iv) pollen presentation (primary or secondary) and (v) pollination mechanism (valvular, pump, brush or explosive).

## MATERIALS AND METHODS

### Study species

The studied species belong to five tribes: Dalbergieae (one species), Crotalarieae (four species), Desmodieae (four species), Indigofereae (two species), and Phaseoleae (10 species; see Table 1). All species are bee-pollinated (Etcheverry *et al.* 2001, 2003, 2008a,b; M. Alemán and T. Figueroa Fleming unpublished data). Voucher specimens are deposited in the Museo de Ciencias Naturales, Universidad Nacional de Salta (MCNS).

### Study area

The study was carried out in the Lerma Valley (Salta Province, Argentina), during 3 consecutive years (2007–2009). The study area (24.34.53°–25.31.38° S and 65.22.30°–

**Table 1.** Flower size, floral rewards, pollination mechanism (PM), pollen presentation type (PP), pollen, ovules, P/O ratio and breeding system (BS) following Cruden (1977), in Papilioinoideae from Northwestern Argentina.

| tribe | genus | species | flower size (mm) | floral reward | PM | PP | pollen | ovules | P/O ratio | BS |
|---|---|---|---|---|---|---|---|---|---|---|
| Crotalarieae | *Crotalaria* | | | | | | 203956.2 ± 105522.3 | 22.9 ± 12.7 | 9299.0 ± 3156.1 | |
| | | *C. incana* | 15.7 ± 0.8 | Nectar + pollen | P | Se | 308850.0 ± 41427.3 | 33.6 ± 5.2 | 9539.5 ± 2836.2 | OX |
| | | *C. micans* | 23.0 ± 2.4 | Nectar + pollen | P | Se | 199694.4 ± 48219.3 | 15.4 ± 1.5 | 13004.3 ± 2978.4 | OX |
| | | *C. pumila* | 8.2 ± 0.5 | Nectar + pollen | P | Se | 61909.1 ± 12448.0 | 8.1 ± 0.5 | 7684.0 ± 1614.2 | OX |
| | | *C. stipularia* | 8.1 ± 2.1 | Nectar + pollen | P | Se | 259150.0 ± 63299.5 | 35.4 ± 7.0 | 7500.2 ± 1939.5 | OX |
| Dalbergieae | *Zornia* | | | | | | | | | |
| | | *Z. contorta* | 9.8 ± 0.5 | Pollen | P | Se | 76750.0 ± 10588.9 | 8.9 ± 0.3 | 8604.5 ± 1071.9 | OX |
| Desmodieae | *Desmodium* | | | | | | 34218.6 ± 12733.5 | 7.1 ± 1.1 | 4962.9 ± 1840.4 | |
| | | *D. incanum* | 8.9 ± 5.5 | Pollen | E | Se | 30563.6 ± 6435.9 | 6.82 ± 1.0 | 4599.1 ± 1317.1 | OX |
| | | *D. pachyrrhizum* | 8.8 ± 0.1 | Pollen | E | Se | 32610.0 ± 4619.1 | 5.7 ± 0.7 | 5771.6 ± 927.4 | OX |
| | | *D. subsericeum* | 9.1 ± 0.7 | Pollen | E | Se | 22290.9 ± 4173.8 | 7.82 ± 1.0 | 2942.11 ± 923.5 | FX |
| | | *D. uncinatum* | 13.5 ± 1.7 | Pollen | E | Se | 51263.6 ± 10468.3 | 7.8 ± 0.4 | 6612.5 ± 1606.1 | OX |
| Indigofereae | *Indigofera* | | | | | | 38904.8 ± 15762.8 | 7.2 ± 1.3 | 5817.71 ± 3070.3 | |
| | | *I. parodiana* | 5.1 ± 0.3 | Nectar + pollen | E | Se | 27272.7 ± 4002.3 | 8.2 ± 0.9 | 3369.9 ± 638.9 | OX |
| | | *I. suffruticosa* | 9.8 ± 2.5 | Nectar + pollen | E | Se | 51700.0 ± 13677.2 | 6.1 ± 0.6 | 8510 ± 2261.9 | OX |
| Phaseoleae | *Cologania* | | | | | | | | | |
| | | *C. broussonetii* | 25.5 ± 1.2 | Nectar + pollen | V | Pr | 165900.0 ± 31656.8 | 14.5 ± 2.0 | 11665.1 ± 2841.4 | OX |
| | *Galactia* | | | | | | | | | |
| | | *G. latisiliqua* | 10.4 ± 0.2 | Nectar + pollen | V | Pr | 41987.5 ± 7274.3 | 8.19 ± 0.7 | 5200.4 ± 1225.9 | OX |
| | *Macroptilium* | | | | | | 15439 ± 7745.5 | 8.9 ± 4.8 | 2025.9 ± 1298.33 | |
| | | *M. erythroloma* | 10.1 ± 3.8 | Nectar | B | Se | 8725.0 ± 1537.0 | 7.7 ± 0.7 | 1134.0 ± 203.8 | FX |
| | | *M. fraternum* | 10.2 ± 0.9 | Nectar | B | Se | 20550.0 ± 8655.3 | 5.6 ± 0.5 | 3676.67 ± 1575.1 | OX |
| | | *M. lathyroides* | 20.2 ± 0.8 | Nectar | B | Se | 22968.7 ± 6357.6 | 18.1 ± 1.2 | 1264.1 ± 321.7 | FX |
| | | *M. panduratum* | 21.34 ± 0.96 | Nectar | B | Se | 1575.0 ± 823.2 | 6.1 ± 0.7 | 2054.8 ± 295.5 | FX |
| | *Phaseolus* | | | | | | | | | |
| | | *P. vulgaris* var. *aborigineus* | 17.15 ± 1.31 | Nectar | B | Se | 2612.0 ± 610.3 | 6.1 ± 0.3 | 427.9 ± 97.6 | FX |
| | *Rhynchosia* | | | | | | 27636 ± 6395.9 | 2.1 ± 0.3 | 13398 ± 3162.1 | |
| | | *R. edulis* | 11.31 ± 1.35 | Nectar + pollen | V | Pr | 27993.3 ± 5925.8 | 2.0 ± 0.0 | 13996.7 ± 2962.9 | OX |
| | | *R. senna* var *texana* | 7.56 ± 0.06 | Nectar + pollen | V | Pr | 27100.0 ± 7343.9 | 2.2 ± 0.4 | 12500.0 ± 3393.2 | OX |
| | *Vigna* | | | | | | | | | |
| | | *V. caracalla* | 48.56 ± 5.25 | Nectar | B | Se | 14033.3 ± 2924.4 | 17.1 ± 2.5 | 844.7 ± 243.7 | FX |

V = valvular; P = pump; E = explosive; B = brush; Pr = primary pollen presentation; Se = secondary pollen presentation; OX = obligate xenogamous; FX = facultative xenogamous.

Values are means ± SD. Means by genus are reported only in those cases where more than one species was studied.

65.39.70° W) extends along the transition zone between Yungas seasonal rain forest and the Chaco dry forest (Olson *et al.* 2001). There is a strong seasonality in the area, with rains concentrated between November and May. Mean annual precipitation is 662.58 mm (Bianchi & Yáñez 1992), whereas mean annual temperature is 17.55 °C (Bianchi 1996).

### Determination of pollen:ovule ratios

For each species, pollen and ovule number were estimated from 10 randomly selected flower buds (from 10 individuals). All anthers from each single flower were softened in a 70% ethanol solution; then anthers were transferred to 0.5 ml ethanol/detergent solution, and macerated with a glass rod. In order to homogenise the mixture, the macerated samples were vortexed for 60 s. Immediately after vortexing, a sample was placed in a haemocytometer and pollen grains were counted. This value was then used to estimate the total number of grains per flower, following Dafni (1992) and Kearns & Inouye (1993). Ovule number was directly determined from dissections of ovaries under a stereoscopic microscope.

### Taxonomic position

Taxa were grouped either into tribes (Crotalarieae, Dalbergieae, Desmodieae, Indigofereae, and Phaseoleae) or into genera (*Crotalaria*, *Cologania*, *Desmodium*, *Galactia*, *Indigofera*, *Macroptilium*, *Phaseolus*, *Rhynchosia*, *Vigna* and *Zornia*) to test association between the P/O and taxon. Tribe classification follows Lavin *et al.* (2005) and the species were named according to Zuloaga *et al.* (2008).

### Floral size and rewards

Flower size and rewards offered to pollinators were studied on January–April 2007–2009. Flower size was determined from 10 randomly collected flowers from 10 individuals per species. All flowers were collected right at the beginning of anthesis. Flowers were preserved in 70% ethanol solution and brought to the laboratory for analyses. Flower size was estimated as the maximum length of the flower on its vexillar side, *i.e.* from the base of the calyx to the tip of the vexillum. Floral measurements were conducted with digital calipers. Flowers from all species were grouped according to three categories of flower length: (i) <10 mm, (ii) >10 mm and ≤20 mm, and (iii) >20 mm. Because the flowers from *Vigna caracalla* were larger than the other species, analyses were conducted including and excluding this species.

Flowers were classified according to the kind of reward they offered: (i) nectar and pollen, (ii) pollen and (iii) nectar. In order to do so, the existence of nectar in flowers from each species was determined. Sampled flowers (25–30 flowers per species; one to two flowers per individual) were bagged at the bud stage to avoid pollinator visits on flowers before determining, qualitatively, the nectar secretion. For this, each flower in anthesis was dissected in the field, recording presence–absence of nectar secretion. A flower with both nectar and pollen rewards had nectar as well as pollen available for pollinators. On the other hand, a flower offering only pollen did not produce nectar. A flower offering only nectar had nectar secretions, but pollen was not available for pollinators (as described for some legume species with the brush mechanism; Yeo 1993).

### Pollen presentation and pollination mechanisms

For each species, pollen presentation and pollination mechanisms were determined in January–April 2007 and 2009. First, we analysed floral morphology and floral parts functioning in pollination mechanism on fresh sample flowers ($n = 10$ flowers from 10 individuals) previously brought to the laboratory, according to Galloni *et al.* (2007). Second, the kinetics of the 20 flowers triggered by animal visitors and the site of pollen deposition on the pollinator body, were observed and recorded in detail in the field. Moreover, we observed whether the pollen was available directly in the anthers (primary pollen presentation) or if it was deposited in another part of the flower previous to the visit (secondary pollen presentation). Based on these observations, we determined whether the species had primary or secondary pollen presentation and their type of pollination mechanism (valvular, explosive, pump and brush mechanisms).

### Statistical analyses

A mixed effect model using tribe as a fixed factor and genera nested within tribes and species nested within genera as random factors, was fitted to the P/O data. We considered heterogeneity of variance among genera, as it achieved a better model fit according to Akaike's information criterion (Pinheiro & Bates 2004). We estimated the relative contribution of each random factor by dividing each component of the variance by the sum of its components (genus within tribe + species within genus + residual). We adjusted the same mixed model, but we replaced the tribe factor either by pollination mechanism, reward type, flower size or pollen presentation as factors affecting the P/O. All statistical analyses were conducted using the statistical program INFOSTAT (2009).

## RESULTS

### Pollen, ovule and pollen:ovule ratios

Mean (±SD) pollen grain number ranged from 2612 ± 610.3 in *Phaseolus vulgaris* var. *aborigineus* to 308850 ± 41427.3 in *Crotalaria incana*; mean ovule number ranged from 2.0 ± 0.0 in *Rhynchosia edulis* to 35.4 ± 7.0 in *Crotalaria stipularia* (Table 1). Mean P/O ranged from 427.9 ± 97.6 in *Phaseolus vulgaris* var. *aborigineus* to 13996.7 ± 2962.9 in *Rhynchosia edulis*. In our sample, there was a 33-fold range in P/O (Table 1). According to Cruden's (1977) classification of breeding systems, the studied species belong to the facultative or obligate xenogamy categories.

Differences in mean values of P/O among tribes were not statistically significant ($F_{4,\ 210} = 0.16$, $P = 0.94$). The P/O variation was attributed to differences among genera (60%).

### Floral size and rewards

Flower size was highly variable among the studied species. The largest flowers were recorded for *Vigna caracalla* (mean ± SD; 48.56 ± 5.25 mm), whereas *Indigofera parodiana* had the smallest (5.09 ± 0.27 mm; Table 1). Flower size did not have a significant effect on the P/O ($F_{2,\ 210} = 1.02$, $P = 0.4$). The greatest variation was related to genera, representing 55% of all variation. When *V. caracalla* was excluded from the analysis, no significant differences were recorded ($F_{2,\ 210} = 0.27$, $P = 0.77$), with the greatest variation also attributed to the genera (55% of all variation).

Of the 21 species studied, five species offered only pollen as floral reward, six species offered only nectar and the remaining 10 offered both nectar and pollen (Table 1). The statistical analysis showed that the P/Os from flowers that offered pollen (mean ± SD; 9187 ± 4148.4), pollen and nectar (5704.7 ± 2256.7) and only nectar as floral reward (1507.5 ± 1222.5) were significantly different ($F_{2,\ 210} = 7.45$, $P < 0.019$; Fig. 1). Species offering only nectar as a reward had a significantly lower P/O than species offering only pollen or nectar and pollen. The greatest variation was attributed to the genera (39.16% of all variation).

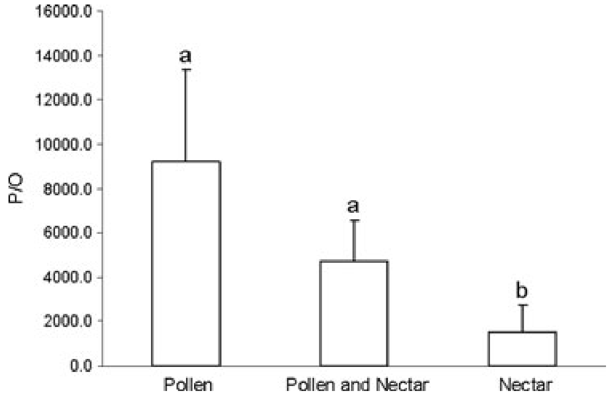

**Fig. 1.** Pollen/ovule ratio (mean ± SD) of different floral reward types within the Papilionoideae (Leguminosae) from Argentina. Different letters indicate significant differences ($P < 0.05$).

### Pollen presentation and pollination mechanisms

Secondary pollen presentation was predominant within the studied species (17 out of the 21 species; Table 1). The P/O was higher in species with primary pollen presentation (mean ± SD; 10486.4 ± 4490.1) than in species with secondary pollen presentation (5001.0 ± 3748.8). However, this difference was not statistically significant ($F_{1,\ 210} = 4.29$, $P = 0.072$). The greatest variation was attributed to the genera (48.6% of all variation). The explosive and brush types were more frequent (six species in both groups). The pump type was recorded within the tribes Dalbergieae and Crotalarieae, whereas the explosive type (sternotribic model) found within the tribes Desmodieae and Indigofereae. The valvular and brush types were recorded in species within the Phaseoleae (Table 1).

There was a significant effect of pollination mechanism on the P/O ($F_{3,\ 210} = 9.26$, $P = 0.011$). Species with valvular and pump pollination mechanisms (10486.4 ± 4490.1 and 9149.2 ± 2842.9, respectively) had higher P/Os than species with the other pollination mechanisms. The smallest P/O was recorded for species with the brush mechanism (1507.4 ± 1222.5). Species with the explosive mechanism had an intermediate P/O (5243.4 ± 2326.9; see Fig. 2). The greatest variation was related to the species, representing 38.9% of all variation.

## DISCUSSION

### Pollen:Ovule ratio and breeding system

The studied species had relatively high P/Os (Table 1). Following Cruden's classification of breeding system based on the P/O, all of the studied species could be classified as either obligate (71%) or facultative (29%) xenogamous. However, experimental studies with some of the studied species (*Collogania ovalifolia*, *Crotalaria incana*, *C. micans*, *C. pumila*, *C. stipularia*, *Desmodium incanum*, *Galactia latisiliqua*, *Macroptilium erythroloma*, *M. fraternum*, *Rhynchosia edulis*, *Vigna caracalla* and *Zornia contorta*) have shown that the species classified as obligate are facultative xenogamous (Etcheverry *et al.* 2001, 2003, 2008a,b). In other Leguminosae, inconsistencies between P/Os and breeding system have also been recorded. For example, P/O is not significantly correlated with autofertility in *Astragalus* (Gallardo *et al.* 1994). Therefore, it seems that the P/O is not always a good indicator of breeding system within the Papilionoideae. Similar discrepancies between breeding system estimated *via* the P/O and realized breeding system have been reported for plants from other families (Vasek & Weng 1988; James & Knox 1993; Ramírez & Seres 1994; Sneddon 1999; Ng & Corlett 2000; Bosch *et al.* 2001; Wang *et al.* 2004; Chouteau *et al.* 2006). For example, the dioecious species *Silene dioica* and *S. latifolia* are obligate outcrossers; however, very low P/Os have been recorded for both of these species (Jürgens *et al.* 2002). Therefore, even when the P/O is useful in plant reproductive biology studies, available evidence suggests that the P/O is not a fully adjusted indicator of breeding system.

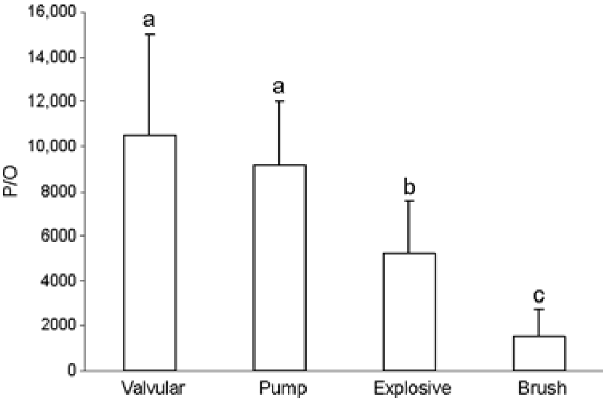

**Fig. 2.** Pollen/ovule ratio (mean ± SD) of different pollination mechanisms within the Papilionoideae (Leguminosae) from Argentina. Different letters indicate significant differences ($P < 0.05$).

### Pollen:Ovule ratio and taxonomic position

Our results show significant relationships between the P/O and taxonomic position, as well as some floral traits (*i.e.* pollination mechanisms and reward type). We found that the P/O variation among species can be explained by their taxonomic position, specifically to genus but not to tribe. This result differs from Galloni *et al.* (2007), who found that the P/O varies among tribes in Mediterranean legumes. Our study recorded significant differences in P/O only when the species were grouped to pollination mechanism or to reward type.

### Floral size and rewards

Our results did not show a relationship between flower size and the P/O. Flower size has been associated with the degree of outbreeding in insect-pollinated species; where plants with large flowers are assumed to be more attractive for pollinators, and so have a high frequency of visits, thus being outcrossed. On the other hand, plants with small flowers are less attractive; therefore they have a low frequency of visits by pollinators and strongly tend to selfing (Kevan 1984; Wyatt 1988). According to this pattern, one might expect that plants with large flowers would have a high P/O, whereas plants with small flowers would have a low P/O, which is not supported in our study. However, positive correlations between P/O and flower size within the Leguminosae have been recorded in other studies (Ortega-Olivencia *et al.* 1997; Rodríguez-Riaño *et al.* 1999; Galloni *et al.* 2007). Small (1988) showed that flowers from inbreeding species are smaller than those from outbreeders, but flower size was not significantly correlated with the P/O.

A variation in P/O has also been attributed to the kind of floral reward offered. For example, it has been suggested that plants offering only pollen will have a higher P/O than flowers offering nectar or any other rewards, because the flowers need to produce extra pollen to compensate for pollen consumed by the pollinators (Vogel 1978; Dulberger 1981; Pellmyr 1985; Mione & Anderson 1992; Cruden 2000). Our results agree with this pattern. We found that species offering pollen or pollen + nectar had a higher P/O than species offering only nectar. However, all of the studied plants offering only nectar had the brush mechanism of pollination. Therefore, differences in P/O between this group and species offering other rewards might be an effect of the pollination mechanism. Further studies will be necessary to unmask these effects.

### Pollen presentation and pollination mechanism

Secondary pollen presentation has been recorded in numerous species of different families of flowering plants (Yeo 1993). This pollen presentation type has traditionally been described as a mechanism that enhances the efficiency and accuracy of pollen export and/or delivery and might be associated with efficient pollen vectors (Howell *et al.* 1993; Yeo 1993). Therefore, species with secondary pollen presentation should have a lower P/O than those having primary pollen presentation (Yeo 1993). Our results agreed with this trend; however, such differences are not significant according to Cruden (2000). Thus, pollen presentation *per se* does not explain the P/O variation, since a higher efficiency in pollen transfer is associated with other floral traits related to pollination mechanism type (*e.g.* floral morphology and kinetics).

We recorded a significant relationship between the P/O and pollination mechanism, as recorded in other studies (Small 1988; López *et al.* 1999; Rodríguez-Riaño *et al.* 1999; Galloni *et al.* 2007). For example, Galloni *et al.* (2007) found significant differences in P/O when comparing 34 Leguminosae species from the Mediterranean flora that differed in pollination mechanism. We found that species with the brush mechanism, in which pollen is transferred from the anthers to the trichomes of the style, presented the lowest P/O. This kind of pollination mechanism was recorded for species with complex, asymmetric flowers: *Macroptilium* spp., *Phaseolus vulgaris* var. *aborigineus* and *Vigna caracalla*, according to Lavin & Delgado (1990). The low P/Os recorded for these species might be associated with an efficient pollination system that assures that conspecific pollen grains are deposited on parts of the pollinators' body that cannot be groomed (Arroyo 1981; Yeo 1993; Galloni *et al.* 2007). This mechanism could therefore minimise pollen wastage and favour reproductive isolation among sympatric congener species with similar pollinators (Etcheverry *et al.* 2008a). As a consequence of this pollination mechanism, pollen is not a resource available to pollinators and the flowers behave typically as nectar flowers.

A high variation in P/O has been recorded for plants with an explosive pollination mechanism (Rodríguez-Riaño *et al.* 1999). For instance, Small (1988) found that *Medicago* species with this mechanism of pollination had the lowest P/O among species within the tribe Trifolieae. In contrast, López *et al.* (1999) recorded the highest P/Os in species with this pollination mechanism. In our study, plants with the explosive pollination mechanism presented an intermediate P/O, agreeing with Galloni *et al.* (2007). Our results, in comparison to those of other authors, indicate that the intermediate position of the explosive mechanism is related to inclusion in the analysis of all the mechanism types present in the legume family. In addition, and according to Rodríguez-Riaño *et al.* (1999), there is great variation in the P/O of species presenting this pollination mechanism within and among different lineages.

Our results showed that species with valvular and pump pollination mechanisms had the highest P/O. Similarly, Galloni *et al.* (2007) found that plants with these pollination mechanisms had the highest P/O within the Mediterranean legumes. The valvular and pump mechanisms are typical of basal clades within Papilionoideae and might be associated with less efficient pollen transfer systems (Arroyo 1981). Arroyo (1981) stated that the simplest pollination mechanism is the valvular one, from which the other three pollination mechanisms have evolved. Leppik (1966) and Arroyo (1981) affirmed that more efficient pollen transfer systems appear to be a major trend in the evolution of the Papilionoideae. Thus, some tribes will be expected to have a more efficient pollination mechanism; such as Phaseoleae, which mainly has the brush mechanism (Lavin & Delgado 1990). Our results showed that species within this tribe had the lowest P/O (Table 1). Likewise, Galloni *et al.* (2007) reported the smallest P/O for species within the Fabeae (Vicieae), which also have the brush mechanism.

In the present study, we studied a group of sympatric legumes that presented variations in the P/O. These variations can mainly be explained in relation to the type of pollination mechanism present in the species. Our results suggest that the P/O has evolved in association with pollination mechanism within this family of plants. Studies with species from different lineages will corroborate this trend. Although the current study has provided new insights on the reproductive biology of Papilionoideae, further comparative studies on the efficiency of pollen transfer, female and male fitness and presence of herkogamy involving related taxa, will allow a better understanding of the selective advantages and disadvantages of the different pollination mechanisms. The large diversity of floral traits involved in pollination mechanisms within the Papilionoideae makes them an excellent system to explore differences in the efficiency of pollen transfer (removal and deposition) and reproductive success among species with different pollination mechanisms.

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