

The relationship between the pollen–ovule ratio and seed size: a comparative test of a sex allocation hypothesis

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ABSTRACT

Hypothesis: Sex allocation theory predicts that the pollen–ovule ratio should increase linearly with increasing seed size among seed plants (Charnov, 1982).

Data examined: We retrieved data for the pollen–ovule ratio, seed size, and possible confounding variables (ovule number, plant height, mating system) from a database and additional literature for 299 gymnosperm plant species of the German flora.

Methods: We analysed uncorrected cross-species data as well as phylogenetically independent contrasts with Model II regressions and (partial) correlations.

Results: A linear positive correlation between pollen–ovule ratio and seed size was found to exist across all plant species analysed and within different mating systems for phylogenetically corrected and uncorrected data. This positive correlation remained valid when we controlled for the effect of possible confounding variables.

Conclusions: The interspecific variation of the pollen–ovule ratio depends, at least partly, on the allocation of resources to female sexual function.

Keywords: comparative analysis, mating system, pollen–ovule ratio, seed size, sex allocation.

INTRODUCTION

The pollen–ovule ratio is an important floral trait that reflects the mating system of a plant (e.g. Cruden, 1977; Preston, 1986; Bennett, 2001; Bosch *et al.*, 2001; Jürgens *et al.*, 2002) (following Neal and Anderson (2005), we will use the term ‘mating system’ instead of ‘breeding system’ throughout this article). Cruden (1977) was the first to observe that outbreeding plants tend to have higher pollen–ovule ratios. He explained this finding by the ‘efficiency of pollination’: self-pollinating plants (i.e. autogamous mating system) need less pollen grains for efficient pollination than plants dependent on agents such as wind or animals for pollination (i.e. xenogamous mating system). Charnov (1982) criticized this view for its bias towards seeds as the means to gain fitness (i.e. that the pollen only serves as a means to

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maximize seed set in a plant). He proposed that seeds and pollen should be equivalent means towards fitness gain. The model he developed based on sex allocation theory hypothesizes an isometric negative relationship between the log pollen–ovule ratio and log pollen grain size, and an isometric positive relationship between the log pollen–ovule ratio and log seed size. For the former relationship, he presented a regression analysis of data from Cruden and Millerward (1981) to support his hypothesis. In contrast, Gallardo *et al.* (1994) found no relationship between pollen–ovule ratio and pollen grain size but held inaccuracy in Charnov’s model responsible for this result. When controlling for additional factors in the model, an increase in the pollen–ovule ratio did indeed coincide with a decrease in pollen size. Both hypothesized relationships received support based on theoretical considerations (Queller, 1984). Also, support for the hypothesized positive relationship between seed size and the pollen–ovule ratio has previously been reported by several authors (Uma Shaanker and Ganeshiah, 1984; Preston, 1986; Mione and Anderson, 1992; Lopez *et al.*, 2000; Bosch *et al.*, 2001). However, these studies were conducted within the taxonomical limitations of single families (Brassicaceae), tribes (Delphinieae, Genisteae), or genera (*Phyllanthus*, *Solanum*).

To test the general applicability of Charnov’s model, a broad taxonomic range of species needs to be analysed. Furthermore, the phylogenetic relationship of the studied species should be accounted for because closely related species are likely to have similar phenotypes and data across species are therefore not independent (Felsenstein, 1985; Harvey and Pagel, 1991). In the present study, we included nearly 300 species of the German flora, belonging to 45 families. The phylogenetic relatedness was incorporated by using phylogenetically independent contrasts (Harvey and Pagel, 1991). The main objectives were: (1) to test the validity of Charnov’s model by conducting a comparative analysis on the relationship between seed size and the pollen–ovule ratio, and (2) to evaluate if this relationship is different among various mating systems and taxa (families).

CHARNOV’S MODEL

Charnov’s model (Fig. 1) is simplified, as it relies only on the number and size of pollen grains and ovules. Any other factors of allocation of resources to male and female functions are omitted. If we let r be the proportion of resource (R) given to pollen, $1 - r$ the proportion of resource (R) given to seeds (as an equivalent for female function and assuming that the number of seeds = the number of ovules), and C_2 and C_1 the cost for one single seed or pollen grain respectively, we can write the number of pollen grains (P) as

$$P = \frac{rR}{C_1} \quad (1)$$

and the number of ovules (or seeds) (O) as

$$O = \frac{(1-r)R}{C_2} \quad (2)$$

Dividing equation (1) by equation (2) and taking the logarithm, it follows that

$$\log\left(\frac{P}{O}\right) = \log\left(\frac{r}{1-r}\right) + \log C_2 - \log C_1 \quad (3)$$

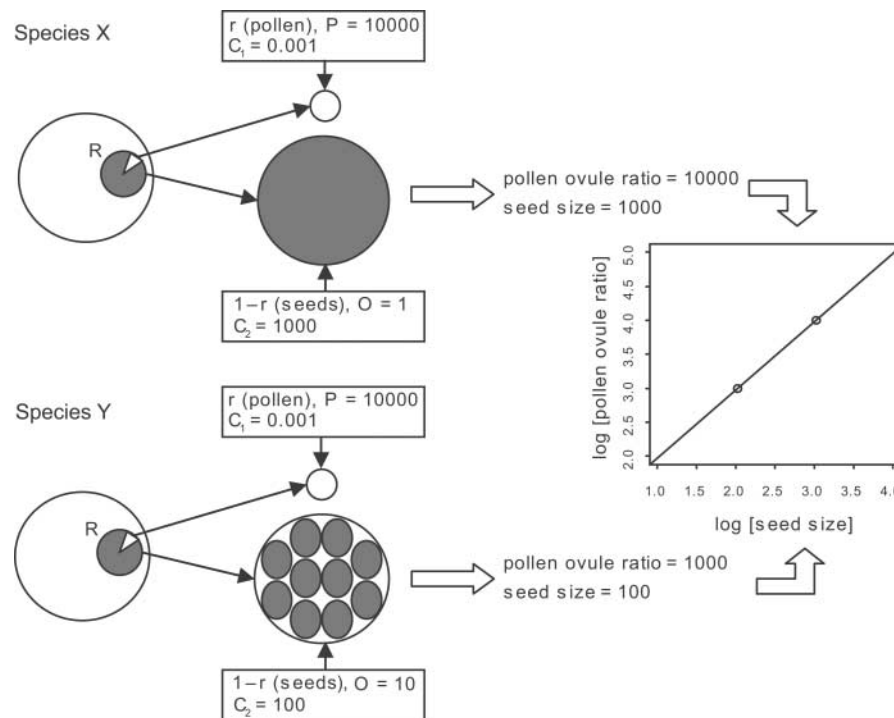


Fig. 1. Application of Charnov's model to two hypothetical species with pollen–ovule ratios of 10,000 and 1000 and seed sizes of 1000 and 100, respectively. The scheme illustrates the assumptions and consequences of the model. See text for further details. Note that the intercept is arbitrary in this example.

Following Charnov's (1982) assumption that the allocated resources (r) and pollen grain size are constant, a linear relationship between \log pollen–ovule ratio and \log seed size is expected. Because the size of a single seed is dependent upon how many seeds can be produced by the plant, the log relationship is directly proportional – that is, the slope of the regression line is the unity slope (see Fig. 1).

Mione and Anderson (1992) pointed out that the positive relationship between seed size and the pollen–ovule ratio could be a spurious correlation. Given negative correlations between seed size and ovule number and between the pollen–ovule ratio and ovule number, an increase in the pollen–ovule ratio with increasing seed size must follow. That plants with large seeds tend to produce few seeds and plants with light seeds produce many seeds is known as the 'seed size/seed number trade-off' (Shiple and Dion, 1992; Jakobsson and Eriksson, 2000). A decrease in the pollen–ovule ratio with decreasing ovule numbers should be found because the ovule number itself is the denominator in the pollen–ovule ratio term. We calculated partial correlations for every combination of the three traits to control for possible inter-correlations.

MATERIALS AND METHODS

Data sources

We obtained data for seed size, pollen–ovule ratio, and mating system from the database BIOLFLOR (Durka, 2002a; Otto, 2002). This database provides ecological and biological data on the vascular plant species of Germany (Klotz *et al.*, 2002; Kühn *et al.*, 2004). We compiled pollen–ovule ratios for 77 additional species from the published literature to supplement the data available in the current version of the database. Germinule length, germinule width, and germinule height were taken from BIOLFLOR and used to calculate seed volume, assuming an ellipsoid shape of the seeds. When used in correlations and for calculating phylogenetically independent contrasts, ‘mating system types’ were recoded to positive integers ordered by increasing xenogamy (obligate autogamous = 1, facultative autogamous = 2, ‘mixed mating system’ = 3, facultative xenogamous = 4, xenogamous = 5). The mating systems are not absolutely identical to those used by Cruden (1977). Cruden’s cleistogamous group is included in the obligate autogamous mating system. The ‘mixed mating system’ is not present in Cruden’s categories. It reflects the idea that mating systems are a continuous trait rather than a characteristic with only two states: predominant selfers and predominant outcrossers (Vogler and Kalisz, 2001; Barrett, 2003). Appendix 1 provides an overview of the families studied, the number of species and genera, and the range of the data within these families. The working phylogeny used in this study is based on a supertree compiled by Durka (2002b) from over 200 literature sources for the 3679 species in the BIOLFLOR database. The 299 species included in the analyses are part of the German flora, but many of them have a wider distributional range. Because plant height is not available in BIOLFLOR, we compiled data for this trait from a national flora source (Bässler *et al.*, 1996) using the given maximal and minimal heights in the descriptions of the species. We present a phylogenetic tree of the studied species accompanied by a data table for seed size, pollen–ovule ratio, plant height, and mating system in the online appendix (http://evolutionary-ecology.com/data/2018_Phylogenetic_Tree.pdf).

Statistical analyses

We tested the relationship between the pollen–ovule ratio and seed size by Pearson’s correlation in the case of (log)normally distributed, continuous variables, and by Kendall’s rank correlation in the non-parametric case (i.e. when mating system was one of the considered variables). Partial Kendall and Pearson correlation were used accordingly to test for the possible confounding effects of covariates that may lead to spurious correlations. We used Model II regression analyses to describe the functional relationship between seed size and the pollen–ovule ratio because Model I regression underestimates the slope coefficient when both X and Y variables are subject to a comparable magnitude of error. From the several available Model II regressions, we chose standardized major axis regression (SMA; also known as reduced major axis regression) because error variances and the ratio thereof were unknown (Sokal and Rohlf, 1995) and are difficult to estimate for phylogenetically independent contrasts (Nunn and Barton, 2000). Under these conditions, standardized major axis regression was found to be better than major axis regression (McArdle, 1987). The r^2 - and P -values presented with the SMA results stem from Pearson correlation tests.

Because the intention of this study was to test Charnov's theory in terms of a functional relationship instead of predicting pollen–ovule ratios from seed size, controlling for the possible effects of the phylogeny of the species was crucial (Harvey and Pagel, 1991; Deschevres *et al.*, 2003). We present results for both cross-species analyses (CSA; i.e. species were treated as independent data points) and phylogenetically independent contrasts (PIC), so that it is possible to evaluate the effect of the phylogenetic relationship of the species on the relationship between the pollen–ovule ratio and seed size.

A well-established approach to correct for the relatedness of the species in comparative analyses is Felsenstein's (1985) independent contrasts, later called phylogenetically independent contrasts by Harvey and Pagel (1991). We calculated phylogenetically independent contrasts with the CAIC software package (Purvis and Rambaut, 1995); branch lengths of the phylogenetic tree were set to unity. Within a given list of species and their phylogenetic tree, CAIC calculates the contrasts (i.e. differences) of trait values between pairs of extant species and pairs of theoretical species at higher nodes of the phylogeny that share a common ancestor. The PIC data values thus cannot be assigned to a distinct species but the contrast values reflect the evolution of a trait within a clade. To compare the CSA data within plant families with the PIC data, we also assigned contrasts to 'families' according to the nodes they have been calculated for. Because CAIC cannot easily handle categorical variables with more than two states, mating system was treated as a continuous variable when calculating contrasts for the correlation analyses. All regression and correlation routines have to be forced through the origin for phylogenetically independent contrasts (Garland *et al.*, 1992), because the direction of subtraction to calculate the contrasts on a given tree is arbitrary. Consequently, the calculation of a full set of contrasts would 'automatically' generate a regression line through the origin.

To compare the relationship among groups of different mating systems and different families, we estimated a common SMA slope for all groups following Warton and Weber (2002). The different slopes among groups were tested for significant heterogeneity by permutation (see Wright *et al.*, 2002, for details), and 95% confidence intervals for the slopes were calculated. Because the hypothesized slope is a unity slope, it is important to note that for SMA regression the default slope is the unity slope given that there is no correlation between the two variables. We also tested for heterogeneity of elevations (intercepts) among groups (Warton and Weber, 2002). This was only meaningful in the case of comparing mating systems for the cross-species analyses because regression of the PIC data was forced through the origin and slopes were heterogeneous among families for the CSA data (see results).

For cross-species analyses, we calculated all the SMA routines and tests with the (S)MATR software (Falster *et al.*, 2003). Because regression with PIC data has to be forced through the origin, contrasts could not be conducted with (S)MATR. Hence we used Microsoft Excel sheets provided by Warton (<http://web.maths.unsw.edu.au/~dwarton/modl2com.xls>; <http://web.maths.unsw.edu.au/~dwarton/model2CI.xls>) to calculate the SMA routines of (S)MATR through the origin. For this, the variances and covariances are calculated by 'subtracting zero' (because the intercept is fixed at the origin) instead of the variable mean from the variable values – that is, $s_y^2 = \Sigma(y^2)/n - 1$, $s_x^2 = \Sigma(x^2)/n - 1$ and $s_{xy}^2 = \Sigma(x * y)/n - 1$, where n is the sample size (Garland *et al.*, 1992; Sokal and Rohlf, 1995). Additionally, there is one more degree of freedom for F -statistics and calculation of confidence limits because the intercept is fixed and does not need to be estimated.

RESULTS

Correlations between seed size, pollen–ovule ratio, ovule numbers, mating system, and plant height

As anticipated, we found a positive correlation between the pollen–ovule ratio and seed size (CSA: $r = 0.66$, d.f. = 297, $P < 0.0001$; PIC: $r = 0.60$, d.f. = 262, $P < 0.0001$), between seed size and mating system (CSA: $\tau = 0.13$, $P < 0.01$; PIC: $r = 0.21$, d.f. = 226, $P < 0.01$), and between the pollen–ovule ratio and mating system (CSA: $\tau = 0.17$, d.f. = 142, $P < 0.0001$; PIC: $r = 0.43$, d.f. = 287, $P < 0.0001$). When controlling for the possible effect of mating system by calculating partial correlations, however, we still found a positive correlation between seed size and the pollen–ovule ratio [CSA: partial $\tau = 0.22$, no test of significance applicable (see Legendre and Legendre, 1998, p. 202); PIC: partial $r = 0.58$, d.f. = 226, $P < 0.0001$]. As it is possible that the overall relationship is driven by tree species, since they tend to have large seeds and are mostly wind pollinated with high pollen–ovule ratios, the possibly confounding effect of plant height was also taken into account. Partial correlation indicates that there is no such effect of plant height (CSA: partial $r = 0.60$, d.f. = 294, $P < 0.0001$; PIC: partial $r = 0.57$, d.f. = 259, $P < 0.0001$). We also found highly significant simple and partial correlations for each pair-wise combination of the traits pollen–ovule ratio, seed volume, and ovule number for both the CSA and PIC analyses (Table 1). All partial correlation coefficients are lower when the third variable is not accounted for.

Functional relationship between seed size and the pollen–ovule ratio

For the CSA and PIC data, there is a significant positive relationship between seed size and the pollen–ovule ratio across all species examined (shown for phylogenetically independent contrasts in Fig. 2), and the confidence intervals of the Model II slopes suggest that the slope is not different from unity (Table 2).

Seed size and the pollen–ovule ratio also showed a positive relationship within each of the five mating systems for both the CSA and PIC data (Table 2). Only the results of the

Table 1. Correlation coefficients (above the diagonals) and partial correlation coefficients (below the diagonals) for the pollen–ovule ratio, seed size, and ovule number for cross-species analyses (CSA) and phylogenetically independent contrasts (PIC).

	Pollen–ovule ratio	Seed size	Ovule number
CSA			
Pollen–ovule ratio	—	0.60***	−0.58***
Seed size	0.43***	—	−0.51***
Ovule number	−0.40***	−0.25**	—
PIC			
Pollen–ovule ratio	—	0.60***	−0.46***
Seed size	0.50***	—	−0.45***
Ovule number	−0.24**	−0.27**	—

Note: **Error probability < 0.01. ***Error probability < 0.001.

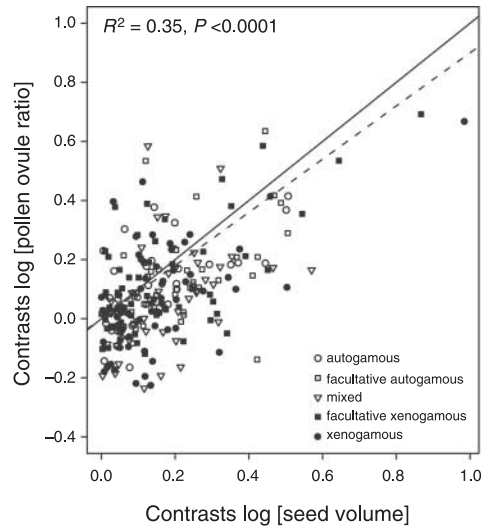


Fig. 2. Phylogenetically independent contrasts of the pollen-ovule ratio and seed volume. Lines depict the unity slope (solid) and the SMA slope (dashed). R^2 - and P -values from ordinary least square regression. Data for the individual slopes and confidence limits within mating systems are given in Table 1.

Table 2. Relationships between the pollen-ovule ratio and seed size within mating systems and across all species for cross-species analyses (CSA) and phylogenetically independent contrasts (PIC)

Mating system	N	r^2	P	SMA intercept	SMA slope	Lower CL	Upper CL
CSA							
All	299	0.43	<0.0001	7.11	0.96	0.88	1.05
Autogamous	37	0.66	<0.0001	6.51	0.92	0.75	1.12
Facultative autogamous	52	0.46	<0.0001	6.48	0.81	0.66	1.00
Mixed	46	0.30	<0.0001	7.13	0.73	0.57	0.93
Facultative xenogamous	61	0.48	<0.0001	6.98	0.80	0.66	0.96
Xenogamous	62	0.25	<0.0001	8.03	1.09	0.88	1.36
PIC							
All	263	0.35	<0.0001	—	0.98	0.89	1.08
Autogamous	31	0.56	<0.0001	—	0.82	0.64	1.05
Facultative autogamous	50	0.47	<0.0001	—	0.93	0.76	1.15
Mixed	45	0.37	<0.0001	—	0.95	0.75	1.21
Facultative xenogamous	57	0.57	<0.0001	—	0.91	0.76	1.08
Xenogamous	55	0.30	<0.0001	—	0.89	0.71	1.12

Note: P - and r^2 -values are from Pearson correlation tests. Intercepts for PIC are not indicated because the regression is forced through the origin. CL = confidence limits.

facultative xenogamous and the mixed mating system species indicated a slope slightly different from unity for the cross-species analyses. A test for heterogeneity among slopes indicated a common slope for both approaches (CSA: test statistic = 7.7, $P = 0.11$; PIC: test

statistic = 0.6, $P = 0.96$). The common slopes estimates were 0.86 (95% confidence interval = 0.79–0.95) and 0.88 (95% confidence interval = 0.80–0.97) for the cross-species analyses and phylogenetically independent contrasts respectively. These slopes are used to make inferences about the heterogeneity and not about the slope *per se*. The elevations of the SMA slopes for the cross-species analyses were significantly heterogeneous among mating systems (test statistic = 29.2, $P < 0.0001$), with intercepts more or less increasing from selfing to outcrossing species (Table 2).

For analysing the relationship within families, only groups with at least five data points for the CSA data were chosen. Only families with a significant relationship between the pollen–ovule ratio and seed size were used to test for heterogeneity among the group slopes. The CSA slopes of these families, ranging from 0.44 to 1.44, were deemed to be heterogeneous (test statistic = 20.6, d.f. = 7, $P < 0.01$) but the PIC slopes (0.83–1.70) were deemed to be homogenous (test statistic = 11.2, d.f. = 7, $P = 0.128$). The common slope estimates were 0.99 (95% confidence interval = 0.89–1.10) for the cross-species analyses and 1.01 (95% confidence interval = 0.89–1.15) for the phylogenetically independent contrasts. The SMA regression of the CSA data showed no significant relationship between seed size and the pollen–ovule ratio for Lamiaceae, Liliaceae, Onagraceae, Ranunculaceae, Rosaceae, and Scrophulariaceae (Table 3). The relationship was only marginally significant ($P < 0.1$) for Ericaceae and Poaceae but significant for Asteraceae, Brassicaceae, Caryophyllaceae, Fabaceae, Juncaceae, and Saxifragaceae, with an extraordinarily strong relationship within the Brassicaceae (Fig. 3) and Saxifragaceae. Among the families that showed a significant linear increase in the pollen–ovule ratio with increasing seed mass, the Asteraceae, Brassicaceae, Fabaceae, and Saxifragaceae had confidence limits that encompass a unity SMA slope.

The results of the phylogenetically independent contrasts were very similar to those of the cross-species analyses (Table 3). The same families that had a significant positive relationship between seed size and the pollen–ovule ratio for the cross-species analyses also

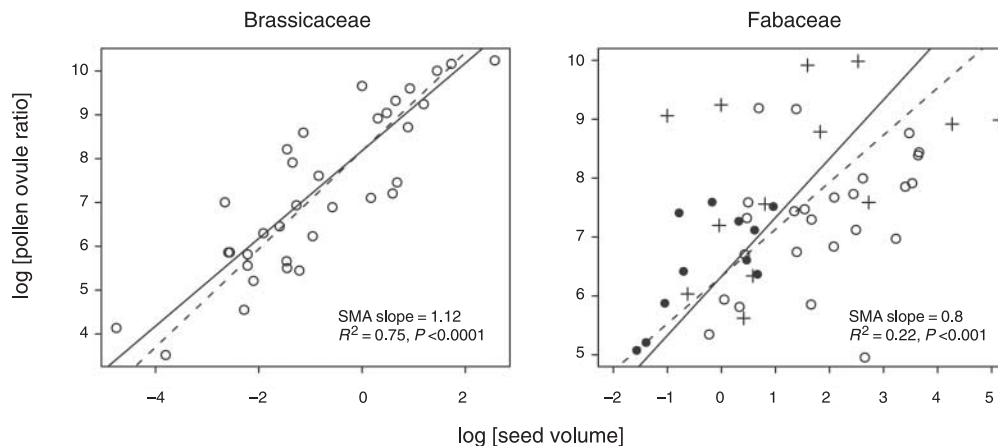


Fig. 3. Relationships between the pollen–ovule ratio and seed volume within the Brassicaceae and Fabaceae. Data points are recent species (CSA). The solid line represents the unity slope and the dashed line represents the SMA slope. For the Fabaceae, the different symbols depict different taxa: ○ = ‘temperate herbaceous clade’ without *Trifolium*, ● = *Trifolium*, + = other Fabaceae.

Table 3. Relationships between the pollen–ovule ratio and seed size within families for cross-species analyses (CSA) and phylogenetically independent contrasts (PIC)

Taxa	<i>N</i>	r^2	<i>P</i>	SMA slope	Lower CL	Upper CL
CSA						
Asteraceae	17	0.35	0.0128	0.90	0.59	1.38
Brassicaceae	34	0.75	<0.0001	1.12	0.93	1.33
Caryophyllaceae	45	0.31	0.0001	0.71	0.55	0.92
Ericaceae	8	0.41	0.0886	0.44	0.22	0.86
Fabaceae	49	0.22	0.0006	0.80	0.62	1.04
Juncaceae	14	0.66	0.0004	1.44	1.01	2.05
Lamiaceae	6	0.00	0.9268	2.20	0.79	6.16
Liliaceae	5	0.10	0.5972	1.69	0.54	5.30
Onagraceae	14	0.16	0.1627	0.92	0.54	1.59
Poaceae	25	0.15	0.0539	1.04	0.71	1.53
Ranunculaceae	11	0.01	0.8135	1.15	0.58	2.26
Rosaceae	10	0.07	0.4721	1.59	0.79	3.21
Saxifragaceae	5	0.91	0.0109	1.21	0.79	1.84
Scrophulariaceae	9	0.29	0.1353	1.73	0.88	3.37
PIC						
Asteraceae	16	0.31	0.0214	1.20	0.77	1.87
Brassicaceae	29	0.70	<0.0001	1.12	0.90	1.38
Caryophyllaceae	36	0.30	0.0005	0.69	0.52	0.91
Ericaceae	7	0.48	0.0552	0.59	0.31	1.12
Fabaceae	38	0.18	0.0067	1.04	0.77	1.40
Juncaceae	7	0.54	0.0390	1.30	0.70	2.42
Lamiaceae	5	0.00	0.9450	2.70	0.93	7.87
Liliaceae	4	0.09	0.6296	1.79	0.53	6.00
Onagraceae	9	0.16	0.2537	0.64	0.32	1.27
Poaceae	22	0.16	0.0597	1.08	0.72	1.62
Ranunculaceae	10	0.02	0.7073	1.10	0.55	2.17
Rosaceae	9	0.02	0.7210	1.26	0.61	2.60
Saxifragaceae	4	0.91	0.0127	1.14	0.71	1.83
Scrophulariaceae	8	0.42	0.0582	1.21	0.65	2.25

Note: *P*- and r^2 -values are from Pearson correlation tests.

showed a significant positive relationship for the phylogenetically independent contrasts. In addition to the families from the cross-species analyses, the Juncaceae also had confidence limits that encompassed a unity SMA slope.

DISCUSSION

As anticipated, seed size and the pollen–ovule ratio were positively correlated. This correlation is also valid when accounting for the phylogenetic relationship of the species under study. Furthermore, the SMA slopes within the mating systems were found not to be different from one. These results are in line with the hypothesis of Charnov and support the

general applicability of the model being tested. Results of partial correlations suggest that none of the suspected covariates (mating system, plant height or ovule number) can completely account for the positive relationship between seed size and the pollen–ovule ratio. In contrast to Mione and Anderson (1992), we still found a negative correlation between ovule number and the pollen–ovule ratio after removing the effect of seed size.

The scatter of the relationship between seed size and the pollen–ovule ratio was moderate to high, even for groups that showed a highly significant correlation. Several factors may have contributed to this scatter. Besides the sample error solely from the measurement procedure of the traits, geographical variation within the traits also might play a role in the unexplained variation. In no case were the data for seed size and the pollen–ovule ratio measured on the same individual or on individuals of the same collection site. Some of the literature used to obtain data on the pollen–ovule ratio was based on plant material collected in the Mediterranean basin instead of Central Europe, where most of the data on seed size were obtained. There is evidence that pollination and mating system can change along latitudes and this change can be accompanied by a change in the pollen–ovule ratio (Thomas and Murray, 1981; Navarro, 1999). The results of the SMA regression of the Fabaceae species, however, contradict a systematic error of the data. Although a large proportion of the data for pollen–ovule ratios in this family was obtained from a study conducted in Spain (Rodríguez-Riano *et al.*, 1999), the model seems to fit the data within this family.

Furthermore, and possibly more importantly, the assumption of constant pollen grain size made by the model is unlikely to be met by real data. We analysed data from Cruden and Millerward (1981), who also reported pollen grain size for a set of insect-pollinated species. In fact, the explained variance was much greater when including the pollen grain size in a multiple linear regression model ($N = 14$, $R^2 = 0.82$, $P < 0.0001$), but there were too few data on pollen grain size to include it in the general analysis. The other constant factor in the model, the proportion of resources r given to sexual function (the $r/1 - r$ term in Charnov's model), cannot be measured directly – that is, it is calculated from the size and number of seeds and pollen grains. Charnov (1982) proposed that as an approximation, r can be considered as an ESS (evolutionarily stable strategy) value within a mating system, thus being a constant. The results of the relationship between seed size and the pollen–ovule ratio for the different mating systems are in line with this proposal as well as the model in general. We could also show that the elevations of the SMA regressions are different among mating systems and that species with a rather outcrossing mating system show higher elevations than selfing species. This corroborates the prediction that outcrossing species should invest relatively more of their resources into pollen (Charlesworth and Charlesworth, 1981; Charnov, 1982). The result, however, might also be influenced by a systematic error of pollen grain size (see above). With regard to the number instead of the sizes of ovules and seeds, there is evidence that Charnov's model is incapable of predicting the correct pollen–ovule ratio for a species when the seed set is low – that is, when not all of the ovules develop into mature seeds (Cruden, 1997). This finding, however, is not a drawback when testing for a functional relationship between the pollen–ovule ratio and seed size. Seed size was only taken as an approximation for investment into female function, and the numbers of seeds produced per ovule by a plant, known as the 'seed–ovule ratio', are not important.

The weak and insignificant correlations between the pollen–ovule ratio and seed size within some of the families studied are most likely due to low sample sizes. The Brassicaceae

and Saxifragaceae showed a particularly strong correlation compared with the other families with a significant relationship. Although morphologically well-distinguished from other families in the order Brassicales, differentiation of the genera within the Brassicaceae is imprecise (Judd *et al.*, 1999). The family shows a low variability in floral and fruit biology compared with other families, and the general pattern that pollen size is less variable than other floral traits (Vonhof and Harder, 1995; Cresswell, 1998) might be enhanced in the Brassicaceae. This could lead to an exceptionally low variation in pollen size so that this factor is more likely to be an approximation of a constant, as assumed by the model.

The high amount of unexplained variation within the remaining families that showed a significant relationship between the pollen–ovule ratio and seed size could be explained by higher variability in pollen sizes and/or the evolution of more specialized pollination syndromes within taxa of lower orders (e.g. tribes or genera) that do not reflect general patterns within the family. Within the Fabaceae, the results of a more detailed analysis support this proposition (see also Fig. 3). When excluding species from the analyses that do not belong to the ‘temperate herbaceous clade’ (Doyle *et al.*, 1997; Wojciechowski *et al.*, 2004), the explained variance increases ($R^2 = 0.29$, $N = 23$, $P = 0.003$). This effect is even stronger when focusing on genera instead of the family. The explained variance (R^2) within the *Trifolium* species is 0.49 ($N = 11$, $P = 0.017$). This effect of focusing on lower taxonomical levels was also found within families that showed no significant relationship whatsoever despite reasonable sample sizes. The Onagraceae had no significant correlation between seed size and the pollen–ovule ratio, although 13 of the 14 studied species belong to *Epilobium*. Excluding the only species that does not belong to the genera (*Epilobium*), however, increased the explained variance ($N = 13$, $R^2 = 0.28$) and resulted in a marginally significant relationship ($P = 0.062$). The Onagraceae are also known to produce polyads in which pollen grains are clumped together. The relationship between seed size and the pollen–ovule ratio is possibly influenced by the fact that polyads – in contrast to single pollen grains – function as the units of pollen dispersal. Besides nectar, many Ranunculaceae species also offer pollen as a reward for pollinators (Durka, 2002a). When we excluded species that only offer nectar from the analysis within Ranunculaceae, the explained variation increased ($N = 7$, $R^2 = 0.47$) and the relationship was marginally significant ($P = 0.09$). These species might show a different pattern from the ones also offering nectar due to selection for bigger pollen size.

The positive relationship found in this study is in accordance with previous studies on the relationship between seed size and the pollen–ovule ratio regarding the outcome of a positive relationship between the two traits. The studies of Uma Shaanker and Ganeshaiiah (1984) and Mione and Anderson (1992) were directly concerned with the validation of Charnov’s model. They both reported a positive correlation between the pollen–ovule ratio and seed size but neither considered the phylogenetic relatedness of the species nor tested the slope of the regression line. In the studies of Lopez *et al.* (2000) and Bosch *et al.* (2001), the model was not explicitly tested and the finding of a positive relationship is only discussed briefly. Preston (1986) studied the largest data set so far, analysing 49 crucifer taxa. He divided the data into autogamous and allogamous taxa and found a positive correlation within both groups.

The theories of Charnov (1982) and Cruden (1977, 1997, 2000) involve different processes responsible for the variation of pollen–ovule ratios across plant species. Finding support for one theory, however, does not imply the mutual exclusion of the other. Our findings suggest that the provisioning of resources to male versus female function is an important

component of shaping the pollen–ovule ratio. But it does not rule out the possibility that certain pollination conditions, such as the pollen-bearing capacity of pollinating insects (Cruden and Millerward, 1981), can be a selective force towards the pollen–ovule ratio. Variation in the pollen–ovule ratio that cannot be attributed to any of the variables and errors discussed above might be due to such conditions. On the other hand, the pollen–ovule ratio is certainly a valuable reflection of the mating system of a plant but cannot completely be explained in terms of this relationship. Only considering the investment in male function (i.e. the number and size of the pollen grains) is inadequate in our opinion. Cruden (1997, p. 33) states, ‘With the exception of ovule number, which responds evolutionarily to selective forces that affect seed number, any given floral trait should respond to changes in other floral traits or functions’. While it can be argued whether or not seed size is a floral trait, it is reasonable to propose that seed size also responds to the selective forces that affect seed number. Furthermore, there is no reason why seed size and number should not be connected to other traits that are just as dependent on the allocation of resources to sexual function. In line with this thinking, Preston (1986, p. 1735) also discussed a possible consequence of the change in the pollen–ovule ratio associated with a change in seed mass. He suggested that ‘if this correlation indicates that resources allocated to male and female function are not independent, then factors influencing variation in seed size, such as seed dispersability and seedling establishment, might also influence the pollen–ovule ratio’. Such associations between the pollen–ovule ratio and other attributes correlated to seed size are conceivable and should be addressed in future research.

Recently, de Jong *et al.* (2005) have developed an ESS model that also predicts bigger seeds for species with high outcrossing rates than for inbreeding species. Seed size is a compromise between mother and offspring in this model because the genotype of the developing seed is thought to partly determine its own growth. Even if this model applies, the mother will still have control of the production of pollen and how many seeds mature, so Charnov’s model remains valid. Thus the prediction of de Jong’s model that seed size is dependent on outcrossing rate does not contradict the hypothesized dependence of the pollen–ovule ratio on seed size in Charnov’s model because the former only applies to the proportion of seed size that is controlled by the offspring itself.

In summary, the results presented here are consistent with the hypothesized linear relationship between seed size and the pollen–ovule ratio, although considerable sources of error were evident in the measurements of traits as well as inherent in the model. While results based on a rather encompassing approach (within mating system and across all species respectively) clearly validate Charnov’s model, results within individual families indicate that patterns within taxa might not be consistent with patterns found on a larger taxonomical scale. For some of the families studied, we believe that this discrepancy is possibly due to differences in the pollination ecology that evolved within taxa. We are aware of the speculative nature of these explanations. But it reflects our opinion that the general pattern – a positive linear relationship between seed size and the pollen–ovule ratio – is influenced by other processes that alter the purely mechanistically provisioning of resources to female and male function as expressed by Charnov’s model. Very similar results for phylogenetically independent contrasts and cross-species analyses lead us to the assumption that the correlation between seed size and the pollen–ovule ratio is not confounded by the phylogeny of the species. The relationship appears to be a fundamental functional relationship that is evolutionarily stable. Thus it can be concluded that the pollen–ovule ratio is at least partly dependent on the allocation of resources to female sex function. It is reasonable

to propose that factors influencing the evolution of seed mass also affect the pollen–ovule ratio and consequently the pollination ecology of plant species.

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APPENDIX 1

Mean, minimum, and maximum values of seed volume and the pollen-ovule ratio within families

Family	No. of species within family	No. of genera within family	Mean seed volume (mm ³)	Minimum seed volume (mm ³)	Maximum seed volume (mm ³)	Mean pollen-ovule ratio	Minimum pollen-ovule ratio	Maximum pollen-ovule ratio
Aceraceae	1	1	137.4	—	—	94 078	—	—
Adoxaceae	1	1	13.8	—	—	3 902	—	—
Alismataceae	1	1	1.3	—	—	175	—	—
Apiaceae	4	4	13.0	1.4	44.8	10 363	645	26 535
Araceae	1	1	33.0	—	—	2 750	—	—
Asclepiadaceae	1	1	10.3	—	—	11	—	—
Asteraceae	17	8	1.4	0.1	10.9	1 939	154	5 224
Balsaminaceae	2	1	8.7	4.3	13.1	80 033	16 066	144 000
Betulaceae	1	1	0.2	—	—	6 734	—	—
Boraginaceae	3	3	7.7	3.9	12.7	18 015	8 731	25 000
Brassicaceae	34	20	1.4	0.0	13.1	5 166	34	28 159
Campanulaceae	1	1	0.1	—	—	647	—	—
Caryophyllaceae	45	15	0.8	0.0	10.4	266	18	1 575
Chenopodiaceae	2	2	0.7	0.6	0.7	18 069	16 950	19 188
Corylaceae	1	1	1043.1	—	—	2 119 717	—	—
Ericaceae	8	5	0.4	0.0	2.2	665	137	1 667
Euphorbiaceae	2	2	2.8	2.1	3.6	122 190	7 179	237 200
Fabaceae	49	16	12.4	0.2	172.0	3 312	142	21 660
Fagaceae	1	1	617.8	—	—	636 594	—	—
Fumariaceae	2	1	4.3	2.6	5.9	1 731	115	3 347
Geraniaceae	2	1	4.5	1.0	8.0	328	117	539
Hippocastanaceae	1	1	7142.2	—	—	451 543	—	—
Juncaceae	14	2	0.1	0.0	0.5	2 747	16	20 440
Lamiaceae	6	5	1.1	0.5	2.1	1 808	402	7 332
Lentibulariaceae	1	1	0.0	—	—	450	—	—
Liliaceae	5	4	7.8	3.2	16.1	5 314	961	18 000

APPENDIX 1—(continued)

Family	No. of species within family	No. of genera within family	Mean seed volume (mm ³)	Minimum seed volume (mm ³)	Maximum seed volume (mm ³)	Mean pollen-ovule ratio	Minimum pollen-ovule ratio	Maximum pollen-ovule ratio
Malvaceae	1	1	1.2	—	—	226	—	—
Onagraceae	14	2	0.2	0.0	1.3	34	7	119
Oxalidaceae	1	1	1.2	—	—	1 632	—	—
Plantaginaceae	4	1	0.6	0.1	1.6	13 157	2 411	32 385
Poaceae	25	20	3.3	0.1	26.9	8 131	61	37 124
Polemoniaceae	1	1	1.0	—	—	441	—	—
Polygonaceae	2	2	8.0	2.9	13.2	3 589	1 500	5 678
Primulaceae	1	1	0.6	—	—	690	—	—
Pyrolaceae	1	1	0.0	—	—	82	—	—
Ranunculaceae	11	6	3.7	1.7	10.0	6 226	1 491	12 700
Rhamnaceae	1	1	20.1	—	—	6 570	—	—
Rosaceae	11	7	9.2	0.2	39.7	23 788	429	168 850
Rubiaceae	2	1	3.8	0.9	6.7	1 991	1 902	2 080
Saxifragaceae	5	2	0.1	0.0	0.2	634	70	1 470
Scrophulariaceae	9	6	0.1	0.0	0.9	709	7	2 575
Solanaceae	1	1	7.3	—	—	184	—	—
Tiliaceae	1	1	76.3	—	—	4 350	—	—
Trilliaceae	1	1	2.6	—	—	4 831	—	—
Ulmaceae	1	1	33.0	—	—	28 367	—	—