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# Effects of habitat disturbance and pollination type on the interspecific variation in pollen-ovule ratios

**Vliv disturbance a způsobu opylení na vnitrodruhovou variabilitu v poměru mezi počtem pylových zrn a vajíček**

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In this study two important factors that are thought to govern interspecific variation in pollen-ovule ratios were examined. First, the effect of habitat disturbance on variation in pollen-ovule ratio was determined. The second factor studied was the pollination type, used as a surrogate for the efficiency of pollination. Because seed mass is known to be strongly correlated with the pollen-ovule ratio it was also included in the analyses to examine if a possible effect of habitat disturbance or pollination type is still valid after accounting for the effect of seed mass. Furthermore, phylogenetically comparative methods were used to investigate whether the correlations between traits were maintained through evolutionary history or are only present in recent species data, i.e. in analyses that do not consider phylogenetic relationships between species. In conflict with the reproductive assurance hypothesis, habitat disturbance did not have a significant effect on interspecific pollen-ovule ratio variation. In contrast, pollination type accounted for a significant proportion of the variation in pollen-ovule ratios, even after taking into account the strong effect of seed mass. General results do not differ between the cross-species and phylogenetic comparative approaches. The results both accord with the predictions of the sex allocation theory and the proposition that the chance of a pollen grain reaching a stigma governs the pollen-ovule ratio.

Keywords: habitat disturbance, mating system, phylogenetic comparative method, pollen-ovule ratio, pollination, seed size

## Introduction

The pollen-ovule ratio is a mathematical construct, calculated by dividing the number of pollen grains by the number of ovules that develop in a flower. However, it is an important floral trait that is correlated with numerous reproductive parameters and can serve as an estimate of sex allocation (Queller 1984). Interspecific variation in pollen-ovule ratios is enormous and this variation is attributed to covariation with the number and size of pollen grains and ovules (Charnov 1982) and also the efficiency of pollination and habitat disturbance (Cruden 1976, 1977, 2000). While there is strong evidence that most of the variation in pollen-ovule ratios is explained by seed size (Uma Shanker & Ganeshaiah 1984, Preston 1986, Götzenberger et al. 2006) the findings and predictions of Cruden have not been thoroughly tested, in particular by using a comparative approach. The idea that habitat disturbance influences pollen-ovule ratios is based on two principles. Firstly, Cruden (1977) and many subsequent studies found that the pollen-ovule ratio is highly correlated with the degree of outcrossing in plant species (reviewed in Cruden 2000). Selfed species tend to have on average lower pollen-ovule ratios than species that outcross. Secondly, it is hypothesized that self-compatible and autogamous species are more successful colonizers than outcrossing species in early successional stages (Baker 1955, 1967, Stebbins 1957). These studies focused on the colonization of islands by plant species. However, this idea can be expanded to other environmental conditions and its generalization in the form of the reproductive assurance hypothesis, dates back to Darwin (1876), and predicts that selfing can evolve if pollinators are unreliable in delivering pollen. Unreliable pollination is not only common during early stages of primary succession but also in disturbed habitats in general (Aizen & Vasquez 2006). Cruden (1977) tested the reproductive assurance hypothesis by using interspecific data on pollen-ovule ratios. Supporting the reproductive assurance hypothesis, he demonstrated an increase in average pollen-ovule ratios from highly disturbed/early successional to undisturbed/late successional habitats, and concluded that species with low pollen-ovule ratios are more common in disturbed habitats than elsewhere.

A drawback of Cruden's study was that it did not account for plant attributes that possibly covary with pollen-ovule ratios, i.e. mating systems and habitat disturbance levels. In this context, longevity, life form, seed mass and the type of pollination are particularly interesting. Barrett et al. (1996) showed using a phylogenetically informed comparative analysis that annuals tend to be inbred. Thus, the association between disturbance and pollen-ovule ratios may stem from the connection with longevity and/or life form. Another strong covariate of the pollen-ovule ratio is seed mass. If one looks at this relationship in the context of mating system biology and seed ecology, an interesting pattern becomes apparent. Species with large seeds, i.e. a high competitive ability but low dispersal potential, tend to be predominantly outcrossing while small seeded species with low competitive ability and higher dispersal potential are predominantly selfing. The latter two attributes are usually associated with high levels of disturbance or early stages of succession (Grime 2001, Lavorel et al. 2007).

Finally, pollination type, i.e. whether a plant is primarily self-pollinated or pollinated by wind or insects, is also an important factor that is associated with both pollen-ovule ratios (reviewed in Erbar & Langlotz 2005), disturbance and successional stage (Rydin & Borgegard 1991, Aizen & Vazquez 2006). The associations among mating system, pollen-ovule ratios, seed mass, longevity, life form and pollination type, and between these traits and habitat disturbance led us to the formulation of three possible hypotheses to account for the influence of disturbance on pollen-ovule ratios:

(i) $H_0$: There is no effect of disturbance on pollen-ovule ratios. (ii) $H_{alt1}$: Disturbance has a direct effect on pollen-ovule ratios. In this case, we should observe that variation in pollen-ovule ratios is explained by disturbance, independent of the other variables included in statistical models. (iii) $H_{alt2}$: Disturbance has an indirect effect on pollen-ovule ratios. Disturbance only correlates with the pollen-ovule ratio because other variables in the model are intercorrelated.

In addition, in determining whether disturbance is linked to the pollen-ovule ratio after accounting for covarying variables, this analysis also provides the framework for insights into interspecific variation in pollen-ovule ratios governed by the efficiency of pollination (Cruden 1977). Cruden argues that selfed species need less pollen per ovule for pollination because there is virtually no hindrance to the transmission of pollen to the stigma in selfed species. For insect and wind pollinated species there is a strong stochastic component, which reduces the probability of a given pollen grain reaching the stigma of a conspecific plant. Contradicting this view, Charnov (1982) argues that the pollen-ovule ratio is governed by the allocation of resources to male and female sex function, as represented by the number and size of pollen grains and seeds. He formulated a mathematical model that predicts a correlation between the pollen-ovule ratio, seed and pollen size. The theories of Charnov and Cruden are not mutually exclusive and some authors (Mione & Anderson 1992, Götzenberger et al. 2006) propose that both, sex allocation and pollination efficiency, contribute to the variation in the pollen-ovule ratio.

In conclusion, our study has two main objectives, both aimed at explaining interspecific variation in pollen-ovule ratios. First, the finding of Cruden that pollen-ovule ratios are correlated with disturbance type is reassessed after accounting for possible effects of covariates. Second, assuming that pollination type represents a measure of pollination efficiency, we determined whether seed mass and pollination efficiency are correlated with the pollen-ovule ratio. For this a comparative approach, analysing the relationships of the variables in a comprehensive dataset, was used. Today it is widely acknowledged that such an analysis should be put into a phylogenetic context because species cannot be viewed as independent data points (Harvey & Pagel 1991). Therefore, we analysed the data using cross species analysis as well as methods that account for phylogenetic dependence.

## Material and methods

### *Data*

We obtained the data used in this study from the BIOLFLOR database (Klotz et al. 2002). BIOLFLOR provides data on many life history traits, including data on seed mass, pollen-ovule ratios, mating system and pollination mode, for the German flora. Additional data on pollen-ovule ratios came from the literature and unpublished data not yet included in the database.

As a measure of disturbance we chose the system of hemerobic levels, which is an indicator of the degree of human influence on vegetation. In this categorization plant species are classified according to the vegetation type in which they occur and the degree to which these vegetation types are subject to anthropogenic alteration. The classification comprises seven different categories from ahemerobic, i.e. no human influence on vegetation, to polyhemerobic, i.e. vegetation that is severely disturbed by human influence, such as deep ploughing or intensive fertilization (Sukopp 1972, Klotz & Kühn 2002). Most species, however, can be assigned to more than one hemeroby category so that using one class per species in the analysis as an ordinal variable would be inadequate. For that reason we reassigned species to only two different classes, one for species of predominantly undisturbed habitats (ahemerob, oligohemerob and mesohemerob species) and one for species of predominantly disturbed habitats (β-euhemerob, α-euhemerob, and polyhemerob species).

When Cruden (1977) assigned species to disturbance levels for his analysis he also considered information on the probability of pollinator occurrence. Such information is not provided by the hemeroby classification. For example, species in the ahemerobic class that are not subject to anthropogenic influences, only occur in subalpine and alpine habitats. These habitats usually have a low pollinator density and species are thought to be self-mating (Price & Waser 1998, Körner 2003). Therefore, a second classification of disturbed and undisturbed habitats was conducted, based on the occurrence of species in vegetation types and the potential availability of pollinators. In the first class were species that grow in either disturbed plant communities or communities expected to have a low pollinator density even if undisturbed in terms of the hemeroby classification (e.g., subalpine and alpine habitats). In the second class were species of undisturbed communities or communities that are expected to have a high pollinator density even if they are classified as disturbed in terms of the hemeroby system (e.g., anthropogenic meadows and pastures; Morris 2000, Steffan-Dewenter & Tscharntke 2002, Moradin et al. 2007).

Although BIOFLOR also contains data for plant strategies sensu Grime (1977), we refrained from using this data as a disturbance indicator, because species are assigned to Grime's competition stress/ruderality scheme on the basis of plant traits like life form, mating system and seed mass. Thus, we would run into the problem of circular reasoning when using this data to study relationships between ruderality (as a measure of disturbance) and the same plant traits that are used to infer ruderality.

Mating system is included in BIOLFLOR by assigning plants to one of the mating system types: obligate autogamous, facultative autogamous, mixed mating system, facultative xenogamous, or xenogamous. For each species this assignment is based on data in the literature.

For the traits that we wanted to study, we retrieved data for 186 species from the BIOLFLOR database, after including additional data on pollen-ovule ratios from the literature. Because of their particular ecological properties we excluded hydrophytes from the data set. Categorical mating system data from BIOLFLOR was recoded in a binary categorization, i.e. species with an obligate or facultative autogamous mating system were considered as selfing species while those with a facultative xenogamous or xenogamous mating system were considered as outcrossing species. Species with mixed mating were omitted. This recoding was especially necessary for the phylogenetic analysis (see below) but also simplified cross-species analysis by avoiding empty cells in contingency tables and undefined contrasts in the multivariable linear model. For this reason we also excluded woody species from the analysis, because woodiness is strongly confounded with pollination type and longevity. All woody plants are perennial and there were no selfers among the woody species in our data. Also, mating system was excluded because of its strong association with pollination type, resulting in empty cells and undefined contrasts.

The data for pollen-ovule ratio, seed mass, mating system, pollination type, longevity and habitat disturbance are given in Electronic Appendix 1 together with the phylogenetic tree that was used for the phylogenetically informed analyses.

### *Cross-species data analyses*

First we conducted a comparative analysis in which species were treated as being independent. Before relating the pollen-ovule ratio to seed mass, mating system, pollination type, longevity and habitat in a multivariable linear model we examined the simple relationships between the explanatory variables. This was done by chi-square tests, ANOVA and regression analysis depending on whether the two variables analysed were both categorical, categorical and continuous, or both continuous, respectively. To achieve normality, pollen-ovule ratios and seed mass were log-transformed for all analyses.

After inspecting the bivariate associations of the variables, a multivariable linear model was set up as a "full" model with log(pollen-ovule ratios) as a continuous response variable and log(seed mass), pollination type, longevity and disturbance level as explanatory variables. The significance of single explanatory variables was assessed by analysing the effect of deleting the variable from the model by type III ANOVA. Type III ANOVA is advocated (Quinn & Keough 2002) when the design is unbalanced, which was the case for our data. Additionally, we set up a model that included all possible pair-wise interaction terms among explanatory variables. Including interaction effects determines whether the main effects collectively explain all of the influence on the dependent variable or that the explanatory variables have an effect that is dependent on another explanatory variable.

Some of the explanatory variables were highly correlated with each other, imposing the problem of multicollinearity when used together in a multivariable linear model (MacNally 1996). In addition to analysing the data with a multivariable linear model, we therefore adopted the method of hierarchical partitioning as proposed by Mac Nally (2000). Hierarchical partitioning helps to identify explanatory variables that have a high correlation with the response variable, independent of the effects of collinearity. It is an alternative to stepwise variable selection procedures, particularly if the study aims to recognize likely causalities of variables instead of finding the one model that best describes the data. The algorithm of hierarchical partitioning first fits a series of models with all possible combinations of the explanatory variables. On the basis of goodness of fit measures for these models an independent and joint effect is estimated for each explanatory variable. The independent effect describes the explained variation in the response variable by a single explanatory variable independent of other explanatory variables in the model. Joint effects describe the explained variance by a response variable that is caused by intercorrelation with other explanatory variables. The joint effect might be negative for variables that mask the effect of other variables. Note that because of the completely different concept, hierarchical partitioning is not comparable to partial correlation. Hierarchical partitioning tries to determine the contribution of a variable in a model relative to the other variables, while partial correlation estimates the variation in the response variable explained by a explanatory variable after the covariation with other explanatory variables is accounted for.

All cross-species analyses were performed in the statistical software R version 2.4.1 (R Development Core Team 2006). For hierarchical partitioning we used the hier.part package for R by Walsh & Mac Nally (2007).

### *Phylogenetic comparative method*

All analyses that incorporated phylogenetic information were based on a phylogenetic tree that we derived from the phylogeny given in BIOLFLOR for the 2769 species in the German flora (Durka 2002). The phylogeny in BIOLFLOR is an informal supertree that was derived by grafting distal clades on basal clades, where phylogenies of the clades where taken from the published literature (see Durka 2002 for details and references). After extracting the species analysed in this study, there were still polytomies left in the tree, i.e. more than two species emerge from a single ancestral node. We used the dichotomous key in a national flora of Germany (Jäger 2005) to resolve these polytomies. Because no information on branch length was available we set all branches to an arbitrary length of one.

Prior to the analysis of trait covariation, we calculated an estimate $\lambda$, which is a parameter for phylogenetic correlation within a trait that ranges from zero to one, where one indicates that the trait under consideration evolves according to a Brownian motion model in the given phylogeny (Pagel 1997, 1999). In addition we used the method of Desdevises et al. (2003) to partition the variation in pollen-ovule ratios among ecological and phylogenetic components. The idea behind this method is to assign the variation in a trait to a part that is explained by phylogeny alone, a part that is explained by ecology alone (i.e., the explanatory variables in the model), and a part that is jointly explained by phylogeny and ecology. The latter part is referred to as niche conservatism (Grafen 1989, Harvey & Pagel 1991) and reflects that related species share similar traits or trait values because they occupy similar niches.

For analysing the pair-wise associations between the variables we had to apply different phylogenetic comparative methods. In the case of correlated evolution between categorical variables we used a method introduced by Pagel (1994) that uses a continuous-time Markov chain approach to model the evolution of two binary variables along a given phylogenetic tree. This process reflects the probability of an evolutionary change along a branch from one state in the character to the other state, in which the probability only depends on the state at the beginning of a branch, not on any earlier event. According to this approach, two models fit the data, one in which the variables are treated as evolving independently and a second in which the variables evolve in correlation with each other. The likelihood of the two models is estimated by maximum likelihood, which compares them using a likelihood ratio test. A method that models the evolution of a discrete variable with more than two character states is not available. Therefore, we recoded the pollination type variable to selfers/non-selfers. We were particularily interested in the transition from a non selfing to selfing pollination type because the reproductive assurance hypothesis predicts proportionally more selfers in disturbed habitats.

To test for correlations between continuous, and between continuous and categorical data we used phylogenetic generalized least square (PGLS) models that account for the phylogenetic relationship of the species by introducing a term for correlated errors (Grafen 1989, Martins & Hansen 1997). The error term is derived from the phylogenetic tree of the analysed species and is in the form of a distance matrix that represents the phylogenetic distances between species. Unlike in ordinary least square regression the errors are allowed to be correlated. To asses the significance of the bivariate models we compared these models to one that was only fit to the intercept. Subsequently, equivalent to the cross-species analysis, we set up a PGLS model in which log(pollen-ovule ratio) was the response variable and log(seed mass), pollination type, longevity and disturbance type explanatory variables. After deletion of single terms, models were compared by likelihood ratio tests. Hierarchical partitioning is able to calculate joint and independent effects based on log-likelihoods, so we could apply this method also to the PGLS model.

The calculation of $\lambda$ and analyses of correlated evolution for pairs of discrete variables were made in the BayesTraits computer programme of Pagel & Meade (2007). All other analyses were conducted using the statistical software R (R Development Core Team 2006) within the package ape (Paradis et al. 2004).

## Results

### *Cross-species analysis*

Results for the data in which disturbance type was derived from the hemeroby level did not differ substantially from that for which we assigned the disturbance type (see Methods, Data), so we only present results for the former. Although there is a large overlap between the pollen-ovule ratio ranges of outcrossed and selfed species the mean pollen-ovule ratios of selfed species is significantly smaller ($F_{1, 184} = 41.6$, $R^2 = 0.18$, $P < 0.0001$). Selfed species do occur proportionally more often in habitats with a low disturbance level ($\chi^2 = 8.51$, df = 1, $P = 0.004$) but the mean pollen-ovule ratio of species in habitats with low disturbance is not significantly smaller than that of species in high disturbance habitats ($F_{1, 184} = 1.24$, $R^2 < 0.01$, $P = 0.27$). Disturbance, however, is negatively associated with seed mass ($F_{1, 184} = 7.66$, $R^2 < 0.03$, $P = 0.006$). In general, there is a high degree of intercorrelation among the variables that were analysed in this study (Table 1).

All variables except disturbance level were significant in the type III ANOVA analysis of the full main effects model (Table 2). In detail, seed mass had a positive and longevity a negative effect on the pollen-ovule ratio. Mean pollen-ovule ratios increased from self-pollinated, to insect-pollinated, to wind-pollinated species. However, when interaction terms were included in the model, the effect of longevity on pollen-ovule ratio was no longer significant (Fig. 1b; $F_{1, 149} = 2.46$, $P = 0.12$). The interaction between longevity and pollination type, however, significantly contributed to the model ($F_{1, 149} = 5.55$, $P < 0.05$), i.e. the effect of longevity on pollen-ovule ratios is dependent on mode of pollination. The main effects of seed mass (Fig 1c) and pollination type (Fig 1a) were both significant in the interaction model. As in the main effects only model, disturbance level was not significant, neither as a main effect nor in its interaction with any other variable.

The joint and independent effects of the variables, based on hierarchical partitioning, agree with those indicated by the multivariable linear model (Fig. 2a). Both, seed mass and pollination type have a strong independent effect on the pollen-ovule ratio, but the effects of longevity and disturbance level appear to be negligible.

### *Phylogenetic analyses*

The estimate of $\lambda$ was 0.93, which supports the Brownian model for the evolution of pollen-ovule ratios. According to the results of the partitioning of ecological and phylogenetic components of the variation in pollen-ovule ratios there is only a small amount of variation that can be attributed to the underlying phylogeny of the species studied. A fifth of the variation in pollen-ovule ratios can be explained by ecology. The greatest part of the variation is explained by a joint effect of phylogeny and ecology (38%), while 27% of the variation remains unexplained. The small effect of pure phylogeny (14%) corroborates the finding that analyses in a phylogenetic context do not differ much from cross-species analyses (see below). However, there were some results that deviated from those of the cross-species analysis. First, several bivariate relationships gave different results for cross species and phylogenetic analyses (Table 1). The negative relationship between disturbance and seed mass is highly significant when analysed across species but insignificant in a phylogenetic context. Showing the opposite pattern, longevity is positively associated to pollen-ovule ratio when analysed with PGLS, but not across species. There were further vari-

Table 1. – Results of $\chi^2$ tests and F tests for cross-species analyses (above diagonal), and log likelihood ratio tests for phylogenetic comparative methods (below diagonal). Significance levels are *** < 0.001, ** < 0.01, * < 0.05, and significant results after sequential Bonferroni are in bold.

| | Pollen-ovule ratio | Seed mass | Habitat disturbance | Pollination type | Longevity | Mating system |
|---|---|---|---|---|---|---|
| Pollen-ovule ratio | — | **F = 111.5*** ** | F = 1.2 | **F = 28.8*** ** | F = 0.02 | **F = 41.6*** ** |
| Seed mass | **LR = 91.6*** ** | — | **F = 7.7** ** | **F = 7.1** ** | F = 3.9* | **F = 7.2** ** |
| Habitat disturbance | LR = 0.4 | LR = 1.9 | — | $\chi^2 = 5.4$ | **$\chi^2$ = 113.3*** ** | **$\chi^2$ = 8.5** ** |
| Pollination type | **LR = 59.5*** ** | LR = 8.9* | LR = 5.6 | — | **$\chi^2$ = 9.9** ** | **$\chi^2$ = 113.3*** ** |

Table 2. – Main effects of seed mass, longevity, pollination type and habitat disturbance on the pollen-ovule ratio. F and P from a type III ANOVA. OLS = ordinary least square; PGLS = phylogenetic generalized least square. Numerator and denominator df are shown.

| | df | F | P |
|---|---|---|---|
| OLS | | | |
| Log10(seed mass) | 1, 179 | 122.51 | < 0.0001 |
| Longevity | 1, 179 | 4.21 | < 0.05 |
| Pollination type | 2, 178 | 41.20 | < 0.0001 |
| Disturbance | 1, 179 | 2.08 | 0.15 |
| PGLS | | | |
| Log10(seed mass) | 1, 179 | 105.98 | < 0.0001 |
| Longevity | 1, 179 | 23.69 | < 0.0001 |
| Pollination type | 2, 178 | 29.42 | < 0.0001 |
| Disturbance | 1, 179 | 1.12 | 0.29 |

able pairs with contrasting results for cross-species analysis and phylogenetic analysis but for those pairs the difference was less clear, because it was only valid after correcting the P values using the sequential Bonferroni method. Secondly, results of both, the PGLS and hierarchical partitioning show that unlike in cross-species analysis, longevity has a significant effect on pollen-ovule ratio independent of other variables in the model. There is no significant interaction effect with pollination type ($F_{2,178} = 1.16$, $P = 0.31$). The independent effect of longevity is about twice the joint effect (see Fig 2b).

## Discussion

### *Reproductive assurance and pollen-ovule ratios*

Although reproductive assurance has a long history in plant ecology, dating back to Darwin (1876), thorough tests of this hypothesis have only been undertaken recently. Most of these studies focus on intraspecific analysis producing ambiguous results (Eckert et al. 2006). Results of several comparative approaches indicate that selfing species occur more often than outcrossers in habitats that are prone to uncertain pollination, which supports the reproductive assurance hypothesis (Jain 1976, Price & Jain 1981). However, Price & Jain (1981) point out that such results must be interpreted with caution because correlations between traits do not necessarily imply causation, and correlation with other traits

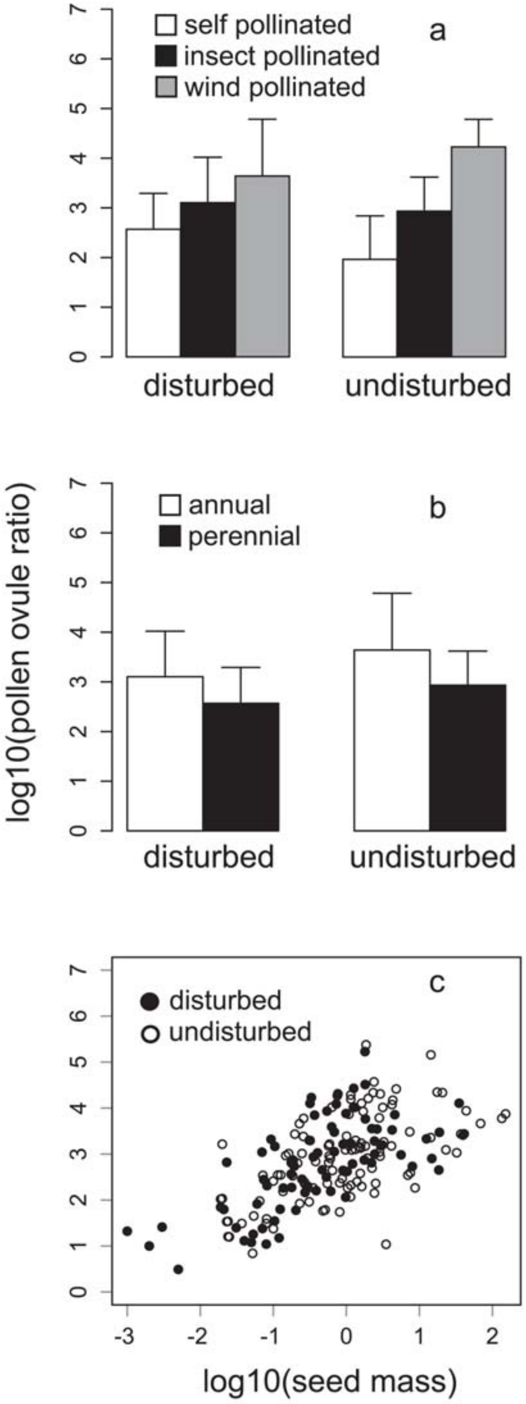

Fig. 1. – Interaction bar plots of the effect of disturbance and pollination type, and habitat disturbance and longevity on the pollen-ovule ratio (a + b), and scatter plot of pollen-ovule ratio and seed size (c).

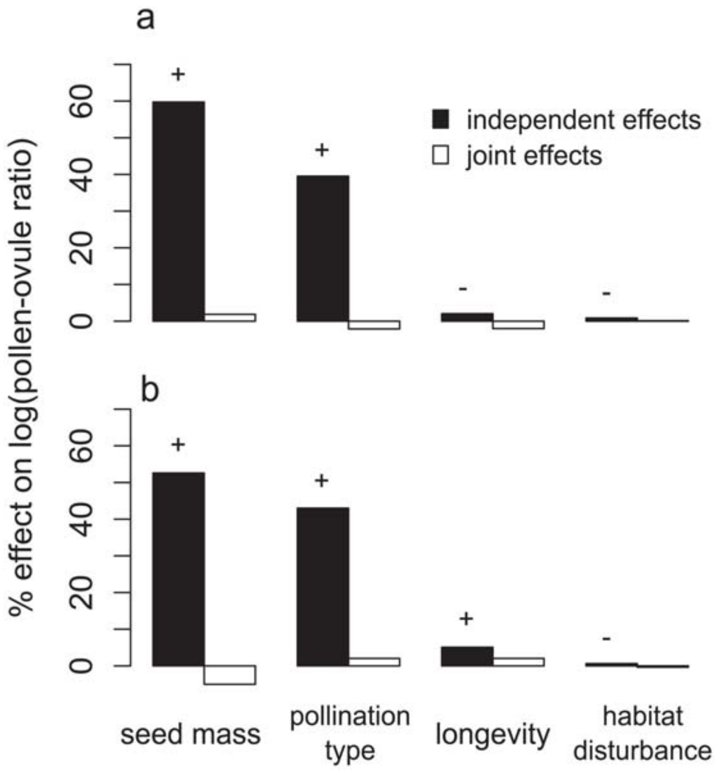

Fig. 2. – Independent and joint effects of seed mass, pollination type, longevity and habitat disturbance on the pollen-ovule ratios obtained by hierarchical partitioning of ordinary least squares (a) and phylogenetic generalized least squares (b).

and phylogeny may confound relationships between traits. We tried to minimize this problem by statistically controlling for several factors that might confound our analysis of the relationship between disturbance and pollen-ovule ratio, including phylogeny.

Contrary to our expectation, we did not detect any correlation between pollen-ovule ratio and disturbance. This result is striking as we found strong correlations between mating system and pollen-ovule ratios, and between mating system and disturbance, at least for the cross-species analysis. Hence, we would expect to find a correlation between habitat type and pollen-ovule ratio at least in form of an indirect relationship mediated via the mating system. However, this expectation is driven by assuming certain directions in the relationships between the variables, which we cannot infer with certainty. For example, we assume that the mating system is one of the variables that causes variation in pollen-ovule ratios. In contrast, it could be the reverse with floral traits and behaviour defining the mating system of a plant (Barrett 1998).

We can state with some certainty that there is no difference in pollen-ovule ratios of the plants growing in disturbed vs undisturbed habitats in our dataset. Plants in both types of habitat seem to have evolved a variety of mating strategies, even when selfed species are more prominent in disturbed habitats. It is unlikely that this result arose from hemeroby being an inadequate measure of disturbance. Though hemeroby may be a “complicated concept” (Hill et al. 2002), there is evidence that this trait reflects disturbance gradients (Fanelli & De Lillis 2004). Also, there was no substantial difference in the results of the analysis that used hemeroby as a disturbance indicator and that which used a classification based on the probability of pollinator occurrence.

These results contradict the findings of Cruden (1976, 1977) who records significant differences in mean pollen-ovule ratios among habitats. Cruden classified habitats as highly disturbed, early successional, late successional with unreliable pollinator activity and late successional with reliable pollinator activity. As described in the introduction, we aimed to apply a similar categorization, though our categories were derived from general information found in floras and do not reflect the actual habitat in which the plant was found. However, it is unlikely that the more general approach affected the results. On the contrary, such a selection of species is more randomized than picking certain species “at random” in their habitats as done by Cruden.

It should also be noted that the hemeroby classification is based on anthropogenic disturbance. Lake and river shores or glacier forefields provide an example of disturbed habitats where disturbance is non-anthropogenic. However, the number of species in our dataset occupying such habitats is relatively low and is unlikely to have affected our general results. Furthermore, our second habitat classification, which incorporated expected pollinator occurrence, also accounts for non-anthropogenic disturbance. If there really is a difference in pollen-ovule ratios among species of differently disturbed habitats, we should have detected it in our comparative analysis of data from the literature.

### *Pollen-ovule ratio variation explained by seed size and pollination type*

A correlation between seed mass and the pollen-ovule ratio is previously reported in several studies (Uma Shaanker & Ganeshaiah 1984, Preston 1986, Götzenberger et al. 2006), supporting the theory of Charnov that pollen-ovule ratios are governed by the allocation of resources to female sex function. In fact, the data in this study on the pollen-ovule ratio and seed size was part of a study that tested Charnov’s hypothesis (Götzenberger et al. 2006) and found a tight correlation between the two traits for some 300 species. Here we show that the pollen-ovule ratio also strongly depends on pollination type even when the effect of seed mass is taken into account. Using the type of pollination as an indicator of the efficiency of pollination, supports our hypothesis that sex allocation and pollination efficiency both govern variation in pollen-ovule ratios and are not mutually exclusive.

Previous studies have reported an association between pollen-ovule ratios and pollination efficiency (see below). As in the present paper, these studies are also affected by pollination efficiency being an ill-defined trait that is not easily measured. Thus, other traits that determine the probability of pollen grains reaching a stigma were used and interpreted in terms of pollination efficiency. For instance, the strikingly low pollen-ovule ratios in *Orchidaceae* are attributed to their very specialized means of pollination, consisting of species-specific insect pollinators and transportation of pollen in dispersal units (Mehrhoff 1983, Lehnebach & Riveros 2003). Similarily, Cruden & Millerward (1981) focused on bee-pollinated species and used the stigma area relative to the pollen-bearing area of the pollinator as a measure of pollination efficiency and found a negative correlation with pollen-ovule ratios.

For our study, it might be argued that pollination type is not a proper indicator of pollination efficiency because it only reflects the mating system and selfing species predominantly self-mate and plants with insect and wind pollination tend to outcross. However, it was the only indicator of pollination efficiency available. Moreover, we repeated the multivariable linear model analysis for only outcrossed species and found the same variables to be significant as for the analysis including all species. Hence, pollen-ovule ratios for wind-pollinated plants are higher than for insect-pollinated plants after accounting for the effects of seed mass, longevity and mating system. This result is reasonable since wind pollination is a far more stochastic process than the transmission of pollen by insects, though a few wind pollinated plant species may have pollen-ovule ratios that are unexpectedly low (Ackermann 2000).

### *The effect of phylogeny*

Phylogenetic comparative methods always depend on a number of evolutionary assumptions. The phylogenetic hypothesis represented by the phylogenetic tree is assumed to reflect the "true" phylogeny. This is rarely accurate for any phylogeny. In our study species were not selected based on their phylogeny but on whether there was data on their pollen-ovule ratio. Thus, the resulting phylogenetic tree is only a subsample of a tree consisting of many more species. However, such subsamples of larger phylogenies are statistically robust estimates of trait correlation for phylogenetically independent contrasts (Ackerly 2000), a method that is conceptually different from PGLS but produces the same estimates when a Brownian model of evolution is assumed (Rohlf 2001). The high value for the estimate of $\lambda$, a measure of phylogenetic correlation within a trait, indicates that pollen-ovule ratios are likely to evolve according to the Brownian model of evolution. Finally we assumed all branches to be of equal length. This assumption is also likely not to be met by the "true" phylogeny but simulation studies show that results from analyses with equal branch lengths are meaningful, especially when no information on branch length is available (Purvis et al. 1994, Ackerly 2000).

Although most of the results of cross-species analyses are confirmed by the results of phylogenetic analyses there are two differences that are worth noting. First, we found a significant difference in seed mass between species of high disturbance and those of low disturbance sites within cross-species analysis, which supports the life strategy hypothesis that species with many small seeds can cope better with high levels of disturbance (Grime 2001, Lavorel et al. 2007). The correlation between seed mass and disturbance was not revealed by PGLS. This suggests that although the present day species in disturbed habitats tend to have smaller seeds compared to present day species in undisturbed habitats, this trait combination has not occurred on many occasions throughout evolutionary history. Secondly, the relationship between longevity and pollen-ovule ratio shows the opposite pattern. While pollen-ovule ratios increased with a change from an annual to a perennial longevity strategy in separate lineages, there is no evidence in our data that current annual species have smaller pollen-ovule ratios than perennial species. Similarly, and probably as a consequence, there is no significant interaction between longevity and pollination type in the multivariate PGLS, unlike in the cross-species analysis. Apart from these two exceptions, it seems that the factors that have driven the evolution of pollen-ovule ratios also affect current species.

## Conclusions

This study did not find that species in disturbed habitats have on average lower pollen-ovule ratios than species in undisturbed habitats with reliable pollinator activity. Such a distinction is still conceivable on an intraspecific level and is recorded for several species (Pellmyr 1985, Affre et al. 1995, Dubois et al. 2003) However, it seems likely that the range of mating systems, and thus the range of pollen-ovule ratios in a particular habitat is too large to detect general interspecific differences among habitats. This becomes even more evident when the wide range of pollen-ovule ratios within mating systems is taken into account. Both seed mass and pollination type have a strong independent effect on pollen-ovule ratios, which corroborates our previous proposition that sex allocation as well as pollination efficiency govern interspecific variation in pollen-ovule ratios.

See http://www.preslia.cz for Electronic Appendix 1.

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## Souhrn

Práce se zabývá dvěma důležitými faktory, o nichž se předpokládá, že určují vnitrodruhovou variabilitu v poměru mezi počtem pylových zrn a vajíček – disturbancí stanoviště a způsobem opylení, který byl využit jako zástupná proměnná pro účinnost opylení. Protože hmotnost semen je silně korelována s poměrem mezi počtem pylových zrn a vajíček, byla také zahrnuta do analýz s cílem zjistit, zda případný vliv disturbance stanoviště zůstane průkazný i po odfiltrování jejího vlivu. Disturbance neměla statisticky průkazný vliv na vnitrodruhovou variabilitu v poměru mezi počtem pylových zrn a vajíček, což je v rozporu s předpokladem, že při nedostatku cizího pylu či opylovačů selekce zvýhodňuje samoopylení („reprodukční pojistka“); způsob opylení naproti tomu vysvětlil značnou část této variability, a to i v modelech beroucích v potaz silný vliv hmotnosti semen. Použití fyloegenetických komparativních metod přineslo tytéž výsledky. Výsledky práce souhlasí s predikcemi teorie směrování zdrojů do samčí a samičí funkce a předpokladem, že poměr mezi počtem pylových zrn a vajíček závisí na pravděpodobnosti, s jakou se pylové zrno dostane na bliznu.

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