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Large pollen at high temperature: an adaptation to increased competition on the stigma?

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Abstract Pollen availability is a major constraint of plant reproductive success. Because pollen size trades-off with the quantity of produced grains, the link between climate characteristics and the determination of pollen size is of fundamental importance. To minimize the rate of water loss due to desiccation, a plant should produce larger grains that also have a lower surface-to-volume ratio. We used a comparative analysis to examine the hypothesis predicting increase in pollen size as a response to desiccation intensity. To test the hypothesis, we correlated the data on pollen size with the climate characteristics, temperature and desiccation intensity of the flowering period, for 232 plant species of 11 taxonomic groups. The analysis showed a positive relationship between the pollen size and temperature, but not with the desiccation intensity. We discuss the potential mechanisms by which increased temperature is an indicator of high competition among pollen grains on the stigma, which in turn is expected to promote large pollen. Our work provides insight into the temperature dependence of pollen production in plants and reveals a link between environmental temperature and the intensity of limitation of plant reproductive success by pollen availability. The result is relevant in the context of global

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climate change. We also discuss why environmental temperature has to be controlled in studies dealing with pollen production, particularly in investigations of size-number trade-off.

Keywords Pollen limitation · Optimal pollen size · Temperature · Desiccation · Size-number trade-off · Competition on stigma

Introduction

Pollen limitation (PL) has been recognized as a considerable constraint of reproductive success in plants. Insufficient pollen delivery to stigmas and poor genetic quality of pollen have been proposed as two major components of PL (Aizen and Harder 2007; Ashman et al. 2004; Hegland and Totland 2008; Knight et al. 2005; Larson and Barrett 2000). The quantitative component of the concept links the reproductive performance of plants with the fundamental trade-off between size and the number of produced pollen grains. The assumption of the size-number trade-off is that resources available for male function are limited, and indeed, several studies confirmed that plants have to compromise between the quantity and volume of produced pollen grains (Mione and Anderson 1992; Sarkissian and Harder 2001; Vonhof and Harder 1995; Yang and Guo 2004). However, empirical support of the existence of the size-number trade-off does not explain which ecological or functional factors determine optimal combinations of size and quantity of pollen produced by a plant growing under given conditions.

The diversity of the pollen sizes produced covers a range of three orders of magnitude, which corresponds to five orders of magnitude regarding the differences in volume (Harder 1998). Such an exceptional diversity is particularly striking in light of the role of pollen size in fertilization, and the size-number trade-off outlined above has not been fully explained. Even the relation between genome size and pollen size appears to be weak when species relatedness is taken into account (see Knight et al. 2010). Certain evidence suggests that pollen size determines reproductive success at several levels. There is a strong positive relationship between pollen grain size and seed-siring success (Cruzan 1990). The size of pollen also

determines the quality of the sired progeny because early fertilized ovules are more generously provisioned by the maternal plant than those that are fertilized later (Delph et al. 1998). Most importantly, large pollen grains have higher chances of success in competition and successful fertilization because their size determines the growth rate of pollen tubes (Gore et al. 1990; Lord and Eckard 1984; Manicacci and Barrett 1995; Vanbreukelen 1982). The relationship corresponds well to the positive trend between pollen size and stigma depth, which is a good measure of the distance that pollen tubes must grow using endogenous resources (Cruden 2009) or between pollen protein content and stigma–ovule distance (Roulston et al. 2000). Kirk (1993) suggested that the evolution of pollen grain size is indirectly driven by the positive selection for seed size, as this is expected to lower the number of seeds per flower and thus intensify the competition between pollen grains. In line with that, pollen size has been shown to correlate positively with seed size over a wide range of species (Kirk 1993).

Low desiccation hypothesis: under high desiccation intensity, pollen grains are large to minimize the rate of water loss

Water balance is an important issue in plant life-histories from the earliest stage of development. Whereas different species represent a wide continuum with respect to the relative water content of produced grains (Franchi et al. 2002; Nepi et al. 2001), plant physiologists classify pollen into two types depending on the water content threshold (Firon et al. 2012). Pollen of recalcitrant type contain more than 30 % water, desiccate quickly during transport and their viability dramatically decreases when the relative water content declines (Aylor 2003; Franchi et al. 2002; Nepi et al. 2001). The fate of pollen with relative water content lower than 30 %, denoted orthodox, is believed to be determined by their ability to keep water content at relatively low levels and, what is more important, as constant as possible during the dispersal phase (Firon et al. 2012). The viability of orthodox pollen dramatically declines, through irreversible damage to the cytoskeleton, when grains are subject to cycles of hydration and dehydration caused by fluctuations of air humidity (Heslop-Harrison and Heslop-Harrison 1992). Thus, in orthodox pollen we can also expect that natural selection promotes traits

that decrease vulnerability to desiccation and reduce fluctuations of relative water content.

The surface-to-volume ratio is one of the major traits that determine the rate of water loss, which in turn translates to viability and chances of successful pollination (Aylor 2003; Ejsmond et al. 2011). Thus, larger grains should have an advantage over smaller ones when desiccation intensity increases. Indeed, as long as we assume that desiccation is the major force determining the viability of pollen grains, we can expect that the optimal strategy for a plant flowering under high desiccation intensity is to produce small numbers of relatively large pollen grains (Ejsmond et al. 2011). Such a tendency has been confirmed by the intraspecific analysis of pollen size of eight species of Rosaceae according to which plants flowering in conditions with high temperatures and high potential evapotranspiration (PET) produced significantly larger pollen grains than conspecifics growing at sites with lower temperatures and lower PET (Ejsmond et al. 2011).

In this paper, we test the "low desiccation" hypothesis that links the desiccation intensity of environment with the size of the pollen produced by insect-pollinated plants. Our expectation supporting the "low desiccation" hypothesis is that high PET during flowering should correlate positively with the size of produced pollen irrespective of temperature. However, the analysis must disentangle the positive correlation between temperature and PET.

Materials and methods

Pollen morphology characteristics

We extracted already published data on the mean polar (P) and equatorial (E) axes of pollen grains for 232 species of 11 taxonomic groups (four tribes and seven genera) published in 10 peer-reviewed articles (Table 1). We then used these measurements to calculate the mean pollen grain volume (V), surface area (S) and the surface-to-volume ratio (S/V). For the geometrical model of a pollen grain, we chose a spheroid (i.e., ellipsoid with two equal radii, 0.5P, 0.5E, 0.5E) with S and V calculated according to standard formulas (e.g., http://mathworld.wolfram.com/Spheroid.html, see also Supplementary material Appendix 1). All characteristics of pollen morphology

used in our analysis as well as the original data that were extracted from the papers and are available in Appendix 2 in Supplementary material. In 92 % of cases, a species was represented by one specimen; in the few species that were represented by two or three specimens, we analyzed the means of the pollen characteristics (given in bold in Supplementary material Appendix 2).

Assignment of coordinates to localities of the place of collection

To determine the collection site for a given specimen and assign geographical coordinates, we used descriptions of localities given in source articles or obtained from herbaria, hardcopy, and digital topographical maps (mainly maps available at The University of Texas Libraries and Google Maps) (see Supplementary material Appendix 3A, B for collection localities). We excluded all cases in which we were unable to find collection sites or the described collection site corresponded to areas with large differences in altitude (up to 500 m). We also excluded all species for which the location referred to a name of an area larger than several dozen square kilometers, e.g., highlands, plains, lowlands. If the collection site was described as a place between cities and villages, we took a midpoint between the given places only in the case of a lack of considerable differences in altitude (up to 500 m) and only if the distance between the mentioned places was not greater than several kilometers; otherwise, the data were excluded.

Climate conditions during flowering periods

Flowering periods for species belonging to *Brunellia*, Cardueae, Centaurinae, and *Muscari* were based on dates of specimen collection. We chose a period of 31 days (1 month) as a time interval for calculation of the mean temperature and PET at flowering, and the day of collection was in the middle of this period. For example, if a plant was collected on May 5, the temperature was calculated as (5 + 15)/31 May temperature +11/31 April temperature. In two cases, i.e., *Muscari coeleste* and *Acantholepis orientalis*, only information on the month of collection was available. In such cases, we used the monthly mean for the temperature and the PET at the collection site in the analysis. We did not have collection dates for

Table 1 General information on the species used in the study

Taxon name	Rank	No. of species investigated	Geographic region	Source
Brunellia	Genus	**16** (0.67)	South America	Orozco (2001)
Cardueae	Tribe	**14** (0.78)	Middle East, North Africa	Garnatje and Martin (2007)
Cayaponia	Genus	**25** (0.83)	Brazil	Barth et al. (2005)
Centaureinae	Subtribe	**16** (0.84)	Middle East, North Africa	Villodre and Garcia-Jacas (2000)
Lessingianthus	Genus	**65** (0.59)	South America	Angulo and Dematteis (2010)
Matthioleae	Tribe	**9** (1.00)	Egypt	Khalik et al. (2002)
Muscari	Genus	**14** (1.00)	Turkey	Pehlivan and Ozler (2003)
Mutisia	Genus	**23** (0.44)	South America	Telleria and Katinas (2009)
Sisymbrieae	Tribe	**9** (0.69)	Egypt	Khalik et al. (2002)
Stachys	Genus	**18** (0.62)	Iran	Salmaki et al. (2008)
Vernonia	Genus	**23** (0.96)	Argentina, Paraguay	Dematteis and Pire (2008)

Because in some cases, we were unable to assign geographic coordinates to collection sites (see. Supplementary material Appendix 2 for details), the number of analyzed species from each taxonomic group (given in bold) was also expressed as a fraction of the total number of species investigated in source articles (given in parentheses)

species belonging to *Cayaponia*, *Lessingianthus*, Matthioleae, Sisymbrieae, *Stachys*, *Vernonia* and the majority of the *Mutisia* specimens. For these groups, the flowering period was assessed from the literature and on-line data sources such as the Turkish Plants Data Service (http://turkherb.ibu.edu.tr), or labels of specimens of the same species as in our study that were collected in the same geographical region, which are available at herbaria on-line collections (main resource of the Missouri Botanical Garden Herbarium). The flowering periods used in the analysis were 4-month periods covering the majority of flowering dates found in literature and on-line resources. We used the following flowering periods: Matthioleae and Sisymbrieae (February–May); *Stachys* (May–August); *Cayaponia*, *Lessingianthus*, *Mutisia*, and *Vernonia* (4 warmest months at the collection site).

To correlate the pollen size with the climatic conditions at the collection sites, we used data on temperature and PET for corresponding places and flowering periods. The temperature and PET at flowering were calculated on the basis of the mean monthly temperature and PET during the period from 1950 to 2000 with a resolution of approximately 0.55×1 km (Hijmans et al. 2005; Trabucco and Zomer 2009). Geographical coordinates, descriptions of the location of the collection sites, and the climate and pollen characteristics are available in Supplementary material: Appendix 2 and 3A, B. All geographic data were processed in ArcGIS 9.3 (Environmental Systems Research Institute, Redlands, USA).

Statistical analysis

To obtain one variable that best characterized both the susceptibility to desiccation and the competitive abilities of the pollen, we performed principal component analysis (PCA) for the five pollen characteristics: polar axis (P), equatorial axis (E), pollen grain volume (V), surface area (S), and surface-to-volume ratio (S/V). To ensure normal distributions, P, E, V, and S were $\log_{10}$ transformed, whereas the S/V ratio was square root transformed. Because the temperature and PET were highly correlated (Pearson correlation coefficient $r = 0.79$, $N = 232$), we also performed PCA to obtain the independent variables best corresponding to temperature and desiccation intensity partly independent of temperature. The temperature and PET in the flowering periods were $\log_{10}$ transformed to assure normal distribution. Principal components calculated for pollen morphology were then analyzed with the general linear model (GLM) with the climate principal component as the continuous predictor and the taxonomic group as the categorical predictor. To take into account the fact that pollen morphology of different taxonomic groups can change in a different manner with climatic conditions, for each analysis, we first verified the GLM model with an interaction term included between the taxonomic group and the climate principal component. If the interaction term turned out to be non-significant, we performed a simple ANCOVA to assess the common slope characterizing the strength of the relationship.

PCA analyses were performed with STATISTICA 10 (StatSoft, Tulsa, USA). To check the robustness of the GLM analyses, we performed Linear Mixed Model fitting with the packages *nlme* and *lme4* implemented in R (Bates et al. 2015; Pinheiro et al. 2015; R Core Team 2015).

We also carried out a complementary analysis based on model selection and Akaike information criterion (AIC). The model selection was undertaken by fitting a series of candidate linear models, including simple regression and GLM with three predictor variables (temperature, PET, and taxonomic group) and interaction terms included. Candidate models were formulated for five dependent variables: polar axis (P), equatorial axis (E), pollen grain volume (V), surface area (S), and surface-to-volume ratio (S/V). Best-fit models were indicated by lowest AIC value and highest Akaike weight w_i providing probability that a given model is the best-fit out of all tested models (Burnham and Anderson 2004). The model selection was performed with R (R Core Team 2015).

In comparative analyses, there is a need to control for phylogenetic signal, i.e., the tendency of closely related taxonomic groups/species to resemble each other (Harvey and Pagel 1998). We were able to construct a composite phylogeny for 60 species of six taxonomic groups: Cardueae, *Cayaponia*, Centaureinae, Matthioleae, *Mutisia*, *Stachys* (Appendix 4 in Supplementary material), with tree topology and branch lengths, based on the outcome of genetic data analyses (for details see Appendix 1 in Supplementary material). Next, using the constructed phylogenetic tree and PDAP (Phenotypic Diversity Analysis Programs version 6.0, Garland and Ives 2000; Garland et al. 1999) we generated variance–covariance matrices used as an input for the software that fit linear statistical models. Results of ordinary (i.e., non-phylogenetic) least squares model (OLS) were compared with the results of the phylogenetic generalized least squares model (PGLS) (Garland and Ives 2000), which provide identical results and is mathematically equivalent to phylogenetic independent contrasts (Garland and Ives 2000; Rohlf 2001). Because OLS and PGLS analyses assume either no or relatively strong phylogenetic signal, we also tested two statistical models in which the strength of phylogenetic signal is estimated simultaneously with regression coefficients (Freckleton et al. 2002). These models are linear statistical models based on an Ornstein–Uhlenbeck process of adaptive evolution (OU) and linear model with Pagel's lambda transformation (PLT) with fitted parameters d (OU) and λ (PLT). The parameters d (OU) and λ (PLT) indicate the strength of the phylogenetic signal (see Blomberg et al. 2003). In the case of both d and λ, the value of 1 indicates that the original candidate tree best fits the data assuming that traits evolved according to the Brownian motion model (PLT, λ) or the Ornstein–Uhlenbeck process of adaptive evolution (OU, d). When the parameters are close to 0, the best-fitting evolutionary model is estimated to be a star phylogeny (no phylogenetic signal). The intermediate values between 0 and 1 indicate that branch lengths that are intermediate between the derived and a star phylogeny provide the best fit (Blomberg et al. 2003; Freckleton et al. 2002; Pagel 1999). As an indicator of the support of models fitted to the data of relatively small number of cases, we report the AICc (Akaike information criterion corrected for finite sample sizes) (Burnham and Anderson 2004; Lavin et al. 2008). When comparing a series of models the one with the lowest AICc were considered to be the best. Apart from AICc, we also present Akaike weights (w_i) that indicate probability that a given model is the best-fit out of all tested models (Burnham and Anderson 2004). Phylogenetically informed statistical linear models were run with the program Regression 2 (Lavin et al. 2008) implemented in Matlab (MathWorks, USA). Whereas the results of the phylogenetically informed analysis were based on a phylogenetic tree with branch lengths calculated on the base of genetic distances, the conclusion did not change when branch lengths were specified by Pagel's (1992) method of branch length manipulation (not shown). Similarly, as in the case of the GLM analyses that were run on the full dataset, we performed PCA to obtain variables that best characterized both the susceptibility to desiccation and the competitive abilities of the pollen, and used these variables in phylogenetically informed analysis.
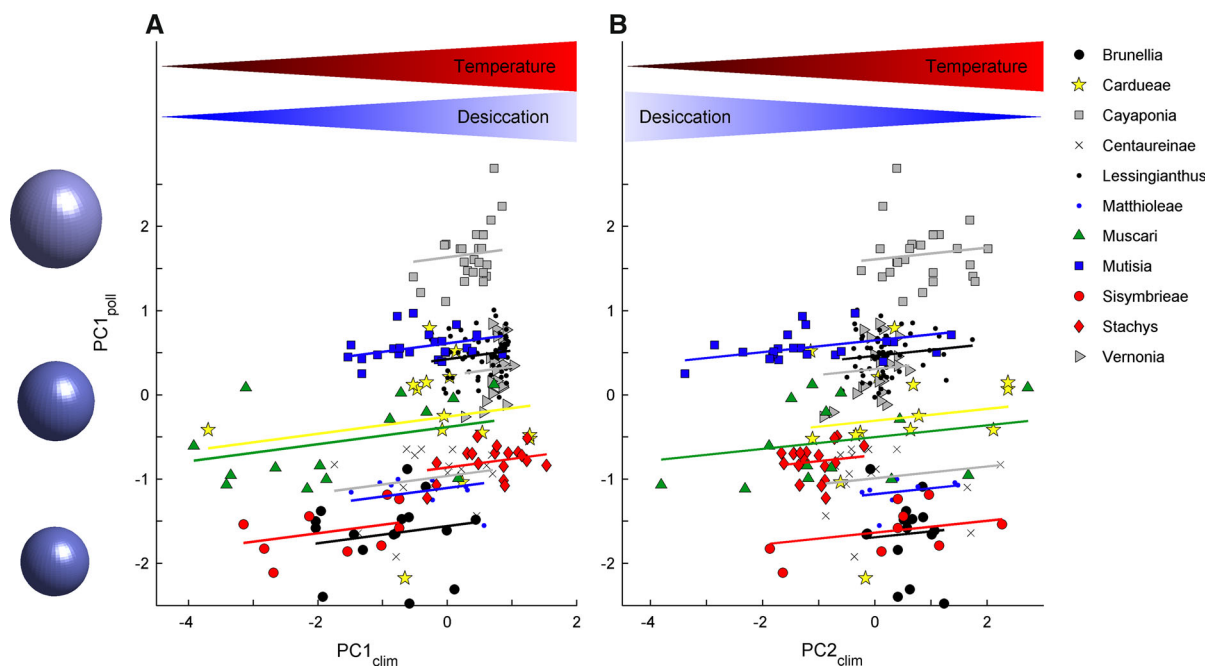
Results

The first component extracted by PCA on the base of characteristics of pollen morphology, hereafter denoted PC1$_{poll}$, explained 98.7 % of the variance and can be interpreted as a measure of pollen size. Both axes lengths, surface area and pollen grain

volume were positively correlated with $PC1_{poll}$ (factor loadings for P, E, S, and V were greater than 0.98). Increasing $PC1_{poll}$ also corresponded with a decrease in the surface-to-volume ratio with a very high determination (factor loading for S/V equal to -0.99). Two components extracted by PCA for the temperature and PET of the flowering period, $PC1_{clim}$ and $PC2_{clim}$, explained 89.6 and 10.4 % of the variance, respectively. Increase in $PC1_{clim}$ indicated raising temperature and PET during flowering period accordingly (factor loadings for temperature and PET were equal to 0.95). With the increase in $PC2_{clim}$, the mean temperature of the flowering period increased while the PET decreased, as noted by the factor loadings of 0.32 for temperature and -0.32 for PET. In other words, the high value of $PC1_{clim}$ indicates places with high temperature and high PET (hot and humid), whereas high value of $PC2_{clim}$ indicates places with high temperature and low PET (hot and arid).

The analyzed taxa did not differ with the response of the pollen size described by $PC1_{poll}$ to PC's describing climate ($PC1_{clim} \times$ taxonomic group:

$F_{10,210} = 1.29$, $p = 0.238$; $PC2_{clim} \times$ taxonomic group: $F_{10,210} = 1.01$, $p = 0.428$), and the interaction term was thus excluded from further analysis. $PC1_{poll}$ was related positively to both $PC1_{clim}$ (Fig. 1a; $PC1_{clim}$: $F_{1,220} = 9.21$, $p = 0.003$) and $PC2_{clim}$ (Fig. 1b; $PC2_{clim}$: $F_{1,220} = 6.14$, $p = 0.014$). Pollen size thus correlated positively with temperature at both places with PET increasing and PET decreasing with temperature, which verifies negatively the 'low desiccation hypothesis'. The relationship between $PC1_{poll}$ and $PC2_{clim}$ was only slightly weaker than between $PC1_{poll}$ and $PC1_{clim}$ (common slope $\pm$ SE: 0.070 ± 0.028 and 0.101 ± 0.033, respectively). In both cases, the taxonomic group turned out to also be a significant predictor of the intercept ($p < 0.0001$ in both PC1poll vs. $PC1_{clim}$ and $PC1_{poll}$ vs. $PC2_{clim}$ analyses), which means that different taxa have different pollen sizes at the same value of $PC1_{clim}$ or $PC2_{clim}$ (Fig. 1a, b). Results derived with General Mixed Model fitting had qualitatively identical results with the GLM analysis (see Appendix 1 in Supplementary material for details).

Fig. 1 The relationship between $PC1_{poll}$, corresponding to pollen grains size and the surface-to-volume ratio, with principal components describing the climatic conditions of the flowering period. *Color arrows* at the top of each panel indicate the direction of the correlation between the climate principal component and temperature and desiccation intensity; high

values of $PC1_{clim}$ and $PC2_{clim}$ correspond to hot-arid and hot-humid climates, respectively. Because, the interaction terms of $PC1_{clim}$ and $PC2_{clim}$ with taxonomic group turned out insignificant (see "Results" section), all *trend lines* for taxa within each panel have a common slope. (Color figure online)

Table 2 Summary of the model selection analysis

Model	Dependent variable Y				
	S/V	V	S	P	E
$Y \sim T*$	0.00 (−408.1)	0.00 (462.6)	0.00 (272.7)	0.00 (−75.6)	0.00 (−29.8)
$Y \sim \text{PET*}$	0.00 (−390.7)	0.00 (485.4)	0.00 (295.3)	0.00 (−60.9)	0.00 (−3.4)
$Y \sim \text{taxon*}$	0.00 (−813.8)	0.00 (17.0)	0.00 (−171.5)	0.00 (−472.5)	0.00 (−492.5)
$Y \sim T* + \text{PET}$	0.00 (−407.1)	0.00 (464.6)	0.00 (274.7)	0.00 (−73.6)	0.00 (−27.8)
$\underline{Y \sim T* + \text{taxon*}}$	0.40 (−827.2)	0.46 (3.3)	0.47 (−185.4)	0.51 (−485.5)	0.43 (−505.8)
$Y \sim \text{PET} + \text{taxon*}$	0.00 (−814.6)	0.00 (15.7)	0.00 (−172.9)	0.00 (−473.9)	0.00 (−493.6)
$\underline{Y \sim T* + \text{PET} + \text{taxon*}}$	0.57 (−827.9)	0.50 (3.1)	0.50 (−185.5)	0.47 (−485.3)	0.51 (−506.1)
$Y \sim T* + \text{taxon} + T \times \text{taxon}$	0.01 (−819.1)	0.01 (11.0)	0.01 (−177.3)	0.00 (−484.6)	0.02 (−499.6)
$Y \sim \text{PET} + \text{taxon} + \text{PET} \times \text{taxon}$	0.00 (−801.6)	0.00 (24.0)	0.00 (−164.2)	0.00 (−465.7)	0.00 (−484.6)
$Y \sim T* + \text{PET} + \text{taxon}^{\#} + T \times \text{taxon}$	0.02 (−820.8)	0.01 (11.0)	0.01 (−177.3)	0.00 (−474.1)	0.02 (−500.0)
$Y \sim T* + \text{PET} + \text{taxon} + \text{PET} \times \text{taxon}$	0.00 (−816.1)	0.02 (9.8)	0.01 (−178.3)	0.01 (−478.3)	0.01 (−498.9)

Best-fit models (underlined) are indicated by lowest AIC value (given in parentheses) and highest Akaike weight w_i (given in italics). Akaike weights w_i indicate probability that a given model provide the best fit out of all tested models. The statistical significance of the predictor variables is indicated by superscript symbols (see table footnotes). For all models, significant effect of a continuous climate variable (T or PET) indicate increase of V, S, P, and E and decrease in S/V with increase in the climate variable. Significant effect of taxon indicates that studied taxa differed with respect to pollen characteristics. Climate variables T and PET and pollen characteristics V, S, P, E, were $\log_{10}$-transformed, V/S was square root transformed

* $p < 0.001$ for all considered Y variables

[#] $0.01 < p < 0.05$ for S/V and $p > 0.05$ for V, S, P, E

According to model selection analysis (see Table 2), inclusion of temperature, PET and taxonomic group provided the best fit for data on pollen volume V, surface-to-volume ratio S/V, surface area S and equatorial axis E. Variation in the polar axis P was best explained by temperature and taxonomic group (Table 2). In contrast to temperature, which turned out to significantly affect pollen characteristics in all models, the effect of PET was significant only when a simple regression was considered (Table 2). When significant, high temperature and PET indicated large pollen grains (high V, S, P and E) with low surface-to-volume ratio S/V.

Phylogenetically informed analysis

The PCA run on the subset of 60 species, for which we were able to resolve the phylogeny, showed very similar pattern to PCA analyses run on the full dataset. The principal component for pollen morphology, hereafter denoted $PC1_{p60}$, explained 98.6 % of the variance with factor loadings for P, E, S and V greater than 0.98 and loading −0.99 for S/V. Two principal components extracted for the temperature and PET of the flowering period, $PC1_{c60}$ and $PC2_{c60}$, explained

82.3 and 17.7 % of the variance, respectively. Increase in $PC1_{c60}$ indicated rising temperature and PET (factor loadings for temperature and PET were equal to 0.91). With the increase in $PC2_{c60}$, the mean temperature of the flowering period increased while the PET decreased (loading 0.42 for temperature and −0.42 for PET).

Out of four candidate models fitted in our phylogenetically informed analysis, the ordinary least squares linear model (OLS) provided the best fit with the data for both $PC1_{c60}$ and $PC2_{c60}$ predictor variables (Table 3). For models with $PC1_{c60}$ predictor variable, the OU model also showed a good fit with the data, but the value of parameter d was close to 0, which indicate a very week phylogenetic signal (Table 3). The results of model fitting showed that for the subset of 60 species, for which we were able to resolve the phylogeny, the residual variation was not explained by taking into account phylogenetic relatedness. Neither Brownian motion model of evolution (PGLS, PLT), nor adaptive evolution model (OU) provided a better fit with the data than ordinary least squares linear model (OLS). This indicates that the phylogenetic signal in the analyzed data were negligible. However, the sample size of 60 species was too little to confirm

Table 3 Summary of the model selection in phylogenetically informed comparative analysis

| Model | PC1$_{p60}$ $\sim$ PC$_{c60}$ + taxon + PC$_{c60}$ × taxon | | PC1$_{p60}$ $\sim$ PC$_{c60}$ + taxon | |
	PC1$_{c60}$	PC2$_{c60}$	PC1$_{c60}$	PC2$_{c60}$
OLS	_0.48_ (99.3)	_0.81_ (98.1)	_0.68_ (87.1)	_0.73_ (85.0)
PGLS	_0.00_ (152.4)*	_0.00_ (164.7)	–	_0.00_ (161.1)
OU d	_0.43_ (99.5), **0.03**	_0.05_ (103.8), **0.00**	_0.15_ (90.2), **0.00**	_0.09_ (89.3), **0.00**
PLT λ	_0.09_ (173.9), **0.91**	_0.15_ (101.5), **0.88**	_0.17_ (89.9), **0.90**	_0.18_ (87.8), **0.88**

Best-fit models (underlined) are indicated by lowest AICc value (given in parentheses) and highest Akaike weight w_i (given in italics). The statistical significance of the interaction term is indicated by a superscript symbol (see table footnotes). In all models with no interaction term included, the effect of climate predictor variables PC$_{c60}$ on pollen morphology was not significant. The AIC and w_i values for OU and PLT models are accompanied with the value of the fitted parameter (given in bold)

* $0.01 < p < 0.05$

the results obtained with GLM analyses performed for 232 species (see above). For all models fitted in our phylogenetically informed analysis, the pollen morphology was not significantly related to climate characteristics. Under the best-fit OLS models, the principal component PC1$_{p60}$ tended to increase with increasing values of climate principal components but the relationship was not significant (common slope ± 95CL: 0.050 ± 0.134 for PC1$_{c60}$ and 0.139 ± 0.177 for PC2$_{c60}$, OLS model).

Discussion

Our work provides evidence that interspecific variability in pollen size is affected by the environmental conditions during the flowering period. As pollen size was related to the climate principal component with opposite trends between temperature and PET, we were able to discriminate between the effects of the two interrelated environmental characteristics: temperature and desiccation intensity. The temperature explained a significant part of the variation in pollen size: large pollen grains were produced by plants growing in places with a high temperature independent of desiccation intensity. Although the surface-to-volume ratio decreased with the increase in pollen volume, we concluded that the variability in S/V, a primary morphological trait characterizing vulnerability to desiccation, was also explained by temperature and not desiccation stress. In other words, a lower surface-to-volume ratio at high temperature is a by-product of selection toward large pollen and not an adaptation for high desiccation stress. The conclusion is consistent with results of model selection analysis.

Whereas temperature and PET were involved in the model providing the best fit to data on surface-to-volume ratio, only the temperature was significantly related to S/V. In contrast to PET, temperature was a significant predictor for both pollen size characteristics and surface-to-volume ratio in all models that provide a good fit to the data (see Table 2). The phylogenetically informed analysis run on a relatively small subsample of the full dataset (the 60 species we were able to construct phylogeny) did not confirm results from the classical statistical analyses performed on the full dataset. Overall, the results obtained do not support the 'desiccation hypothesis' proposed in our earlier work (Ejsmond et al. 2011). According to the hypothesis, plants should respond to increased desiccation intensity in the environment by producing grains that have low surface-to-volume ratio. In the previous work, we showed that at the intraspecific level, plants may produce larger pollen in response to the combined effects of temperature and desiccation (Ejsmond et al. 2011). However, we were unable to separate the effects of these two environmental characteristics, perhaps due to the relatively small geographical region considered in the study, with a relatively narrow range of PET and temperature at the collection sites (see Figure Appendix 1 in Ejsmond et al. 2011).

Temperature has to be controlled in studies on pollen production

The fundamental assumption of the expected trade-off between the size and the number of produced pollen states that the resources available for male function are limited. However, in the majority of studies in which

the size-quantity trade-offs in pollen production were investigated, the potential diversity in the amount of resources available for a plant was not controlled for by the authors (but see Vonhof and Harder 1995). Increased temperature affects not only the size of produced pollen, as shown by our results, but is also likely to be an important determinant of the production rate in plants. Perhaps the lack of a relationship between size and the quantity of produced pollen reported by some authors (e.g., Aguilar et al. 2002; Lopez et al. 2005) can result from ignoring the effect of temperature on both pollen size and the resource pool available for male function.

Pollen size, competition on stigma and temperature

We found that pollen size correlates with the temperature during florescence, regardless of PET. We discuss below the mechanisms given as potential explanation for the observed pattern and based on the fact, that the size determines the competitive ability of the pollen (Gore et al. 1990; Lord and Eckard 1984; Manicacci and Barrett 1995; Vanbreukelen 1982).

Pollen grain competition is expected to be strong when numerous pollen grains are competing to be the parents of only few seeds. Pollen size has been shown to correlate positively with seed size over a wide range of species. At the same time, it is hypothesized that the selection for large seeds imposes a strong competition among pollen grains, which in turn could favor larger pollen due to the larger seed size (Kirk 1993). Environmental temperature is one of the factors associated with a change in seed mass during seed plant evolution (Moles et al. 2005), and several studies based on interspecific comparative analyses provide evidence that across plant taxa, seed size increases with temperature (Dainese and Sitzia 2013; De Frenne et al. 2013; Moles et al. 2007). Thus, it is possible that the evolution of pollen grain size can be driven by the temperature-seed size dependence.

Seed size correlates with genome size in a broad range of plant taxa (Beaulieu et al. 2007). As there are several reasons why genome size and pollen size should be positively correlated, the comparative analyses based on results of phylogenetic independent contrasts did not support this hypothesis (Knight et al. 2010). We are far from understanding the mechanism

driving the evolution of DNA content. Dozens of hypotheses have been proposed to explain the ultimate drivers of genome size variability observed across the tree of life (for example see Knight and Ackerly 2002; Kozłowski et al. 2003), with few influential non-adaptive hypotheses (e.g., Lynch 2007). Hence, in our opinion the role of genome size in the observed relationship between pollen size, temperature and seed size is unknown.

The foraging activity of insect floral visitors strongly depends on ambient temperature. There are studies suggesting that temperature, considered within a given plant community or an altitudinal gradient, is a strong determinant on the activity of insect pollinators and flower visitation rates (e.g., Arroyo et al. 1985; McCall and Primack 1992; Totland 2001; Wikstrom et al. 2009). The activity of hummingbirds, a second important group of pollinators, also decreases considerably at low temperatures (Elphick et al. 2001; Fernandez et al. 2002). According to this mechanism, increased temperature is an indicator of the high visitation rates of pollinators, under which the optimal strategy for a plant is to produce large grains that are able to compete with rival pollen delivered to stigmas.

The proposed mechanisms provide a link between the environmental temperature and the intensity of pollen competition, which is expected to promote the production of large pollen grains. If the strength of pollen competition rises together with temperature, we can expect a long-term change in pollen morphology towards a larger size. We can also expect intensification of PL with increasing environmental temperature.

According to the results presented, pollen size increases with temperature in several plant taxa. This suggests that pollen production and the intensity of PL can be indirectly determined by environmental temperature through the trade-off between size and the number of produced grains. The most likely explanation for the observed pattern is that the strength of the pollen competition increases with the temperature of the flowering period, which in turn is expected to promote large pollen grains.

provided detailed descriptions of the collection sites of some of the specimens included in our study: A.P. Clark (U.S. National Herbarium, Department of Botany, Smithsonian Institute), J.C. Solomon (Missouri Botanical Garden), T. Zanoni (New York Botanical Garden), M. Assadi (Research Institute of Forests and Rangelands Herbarium) and M.C. Telleria.

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