How much of morphological variability in pollen from genus *Rubus* L. might be explained by climate variability

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Abstract. The aim of this study was to assess the influence of 19 climatic factors during flowering periods and taxonomic proximity on the morphological features of the pollen from the genus *Rubus* L., which comprises numerous species, often with small ranges of natural occurrence.. It was hypothesized that the pollen morphology would be driven more by the effects of taxonomic proximity than by climatic variables, due to the conservatism of the pollen features, connected with a shared evolutionary history. The analyses revealed that climatic variability can explain an additional 2.5% to 14.0% of pollen morphology. The majority of the modelled pollen features were not correlated with the bioclimatic factors studied, except for the P/E ratio, which was positively correlated, and E, which was negatively correlated with PC3. However, most of the variability was explained by random effects connected with the taxonomic affiliation of the studied species to the genus *Rubus* L., which is very difficult in taxonomic terms. The study, therefore, showed how much additional interspecific variability in pollen morphology might be explained by the climatic variability of the species distributions.

Keywords: Intraspecific variability, interspecific variability, bioclimatic variables, mixed-effects models, variance partitioning, palynology, *Rubus.*

INTRODUCTION

Environmental factors which affect plant growth can be classified as abiotic factors and biotic factors. The abiotic factors that affect plant characteristics include topography, soil, and climatic factors (light, temperature, moisture etc.). They are the non-living components of the environment. They also affect plant adaptation (Eyduran et al. 2015; Unlukara 2019; Marsic et al. 2019).

Pollen production and pollen morphology may be strongly constrained by environmental factors, including climate (Charlesworth et al. 1987; Murcia 1990; Delph et al. 1997; Walther et al. 2002; Rao et al. 2019). Nevertheless, knowledge of their impact on the morphological features of pollen is scarce (Ejsmond et al. 2011, 2015). The most important climatic factors affecting the

growth and development of pollen are air temperature and humidity (Delph et al. 1997; Harder and Aizen 2010; Zinn et al. 2010; Ejsmond et al. 2011, 2015; Hinojosa et al. 2018). Furthermore, pollen grains are sensitive to abiotic stresses such as high temperature (Paupière 2014). Pollen reaction to heat stress during the flowering period was also observed by Prasad et al. (2011) and Omidi et al. (2014).

Several studies have confirmed that plants have to choose between the quantity and size of pollen grains produced (Mione and Anderson 1992; Vonhof and Harder 1995; Cruden 2000; Sarkissian and Harder 2001; Ashman et al. 2004; Yang and Guo 2004; Knight et al. 2005). However, empirical support of this compromise does not explain which ecological or functional factors determine the optimal combination of pollen size and quantity produced by a plant growing under given conditions (Ashman et al. 2004; Ejsmond et al. 2011, 2015). Therefore, there is a need for a new trend in palyno-climatic research, the results of which may be helpful in solving the abovementioned research problems. Ejsmond et al. (2011) proved that pollen production is closely connected to environmental temperature through the optimization of the number and size of the pollen grains produced. In the opinion of these authors, temperature does not significantly affect pollen shape. According to Ejsmond et al. (2015), pollen size increases with temperature. Indeed, it is likely that the intensity of the pollen competition on stigma increases the optimal temperature of the flowering period, which in turn is expected to promote large pollen grains.

The genus *Rubus* L. is species rich and includes from 750 to more than 1000 species distributed worldwide (Weber 1995); The genus includes 108 species in Poland (Kosiński et al. 2018). Genus *Rubus* is highly complex and is one of the most taxonomically challenging genera of flowering plants (Robertson 1974; Ling-Ti 1983; Richards et al. 1996) and circumscription of the species is complicated by hybridization, polyploidy, agamospermy, and the lack of a universal species concept (Gustafsson 1943; Weber 1996; Zieliński 2004; Zieliński et al. 2004). The very large and growing number of *Rubus* species are resulted from the small and local geographic distribution of their natural occurrence. A recent species concept for European *Rubus* agamosperms, only allows as species those biotypes whose distribution exceeds an area of 50 km in diameter (Weber 1996).

In this study, for the first time, 11 morphological features of the pollen from 57 *Rubus* species were tested for their correlation with 19 climatic factors during flowering periods. The aim was to assess the influence of bioclimatic variables and taxonomic proximity on the morphological features of the pollen in the genus *Rubus*, which comprises numerous species, often with small ranges of natural occurrence. It was hypothesized that the pollen morphology would be driven more by of taxonomic proximity than by climatic variables, due to the conservatism of the pollen features, connected with a shared evolutionary history.

STUDIED TAXA

The study was conducted on 57 *Rubus* species, representing four out of five subgenera, all three sections and 22 series found in Poland, including six endemic species (*R. capitulatus* Utsch*, R. chaerophylloides* Sprib.*, R. ostroviensis* Sprib.*, R. posnaniensis* Sprib.*, R. seebergensis* Pfuhl ex Sprib. and *R. spribillei* Sprib.) (Table 1). The taxonomic classification of the studied taxa followed Zieliński (2004). The verification of the taxa was made by Prof. Jerzy Zieliński (from the Institute of Dendrology, the Polish Academy of Sciences in Kórnik), an outstanding taxonomist and specialist of the genus *Rubus*.

Pollen sampling and preparation

Several randomly-selected inflorescences (flowers) were collected from 57 natural bramble localities in Poland (Table 1). The plant material was stored in the herbarium of the Department of Forest Botany, Poznan University of Life Sciences (PZNF).

Acetolysis was carried out on the pollen grains according to the method used by Erdtman (1952, 1960). The grains were mixed with the acetolysis solution, which consisted of nine parts acetic anhydrite and one part concentrated sulphuric acid. The mixture was then heated to boiling point and kept in a water bath for 2-3 min. The samples were centrifuged in the acetolysis mixture, washed with acetic acid and centrifuged again. The pollen grain samples were then mixed with 96% alcohol and centrifuged 4 times, with the processed grains subsequently divided into two groups. One half of the processed sample was immersed in an alcohol-based solution of glycerin for LM, while the other was placed in 96% ethyl alcohol in preparation for scanning electron microscopy (SEM). The SEM observations were made using a Zeiss Evo 40 and the LM measurements of the acetolysed pollen grains were taken using a Biolar 2308 microscope at a magnification of 640x. The pollen grains were immersed in glycerin jelly and measured using an ocular eyepiece with a scale. The measurement results were converted into micrometers by multiplying each measurement by two. Each sample consisted of 30 mature, randomly selected, properly developed pollen grains. In total, 1710 pollen grains were measured. This

Table 1. List of localities of the *Rubus* species studied.

No	Species	Localities	Geographical coordinates	Collector, herbarium
1	*R. acanthodes*	Poland, Dolnośląskie, Nowe Łąki near Pielgrzymka	51°07′06,1″N, 15°46′37,5″E	Boratyńska, Dolatowska, Tomlik, Zieliński; KOR
2	*R. allegheniensis*	Poland, Zachodniopomorskie, Łukęcin near Świnoujście	54°02′34,9″N, 14°52′23,8″E	Boratyńska, Dolatowska, Zieliński; KOR
3	*R. angustipaniculatus*	Poland, Mazowieckie, Zakrzew near Radom	50°26′27,3″N, 21°00′02,4″E	Maliński, Zieliński; POZNF
4	*R. apricus*	Poland, Wielkopolskie, Bachorzew near Jarocin	51°59′39,9″N, 17°33′49,9″E	Maliński, Zieliński; POZNF
5	*R. bavaricus*	Poland, Wielkopolskie, Robczysko near Leszno	51°48′41,4″N, 16°45′38,6″E	Danielewicz, Maliński; POZNF
6	*R. bifrons*	Poland, Podkarpackie, Łukowe near Sanok	49°25′20,1″N, 22°14′14,1″E	Oklejewicz; KOR
7	*R. caesius*	Poland, Lubuskie, Osiecznica near Krosno Odrzańskie	52°04′45,0″N, 15°03′11,0″E	Maliński, Zieliński; POZNF
8	*R. camptostachys*	Poland, Wielkopolskie, Raków near Kępno	51°11′16,8″N, 18°05′54,1″E	Zieliński; KOR
9	*R. canadensis*	Poland, Dolnośląskie, Bialskie Mts. near Stronie Śląskie	50°14′59,9″N, 16°57′45,7″E	Kosiński; KOR
10	*R. capitulatus*	Poland, Wielkopolskie, Psienie-Ostrów near Pleszew	51°57′48,2″N, 17°45′15,5″E	Danielewicz, Maliński; POZNF
11	*R. chaerophylloides*	Poland, Wielkopolskie, Laskowo near Chodzież	53°01′19,2″N, 17°05′45,4″E	Maliński, Zieliński; POZNF
12	*R. chlorothyrsos*	Poland, Pomorskie, Bargędzino near Łeba	54°43′53,4″N, 17°43′19,3″E	Boratyńska, Dolatowska, Zieliński; KOR
13	*R. circipanicus*	Poland, Zachodniopomorskie, Jarosławiec near Ustka	54°32′21,3″N, 16°32′31,6″E	Zieliński; KOR
14	*R. clusii*	Poland, Małopolskie, Dobronków near Tarnów	49°59′28,2″N, 21°20′37,5″E	Maliński, Zieliński; POZNF
15	*R. constrictus*	Poland, Małopolskie, Lipinki near Gorlice	49°40′20,4″N, 21°17′31,6″E	Oklejewicz; KOR
16	*R. corylifolius*	Poland, Lubuskie, Różanówka near Bytom Odrzański	51°46′05,4″N, 15°52′29,5″E	Maliński, Zieliński; POZNF
17	*R. czarnunensis*	Poland, Pomorskie, Drzewicz, Bory Tucholskie National Park	53°51′07,3″N, 17°34′08,4″E	Tomlik, KOR
18	*R. divaricatus*	Poland, Lubuskie, Bielawy near Bytom Odrzański	51°46′21,3″N, 15°55′09,6″E	Maliński, Zieliński; POZNF
19	*R. dollnensis*	Poland, Dolnośląskie, Młynowiec near Stronie Śląskie	50°16′36,1″N, 16°54′04,8″E	Kosiński, Tomaszewski, Zieliński; KOR
20	*R. fabrimontanus*	Poland, Lubuskie, Tarnów Jezierny Nowa Sól	51°51′45,1″N, 15°59′07,7″E	Maliński, Zieliński; POZNF
21	*R. fasciculatus*	Poland, Podkarpackie, Gruszowa near Przemyśl	49°40′57,4″N, 22°40′47,2″E	Maliński, Zieliński; POZNF
22	*R. glivicensis*	Poland, Małopolskie, Maga near Tarnów	50°00′09,8″N, 21°20′24,7″E	Maliński, Zieliński; POZNF
23	*R. gothicus*	Poland, Wielkopolskie, Pakówka near Bojanowo	51°40′20,7″N, 16°46′07,9″E	Maliński, Zieliński; POZNF
24	*R. grabowskii*	Poland, Lubuskie, Tarnów Jezierny Nowa Sól	51°51′45,1″N, 15°59′07,7″E	Maliński, Zieliński; POZNF
25	*R. gracilis*	Poland, Podkarpackie, Pod Lasem, near Rzeszów	49°53′42,5″N, 21°35′52,1″E	Maliński, Zieliński; POZNF
26	*R. hemrici-egonis*	Poland, Opolskie, Barnice near Głubczyce	50°03′02,5″N, 17°47′38,5″E	Kosiński, Tomaszewski, Zieliński; KOR
27	*R. hercynicus*	Poland, Dolnośląskie, Stare Bogaczowice near Wałbrzych	50°50′53,7″N, 16°11′37,4″E	Boratyński, Zieliński; KOR
28	*R. hevellicus*	Poland, Wielkopolskie, Tarce near Jarocin	52°00′02,4″N, 17°35′26,1″E	Maliński, Zieliński; POZNF
29	*R. idaeus*	Poland, Kujawsko-Pomorskie, Brodnica near Bydgoszcz	53°15′29,2″N, 19°23′57,9″E	Tomlik; KOR
30	*R. koehleri*	Poland, Dolnośląskie, Mirsk near Świeradów-Zdrój	50°58′19,9″N, 15°23′08,9″E	Boratyński; KOR
31	*R. lamprocaulos*	Poland, Dolnośląskie, Serby near Głogów	51°41′04,1″N, 16°06′42,9″E	Maliński, Zieliński; POZNF
32	*R. macrophyllus*	Poland, Dolnośląskie, Przywsie near Rawicz	51°34′37,1″N, 16°52′36,1″E	Maliński, Zieliński; POZNF
33	*R. marssonianus*	Poland, Pomorskie, near Kartuzy	54°20′03,2″N, 18°11′50,5″E	Boratyński; KOR
34	*R. micans*	Poland, Opolskie, Wieszczyna near Prudnik	50°19′18,2″N, 17°34′48,4″E	Kosiński, Tomaszewski, Zieliński; KOR
35	*R. mollis*	Poland, Dolnośląskie, Lądek-Zdrój, Trzykrzyska Mt.	50°20′54,6″N, 16°52′39,9″E	Kosiński, Tomaszewski, Zieliński; KOR
36	*R. montanus*	Poland, Dolnośląskie, Kowary near Kostrzyca	50°47′37,5″N, 15°50′01,8″E	Zieliński; KOR
37	*R. nessensis*	Poland, Dolnośląskie, Karczmisko near Kłodzko	50°17′56,7″N, 16°49′32,8″E	Kosiński; KOR

(Continued)

No	Species	Localities	Geographical coordinates	Collector, herbarium
38	*R. odoratus*	Poland, Lubelskie, Niedrzwica Duża near Lublin	51°06'51,3"N, 22°23'16,2"E	illegible name; KOR
39	*R. opacus*	Poland, Wielkopolskie, Starkowo near Leszno	51°58'37,7"N, 16°18'35,7"E	Zieliński; KOR
40	*R. orthostachys*	Poland, Wielkopolskie, Ostatni Grosz near Krotoszyn	50°39'54,4"N, 17°21'18,9"E	Maliński, Zieliński; POZNF
41	*R. ostroviensis*	Poland, Wielkopolskie, Wielkopolski National Park near Poznań	52°16'26,5"N, 16°46'50,1"E	Zieliński, Maliński; POZNF
42	*R. parthenocissus*	Poland, Podkarpackie, Koniusza near Przemyśl	49°40'57,4"N, 22°40'47,2"E	Maliński, Zieliński; POZNF
43	*R. pedemontanus*	Poland, Dolnośląskie, Nowy Kościół near Złotoryja	51°04'20,1"N, 15°52'05,3"E	Boratyński, Zieliński; KOR
44	*R. perrobustus*	Poland, Podkarpackie, Dudyńce near Sanok	49°39'04,9"N, 22°04'31,9"E	Oklejewicz; KOR
45	*R. pfuhlianus*	Poland, Wielkopolskie, Mieczewo near Kórnik	52°14'20,8"N, 17°00'27,8"E	Zieliński; KOR
46	*R. plicatus*	Poland, Lubuskie, Różanówka near Bytom Odrzański	51°46'05,4"N, 15°52'29,5"E	Maliński, Zieliński; POZNF
47	*R. posnaniensis*	Poland, Opolskie, Szybowice near Prudnik	50°21'09,5"N, 17°29'11,9"E	Kosiński, Tomaszewski, Zieliński; KOR
48	*R. pyramidalis*	Poalnd, Wielkopolskie, Chruszczyny near Ostrów Wielkopolski	51°38'41,4"N, 17°35'42,6"E	Maliński, Zieliński; POZNF
49	*R. radula*	Poland, Podkarpackie, Hermanowa near Rzeszów	49°56'07,4"N, 22°00'40,4"E	Maliński, Zieliński; POZNF
50	*R. schleicheri*	Poland, Wielkopolskie, Kościan	52°05'10,7"N, 16°38'41,9"E	Maliński, Zieliński; POZNF
51	*R. scissus*	Poland, Śląskie, Rudniki near Częstochowa	50°52'33,6"N, 19°14'28,5"E	Zieliński; KOR
52	*R. seebergensis*	Poland, Wielkopolskie, Wielkopolski National Park near Poznań	52°16'26,5"N, 16°46'50,1"E	Danielewicz; POZNF
53	*R. siemianicensis*	Poland, Wielkopolskie, Psienie-Ostrów near Pleszew	51°57'48,2"N, 17°45'51,5"E	Danielewicz, Maliński; POZNF
54	*R. sprengelii*	Poland, Wielkopolskie, Borownica near Zduny	51°38'20,8"N, 17°24'23,3"E	Maliński, Zieliński; POZNF
55	*R. spribillei*	Poland, Wielkopolskie, Gądki near Kórnik	52°18'45,4"N, 17°02'47,8"E	Zieliński; POZNF
56	*R. wimmerianus*	Poland, Podkarpackie, Gniewczyna Łańcucka near Przeworsk	50°06'19,5"N, 22°29'43,7"E	Oklejewicz, Zatorski; POZNF
57	*R. xanthocarpus*	Poland, Świętokrzyskie, Miedzianka near Kielce	50°50'22,5"N, 20°22'03,3"E	Maciejczak, Bróz, Zieliński; KOR

KOR - Herbarium of the Institute of Dendrology, Polish Academy of Sciences, Kórnik, Poland; PZNF - Herbarium of the Department of Forest Botany, Poznań University of Life Science.

study uses the results of biometric pollen measurements made for earlier, already published (Lechowicz et al. 2020) studies on morphology and variability pollen of Polish and European *Rubus* species.

Features analyzed

The pollen grains were analyzed for 11 quantitative characters: length of the polar axis (P) and equatorial diameter (E), length of the ectoaperture (Le), thickness of the exine along the polar axis and equatorial diameter (Exp and Exe), distance between apices of two ectocolpi (d) and P/E, Le/P, Exp/P, Exe/E, d/E (apocolpium index P.A.I) ratios.

The descriptive palynological terminology followed Punt et al. (2007) and Halbritter et al. (2018).

Climatic data

In the analysis, 19 bioclimatic variables were used (Table 2), developed for species distribution models BIO-CLIM (Booth 2018; Booth et al. 2014)SDM is one of the most active areas of global ecology. Three books published in 2009, 2011 and 2017 have reviewed SDM, and the closely related areas of ecological niche modelling and habitat suitability modelling. All three books provide excellent introductions to these topics, but give very little information on the role that BIOCLIM played in laying the foundation for these research areas. Understanding the history of BIOCLIM is vital because it was the first package to implement the basic SDM process in an easy-to-use integrated system. It provided what are still the most commonly used set of 19 bioclimatic variables and contributed to the development of the interpolation routines used to prepare the most commonly used source of bioclimatic data (WorldClim. These variables were obtained from the WorldClim 1.4 database (Hijmans et al. 2005) using *raster::getData()* function in 2.5' resolution (~5 km in the study area). Due to intercorrelations between the variables, Principal Component Analysis (PCA) was used to use the main gradients of the bioclimatic variables in the reduced space (Fig. 1 a-c). Prior to PCA, the variables were scaled and centered to avoid artifacts connected with differences in ranges and units. PCA was performed using *vegan::rda()* function. Analysis of the inertia shared by particular principal components (screeplot, Fig. 1 d) revealed that the PC1-PC3 axes explained more variance than the null model of random variance distribution (broken stick model). Thus, all of them were used in further analyses. PC1 described the transition between the wetter and colder parts of the

Table 2. Overview of bioclimatic variables used in this study.

Abbreviation	Variable	Unit
bio1	Annual Mean Temperature	°C
bio2	Mean Diurnal Range (Mean of monthly (max temp - min temp)	°C
bio3	Isothermality (BIO2/BIO7) (* 100)	°C
bio4	Temperature Seasonality (standard deviation *100)	°C
bio5	Max Temperature of Warmest Month	°C
bio6	Min Temperature of Coldest Month	°C
bio7	Temperature Annual Range (BIO5-BIO6)	°C
bio8	Mean Temperature of Wettest Quarter	°C
bio9	Mean Temperature of Driest Quarter	°C
bio10	Mean Temperature of Warmest Quarter	°C
bio11	Mean Temperature of Coldest Quarter	°C
bio12	Annual Precipitation	mm
bio13	Precipitation of Wettest Month	mm
bio14	Precipitation of Driest Month	mm
bio15	Precipitation Seasonality (Coefficient of Variation: mean/SD*100)	%
bio16	Precipitation of Wettest Quarter	mm
bio17	Precipitation of Driest Quarter	mm
bio18	Precipitation of Warmest Quarter	mm
bio19	Precipitation of Coldest Quarter	mm

study area (a positive correlation with mean annual temperature) and the warmer and drier parts (a negative correlation with precipitation in both cold and warm periods). Therefore, PC1 was seen as representing aridity and temperature gradient. PC2 increased as the temperature of the driest and coldest month rose, as well as the precipitation in the coldest quarter, but decreased with the increasing temperature range, isothermality, precipitation in the wettest month and precipitation seasonality. Thus, PC2 was considered to represent aridity and climate variability. PC3 described seasonal variation in the climate, showing a positive correlation with temperature isothermality and a negative correlation with seasonality. Therefore, PC3 represented continentality – the seasonal variation gradient from a maritime to a continental climate (high PC3 values indicated a low seasonality of temperature). These three axes of PCA explained 53.96%, 22.24% and 15.97% of the variability, respectively. A lack of variance inflation in the models was also ensured by calculating the variance inflation factors.

Data analysis

To assess the studied relationships which acknowledged intraspecific variability and species-specific
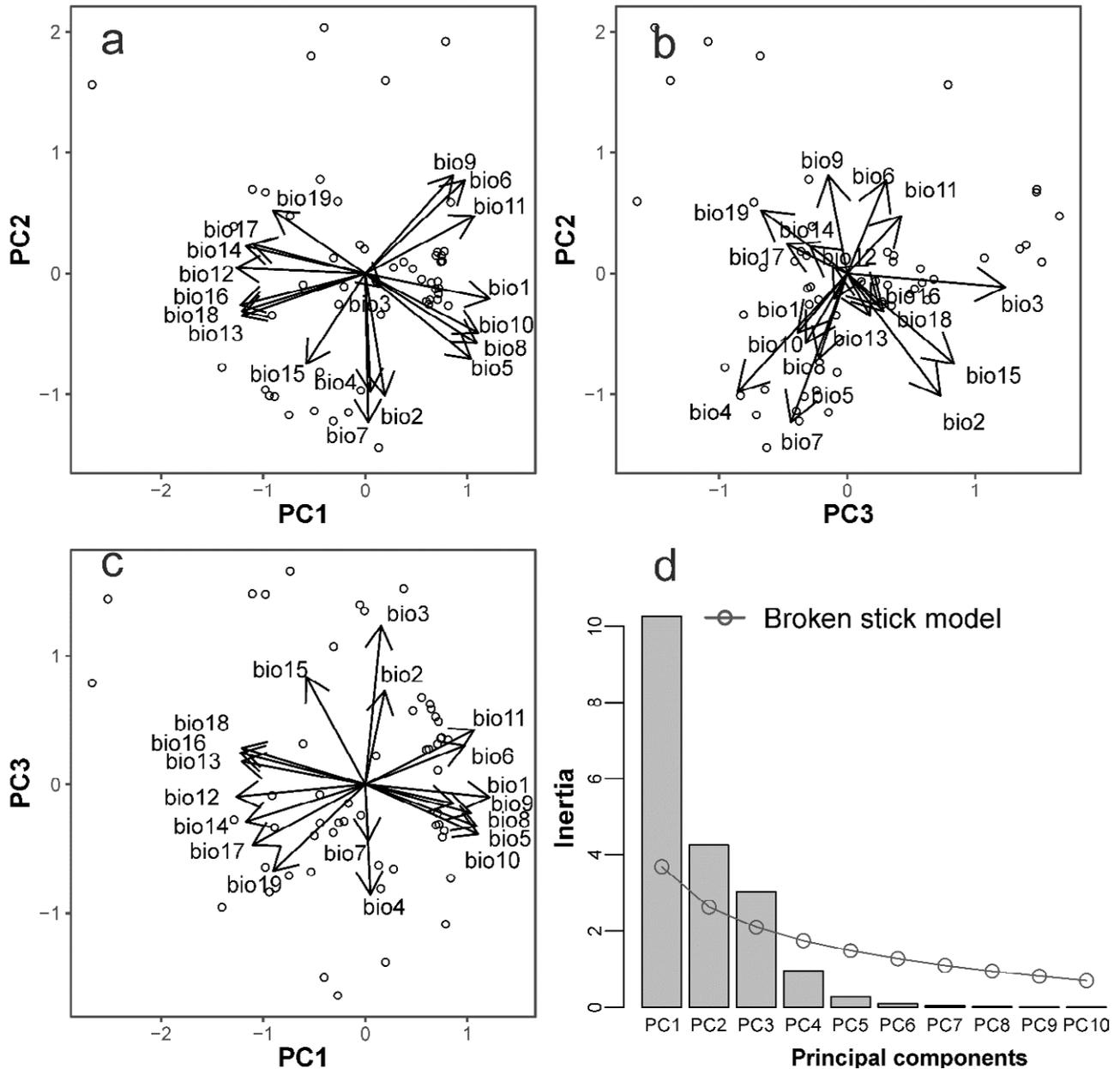
Figure 1. Result of principal components analysis for 19 bioclimatic variables (see: Table 2).

effects, we used linear mixed-effects models (LMM) would be used. For data aggregated at species level, full models were used (eq. 1)

$$Y = \beta_0 + \Sigma^n_{i=1} \beta_i X_i + u_{p:q:r:s} + u_{q:r:s} + u_{r:s} + u_s + \varepsilon_{p,q,r,s,j} \quad (1)$$
$$u_{p:q:r:s} \sim N(0,\sigma^2)$$
$$u_{q:r:s} \sim N(0,\sigma^2)$$
$$u_{r:s} \sim N(0,\sigma^2)$$
$$u_s \sim N(0,\sigma^2)$$
$$\varepsilon_{p,q,r,s,j} \sim N(0,\sigma^2)$$

where Y = dependent variable (pollen morphological feature), X = predictors (particular climate PCA axes and their interactions), $u_{p:q:r:s}$ = random effects connected with series (nested in subsection, section and subgenus), $u_{q:r:s}$ = random effects connected with subsection (nested in section and subgenus), $u_{r:s}$ = random effects connected with section (nested in subgenus), u_s = random effects connected with subgenus, and $\varepsilon_{p,q,r,s,j}$ = residual error of particular samples.

A mixed-effects models *lmerTest* package (Bates et al. 2015; Kuznetsova et al. 2017) was developed. Firstly,

a model compromising all three main bioclimatic components (PC1-PC3) was developed and then reduced according to Akaike Information Criterium corrected for small samples (AICc) using *MuMIn::dredge()* function (Bartoń 2017). From the list of candidate models the best fit was chosen, according to AICc and Akaike weights. In the case that the best fit was a null model (intercept-only), the second best model was used as the final model. Metrics (AICc and Akaike weights) were also provided for the null and full models in order to show the increase in information in the models. Information was provided on the amount of variance explained by the fixed effects using only the marginal coefficient of determination (R^2_m) and by both the random and fixed effects using the conditional coefficient of determination (R^2_c), calculated following Nakagawa and Schielzeth (2013), using the *MuMIn::r.squaredGLMM()* function (Bartoń 2017).

We assumed random effects connected with taxonomic nestedness as a proxy of interspecific variability, to compare with effect size of climatic variables. This made it possible to acknowledge phylogenetic non-independence in the data using random effects in the models. We ignored p-values as a measure of statistical significance since these can be biased by sample size or not connected with biologically meaningful effects (Wasserstein and Lazar 2016).

RESULTS

The two most important climatic factors – temperature and humidity – were analyzed based on 19 bioclimatic variables (Table 2). Analyses of the mixed-effects models revealed that in the case of the majority of the modeled pollen features, the null model had the lowest AIC, which means that these traits were not correlated with the bioclimatic variables studied (Table 3). However, it was found that the P/E ratio was positively correlated and E was negatively correlated with PC3 (Table 4). Nevertheless, the estimates indicated low effect sizes. In the case of these morphological features of the pollen, the climatic data explained 14.0% and 2.8% of the variability, respectively. In contrast, the taxonomic affiliation, included in the models as a random effect, explained from 2.5% to 75.2% of the variability (Table 3).

Among the taxonomic random effects, the most important were these of the subgenus and series, while the least were those of the section (Table 4).

DISCUSSION

The results confirmed the hypothesis that pollen morphology is driven more by taxonomic proximity effects than by climatic variables, due to pollen feature conservatism associated with shared evolutionary history. The reproductive parts of plants (pollen grains and seeds) characters are more conservative and constant than their vegetative ones (Cruden 1977, 2009). Therefore, pollen grains of related taxa usually have similar morphology, as it is the case also with pollen grains of the majority of the genera of the Rosaceae family (e.g. *Crataegus, Malus, Rosa, Rubus,* and *Spiraea*). They have isopolar monads, are generally medium-sized (rarely

Table 3. Summary of mixed-effects models comparison for studied pollen morphological features.

Pollen feature	Full model AICc	Full model AW	Null model AICc	Null model AW	Final model AICc	Final model AW	Final model dependent variables	Final model R^2_c	Final model R^2_m
P	258.9	0.010	**251.2**	**0.450**	**251.2**	**0.450**	null	0.000	0.738
E	237.2	0.019	231.7	0.297	**231.4**	**0.348**	PC3	0.025	0.744
P/E	-122.7	0.000	-142.6	0.486	**-142.7**	**0.499**	PC3	0.140	0.327
Exe	22.8	0.000	**-11.0**	**0.933**	**-11.0**	**0.933**	null	0.000	0.133
Exp	21.1	0.000	**-3.0**	**0.908**	**-3.0**	**0.908**	null	0.000	0.025
Le	246.4	0.006	**237.4**	**0.527**	237.4	0.527	null	0.000	0.752
d	166.6	0.001	**154.4**	**0.667**	154.4	0.667	null	0.000	0.255
Le/P	-244.6	0.000	**-280.6**	**0.992**	-280.6	0.992	null	0.000	0.459
Exp/P	-313.5	0.000	**-354.2**	**0.996**	-354.2	0.996	null	0.000	0.395
Exe/E	-295.7	0.000	**-334.4**	**0.995**	-334.4	0.995	null	0.000	0.415
d/E	-181.7	0.000	**-214.1**	**0.985**	-214.1	0.985	null	0.000	0.539

AICc – Akaike Information Criterion corrected for small samples; AW – Akaike weights; Full model refer to model with three variables – PC1, PC2 and PC3, null model – to model with intercept and random effects only and final model – to best fit model (parameters – Table 4).

Table 4. Parameters of mixed-effects models of studied pollen morphological features.

Pollen feature	SD $u_{p:q:r:s}$	SD $u_{q:r:s}$	SD $u_{r:s}$	SD u_s	SD $\varepsilon_{p,q,r,s,j}$	Term	Estimate	SE	df	t	p
P	1.20042	0.80436	0.00112	2.14196	1.55210	Intercept	23.6510	1.2610	3.0920	18.7600	0.0003
E	0.89480	0.18510	0.57330	3.01650	1.66410	Intercept	20.1160	1.1449	3.2811	17.5700	0.0002
						PC3	-0.5264	0.2677	49.4355	-1.9670	0.0549
P/E	<0.00001	0.00073	<0.00001	<0.00001	0.00262	Intercept	1.1792	0.0150	5.3231	78.7140	<0.0001
						PC3	0.0306	0.0092	53.8903	3.3290	0.0016
Exe	0.06843	<0.00001	<0.00001	0.00006	0.17757	Intercept	1.7828	0.0281	19.1669	63.5500	<0.0001
Exp	<0.00001	<0.00001	<0.00001	<0.00001	0.20037	Intercept	1.8058	0.0266	51.2241	67.7900	<0.0001
Le	1.02893	0.62121	<0.00001	2.09733	1.39485	Intercept	19.1220	1.2080	3.3490	15.8200	0.0003
d	0.18280	<0.00001	<0.00001	<0.00001	0.53320	Intercept	4.4877	0.1348	19.4111	33.3000	<0.0001
Le/P	0.00348	<0.00001	<0.00001	0.01428	0.01616	Intercept	0.8126	0.0104	2.7305	77.9400	0.0000
Exp/P	0.00463	<0.00001	<0.00001	0.00412	0.00773	Intercept	0.0736	0.0036	0.9695	20.6100	0.0337
Exe/E	0.00003	<0.00001	<0.00001	0.00003	0.00008	Intercept	0.0858	0.0044	0.9280	19.4300	0.0400
d/E	0.00004	<0.00001	<0.00001	0.00094	0.00084	Intercept	0.2250	0.0193	2.2835	11.6700	0.0044

SD – standard deviations of random effects: $u_{p:q:r:s}$ – random effects connected with series (nested in subsection, section and subgenus); $u_{q:r:s}$ – random effects connected with subsection (nested in section and subgenus); $u_{r:s}$ – random effects connected with section (nested in subgenus); u_s – random effects connected with subgenus; $\varepsilon_{p,q,r,s,j}$ – residual error of particular samples.

small-sized), with tricolporate or tricolpate pollen grains and mostly striate exine ornamentation (Nazeri 2008; Polyakova and Gataulina 2008; Wrońska-Pilarek and Jagodziński 2011; Wrońska-Pilarek et al. 2013, 2019; Lechowicz et al. 2020). Moreover, intra-generic studies indicated that pollen grains were so similar that usually it was possible to distinguish only a few sections or series and from a few to several individual species, and most often there were groups of taxa with similar pollen characteristics. In this research, among the random effects describing the phylogenetic relatedness of the species studied, the most important ranks were those of subgenus and series, while the least were those of section (Table 4). In contrast, the latest palynological study on *Rubus* (Lechowicz et al. 2020) revealed a low agreement between pollen morphological differentiation and taxonomic division. Pollen traits were most useful at the species level. In the case of the subgenus and series, it was observed that species belonging to these taxa did not generally form separate groups. Other genera of the Rosaceae family (e.g. *Spiraea, Rosa,* and *Crataegus*) showed a greater correlation between pollen morphology and infrageneric taxonomic classification (Wrońska-Pilarek and Jagodziński 2011; Wrońska-Pilarek et al. 2013, 2019). In *Rubus* this may be the result of apomixes, that is the replacement of the normal sexual reproduction by asexual reproduction, without fertilization, which could reduce natural variability (Weber 1996; Zieliński 2004).

In older papers, Bell (1959) and Aizen and Raffaele (1998) indicated differences in pollen size due to fluctuations in the temperature occurring under the influence of different climatic conditions. According to Déri's (2011) modern studies on *Cydonia oblonga*, the pollen size and shape of this species were dependent on different climatic factors, such as temperature and humidity. Ejsmond et al. (2011), based on theoretical findings, showed a general trend for plants in environments with higher temperatures and potential evapotranspiration (PET) to produce less numerous and larger pollen grains (pollen with larger values of P and E) and to exhibit a slight change in grain shape, which may be more difficult to detect. Dainese and Sitzia (2013) as well as Maiti and Rodriguez (2015) and Azzazy (2016) also confirmed this opinion. Ejsmond et al. (2011) claimed that temperature does not significantly affect pollen shape. The research presented here did not fully confirm these results. Our study shows that pollen shape (P/E ratio) was positively correlated and equatorial diameter (E) was negatively correlated with PC3 (Table 4). This means that the less seasonal variability of the climate (higher PC3 values), the shorter the equatorial diameter of the pollen (E) and the larger the value of the P/E ratio , that is, the larger the share of elongated pollen grains.

Lawrence and Campbell (1999) sampled 57 *Rubus* taxa including 20 species of subgenus *Rubus*, one to seven species from other 11 subgenera. Their genetic analyzes indicated that species from this genus were generally consistent with biogeography and ploidy, but traditionally important morphological characters, such as stem armature and leaf type, appeared to have a limited phylogenetic value in *Rubus*. This confirms

the results of our earlier palynological studies (Lechowicz et al. 2020), which also showed that the morphological features of the pollen had considerable but limited impact on the taxonomy of genus *Rubus*. Lawrence and Campbell (1999) proved that ITS sequences were most informative among subgenera, and variability was low between closely related *Rubus* species. They distinguished three large clades in the genus *Rubus*. The first one contained all the sampled species of nine of the 12 studied subgenera, including subgenera *Cylactis*, *Anoplobatus* and *Idaeobatus* analyzed in this paper. The second clade included extreme Southern Hemisphere species of subgenera *Comaropsis* and *Lampobatus*, and the third consisted of subgenus *Rubus* (from which came 54 of the 57 examined species) and *R. alpinus* of subgenus *Lampobatus*. Such research results seem to confirm the hypothesis presented in this study that in *Rubus* pollen, the "impulse" caused by taxonomic and genetic factors is stronger than the influence of climatic factors. The cited authors showed the compatibility of the studied species with biogeography, which would indicate the great importance of the ranges of natural occurrence of the individual blackberry species for their diagnosis. The cited studies also indicate that the impact of geographical factors associated with climate factors on pollen morphology could perhaps be greater than demonstrated in this paper. It cannot be excluded that the results obtained in this study were influenced by the fact that the pollen grains (pollen samples) were collected from one natural site of a given blackberry species.

In contrast to palyno-climatic studies, research on the relationships between leaf traits and climatic data have been conducted more often. Wright et al. (2017) analyzed leaf data for 7670 plant species, along with climatic data from 682 sites worldwide. The authors provided a fully quantitative explanation for the latitudinal gradient in leaf size, with implications for plant ecology and physiology, vegetation modelling, and paleobotany. Large-leaved species predominate in wet, hot, sunny environments; small-leaved species typify hot, sunny environments only in arid conditions; small leaves are also found in high latitudes and at high elevations. By modelling the balance of leaf energy inputs and outputs, they showed that daytime and nighttime leaf-to-air temperature differences were key to geographic gradients in leaf size. Midolo et al. (2019) performed a global meta-analysis of leaf traits in 109 plant species located in four continents and demonstrated that there were common cross-species patterns of intraspecific leaf trait variation across elevational gradients worldwide. Irrespective of whether such variation is genetically determined via local adaptation or attributed to phenotypic plasticity,

the leaf trait patterns quantified here suggest that plant species are adapted to living in a range of temperature conditions. The comprehensive studies cited above showed clear relationships between the morphological characteristics of leaves (e.g. leaf size) and climatic factors. It is therefore probable that in the case of a much larger pollen grain sample of different species from different geographical locations, the relationships between the pollen features and climatic factors would be much more pronounced.

CONCLUSIONS

The study revealed than climatic variability can explain an additional 2.5% to 14.0% of pollen morphology. However, most of the variability was explained by random effects connected with the taxonomic affiliation of the studied species to the genus *Rubus* L.*,* which is very difficult in taxonomic terms.

Although the study analysed data concerning the interactions between the study site climate and the taxonomic affinity of the species on the pollen morphology, the results are biased due to the lack of species-specific replications. For this reason, phylogenic differences in the pollen morphology may have been masked by site specific effects. Despite this, as previously no attempts had been made to differentiate these two effects in the case of apomictic genus comprised of species often with small geographic ranges, it is assumed that these results might be a preliminary finding in the further exploration of such relationships. Further studies are required in order to determine whether knowing the biometric features of the pollen grains can significantly improve predictions of the impact of climate change on plant populations and to reconstruct past environmental conditions.

REFERENCES

Aizen MA, Raffaele E. 1998. Flowering - shoot defoliation affects pollen grain size and post-pollination pollen performance in *Alstroemeria aurea*. Ecology. 79: 2133–2142.

Ashman TL, Knight TM, Steets JA, Amarasekare P, Burd M, Campbell DR, Dudash MR, Johnston MO, Mazer SJ, Mitchell RJ, et al. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. Ecology. 85: 2408–2421.

Azzazy M. 2016. Environmental impacts of industrial pollution on pollen morphology of *Eucalyptus glob-*

ulus Labill. (Myrtaceae). J Appl Biol Biotechnol. 4: 57–62.

Bartoń K. 2017. MuMIn: Multi-Model Inference (Version 1.40.0). Retrieved from https://cran.r-project.org/web/packages/MuMIn/index.html.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw. 67: 1–48.

Bell CR. 1959. Mineral nutrition and flower to flower pollen size variation. Am J Bot. 46: 621–624.

Booth TH. 2018. Why understanding the pioneering and continuing contributions of Bioclim to species distribution modelling is important. Austral Ecol. 43: 852–860.

Booth TH, Nix HA, Busby JR, Hutchinson MF. 2014. Bioclim: the first species distribution modelling package, its early applications and relevance to most current MaxEnt studies. Divers Distrib. 20: 1–9.

Charlesworth D, Schemske DW, Sork VL. 1987. The evolution of plant reproductive characters; sexual versus natural selection. In: Stearns SC (ed.) The evolution of sex and its consequences. Springer.

Cruden RW. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution. 31: 32–46.

Cruden RW. 2000. Pollen grains: why so many? Plant Syst Evol. 222: 143–165.

Cruden RW. 2009. Pollen grain size, stigma depth, and style length: the relationships revisited. Plant Syst Evol. 278: 223–238.

Dainese M, Sitzia T. 2013. Assessing the influence of environmental gradients on seed mass variation in mountain grasslands using a spatial phylogenetic filtering approach. Perspect. Plant Ecol Evol Syst. 15: 12–19.

Delph LF, Johannsson MH, Stephenson AG. 1997. How environmental factors affect pollen performance: ecological and evolutionary perspectives. Ecology. 78: 1632–1639.

Déri HNT. 2011. Morphological investigations on anthers and pollen grains of some quince cultivars. Acta Biol Szeged. 55: 231–235.

Ejsmond MJ, Wrońska-Pilarek D, Ejsmond A, Dragosz-Kluska D, Karpińska-Kołaczek M, Kołaczek P, Kozłowski J. 2011. Does climate affect pollen morphology? Optimal size and shape of pollen grains under various desiccation intensity. Ecosphere. 2: 1–15.

Ejsmond MJ, Ejsmond A, Banasiak Ł, Karpińska-Kołaczek M, Kozłowski J, Kołaczek P. 2015. Large pollen at high temperature: an adaptation to increased competition on the stigma? Plant Ecol. 216: 1407–1417.

Erdtman G. 1952. Pollen morphology and plant taxonomy. Angiosperms. An introduction to palynology. Almquist and Wiksell.

Erdtman G. 1960. The acetolysis method. A revised description. Sven Bot Tidskr. 54: 561–564.

Eyduran SP, Ercisli S, Akin M, Beyhan Ö, Geçer AEK. 2015. Organic Acids, sugars, vitamin c, antioxidant capacity, and phenolic compounds in fruits of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry genotypes. J Appl Bot Food Qual. 88: 134–138.

Gustafsson A. 1943. The genesis of the European blackberry flora. Otto Harrassowitz.

Halbritter H, Ulrich S, Grímsson F, Weber M, Zetter R, Hesse M, Buchner R, Svojtka M, Frosch-Radivo A. 2018. Illustrated Pollen Terminology. 2nd ed. Springer.

Harder LD, Aizen MA. 2010. Floral adaptation and diversification under pollen limitation. Philos. Trans R Soc Lond, B, Biol Sci. 365:529–543.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. Int J Climatol. 25: 1965–1978.

Hinojosa L, Matanguihan JB, Murphy KM. 2018. Effect of high temperature on pollen morphology, plant growth and seed yield in quinoa (*Chenopodium quinoa* Willd.). J Agron Crop Sc. 205: 33–45.

Knight TM, Steets JA, Vamosi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnston MO, Mitchell RJ, Ashman TL, et al. 2005. Pollen limitation of plant reproduction: pattern and process. Annu Rev Ecol Evol Syst. 36: 467–497.

Kosiński P, Maliński T, Sliwinska E, Zieliński J. 2018. *Rubus prissanicus* (Rosaceae), a new bramble species from North West Poland. Phytotaxa 344: 239–247.

Kuznetsova A, Brockhoff PB, Christensen RHB. 2017. LmerTest: Tests in Linear Mixed Effects Models. J Stat Softw. 82: 26.

Lawrence A, Campbell CS. 1999. Phylogeny of *Rubus* (Rosaceae) Based on Nuclear Ribosomal DNA Internal Transcribed Spacer Region Sequences. Am J Bot. 86: 81–97.

Lechowicz K, Wrońska-Pilarek D, Bocianowski J, Maliński T. 2020. Pollen morphology of Polish species from the genus *Rubus* L. (Rosaceae) and its systematic importance. PLoS ONE. 15: 1-23.

Ling-Ti L. 1983. A study on the genus *Rubus* of China. J Syst Evol. 21: 13–25.

Maiti R, Rodriguez HG. 2015. Phenology, morphology and variability in pollen viability of four woody trees (*Cordia boissieri*, *Parkinsonia texana*, *Parkinsonia aculeate* and *Leucophyllum frutescens* exposed to

environmental temperatures in Linares, Northeastern Mexico: Open Access.

Marsic NK, Necemer M, Veberic R, Ulrih NP, Skrt M. 2019. Effect of cultivar and fertilization on garlic yield and allicin content in bulbs at harvest and during storage. Turk J Agric For. 43: 414–429.

Midolo G, Frenne PDe, Hölzel N, Wellstein C. 2019. Global patterns of intraspecific leaf trait responses to elevation. Glob Chang Biol. 25: 2485–2498.

Mione T, Anderson GJ. 1992. Pollen-ovule ratios and breeding system evolution in solanum section Basarthrum (Solanaceae). Am J Bot. 79: 279–287.

Murcia C. 1990. Effect of floral morphology and temperature on pollen receipt and removal in *Ipomoea trichocarpa*. Ecology. 71: 1098–1109.

Nakagawa S, Schielzeth H. 2013. A general and simple method for obtaining R2 from generalized linear mixed-effects models. Methods Ecol Evol. 4: 133–142.

Nazeri JV. 2008. Pollen morphology of the genus *Malus* (Rosaceae). Iran J Sci Technol Trans. A Sci. 32: 90–97.

Omidi M, Siahpoosh MR, Mamghani R, Modarresi M. 2014. The influence of terminal heat stress on meiosis abnormalities in pollen mother cells of wheat. Cytologia. 79: 49–58.

Paupière MJ, Van Heusden AW, Bovy AG. 2014. The metabolic basis of pollen thermo-tolerance: perspectives for breeding. Metabolites. 4: 889–920.

Polyakova TA, Gataulina GN. 2008. Morphology and variability of pollen of the genus *Spiraea* L. (Rosaceae) in Siberia and the Far East. Contemp Probl Ecol. 1: 420–424.

Prasad PVV, Booteb KJ, Allen JrLH. 2011. Longevity and temperature response of pollen as affected by elevated growth temperature and carbon dioxide in peanut and grain sorghum. Environ Exp Bot. 70: 51–57.

Punt W, Hoen PP, Blackmore S, Nilsson S, Thomas ALe. 2007. Glossary of pollen and spore terminology. Rev Palaeobot Palynol. 1431: 1–81.

Rao G, Ashraf U, Kong L, Mo Z, Xiao L, Zhong K, Rasul F, Tang X. 2019. Low soil temperature and drought stress conditions at flowering stage affect physiology and pollen traits of rice. J Integr Agric. 18: 1859–1870.

Richards AJ, Kirschner J, Stepanek J, Marhold K. 1996. Apomixis and taxonomy: An introduction. Folia Geobot. 31: 281–282.

Robertson KR. 1974. The genera of Rosaceae in the southeastern United States. J Arnold Arbor. 55: 344–401.

Sarkissian TS, Harder LD. 2001. Direct and indirect responses to selection on pollen size in *Brassica rapa* L. J Evol Biol. 14: 456–468.

Unlukara A. 2019. Effects of depth-dependent irrigation regimes and organomineral fertilizers on water use and quality attributes of sugar beet. Turk J Agric For. 43: 492–499.

Vonhof MJ, Harder LD. 1995. Size-number trade-offs and pollen production by Papilionaceous Legumes. Am J Bot. 82: 230–238.

Walther GR, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin JM, Hoegh-Guldberg O, Bairlein F. 2002. Ecological responses to recent climate change. Nature. 416: 389–395.

Wasserstein RL, Lazar NA. 2016. The ASA's Statement on p-Values: Context, Process, and Purpose. Am Stat. 70: 129–133.

Weber HE. 1995. *Rubus* L. In: Weber, H. E. (ed.), Gustav Hegi Illustrierte Flora von Mitteleuropa. 3nd ed., vol. 4/2A. – Berlin and Oxford, Blackwell Wissenschafts–Verlag: p. 284–595.

Weber HE. 1996. Formed and Modern Taxonomic Treatment of the Apomictic *Rubus* Complex. Folia Geobot. 31: 373–380.

Wright IJ, Dong N, Maire V, Prentice C, Westoby M, Díaz S, Gallagher1 RV, Jacobs BF, Kooyman1 R, Law EA, et al. 2017. Global climatic drivers of leaf size. Science. 357: 917–921.

Wrońska-Pilarek D, Jagodziński AM. 2011. Systematic importance of pollen morphological features of selected species from the genus *Rosa* (Rosaceae). Plant Syst Evol. 295: 55–72.

Wrońska-Pilarek D, Bocianowski J, Jagodziński AM. 2013. Comparison of pollen grain morphological features of selected species of the genus *Crataegus* L. (Rosaceae) and their spontaneous, interspecific hybrids. Bot J Linn Soc. 172: 555–571.

Wrońska-Pilarek D, Wiatrowska B, Bocianowski J. 2019. Pollen morphology and variability of invasive *Spiraea tomentosa* L. (Rosaceae) from populations in Poland. PLoS One. 14: 1–19.

Yang CF, Guo YH. 2004. Pollen size-number trade-off and pollen-pistil relationships in Pedicularis (Orobanchaceae). Plant Syst Evol. 247: 177–185.

Zieliński J. 2004. The genus *Rubus* (Rosaceae) in Poland. Pol Bot Stud. 16: 1–300.

Zieliński J, Kosiński P, Tomaszewski D. 2004. The genus *Rubus* (Rosaceae) in southeastern Lower Silesia (Poland). Pol Bot J. 49: 161–180.

Zinn KE, Tunc-Ozdemir M, Harper JF. 2010. Temperature stress and plant sexual reproduction: uncovering the weakest links. J Exp Bot. 61: 1959–1968.