Wulfenia **29** (2022): 47–60

Wulfenia
Mitteilungen des
Kärntner Botanikzentrums
Klagenfurt

Intraspecific morphological variability in the genus *Ophrys* (Orchidaceae) is driven by changing environments

Igor Paušič, Danijel Ivajnšič, Nataša Pipenbaher & Marcela Bešter

Summary: Species are not static; they are subject to dynamic processes that continue to operate. Floral morphology of the nominate form, *Ophrys sphegodes* Mill. s. str. (Orchidaceae) was evaluated and described using classical morphology and related to site-specific microclimatic conditions. Specimens from each locality in this study are represented in a set of 21 measured macromorphological character values, obtained from a single flower. In total, 253 specimens, belonging to 12 populations, all of them from Slovenia, were morphologically evaluated. Climatic, submediterranean – continental gradient was followed and related to floral macromorphological character values. Correlation tests were performed based on 21 bioclimatic variables and a full set of measured macromorphological character values. Six floral characters express statistically significant relation to site-specific bioclimatic variable values. Consequently, active components (characters) of the flower, such as labellum length, macula proportions, connective (gynostemium) length and lateral swellings of the labellum, are all important characters for the correct alignment between the body of the pollinator and the reproductive parts of the plant are clearly affected by the local climatic conditions. An integrative ecological, but also taxonomical approach to the genus *Ophrys* should take into account site-specific ecological conditions to understand drivers causing enormous variability among *Ophrys* flowers, since pollinator-mediated selection alone does not prove to be the only cause.

Keywords: *Ophrys*, intraspecific variability, traditional morphometrics, bioclimatic variables

Plants belonging to different genera exhibit substantial intraspecific variation in floral traits (Knudsen et al. 2006). One of the most striking features of angiosperms – orchids in particular – is the enormous diversity of their floral traits. Pollinator-mediated selection is considered the main factor that is shaping changes in phenotypic floral trait distributions (Fenster et al. 2004). The colour, size and shape of flowers are important visual signals that attract pollinators searching for rewards (Dormont et al. 2019; Valenta et al. 2017). Early studies dealing with intraspecific variation in floral traits have focused on floral colour polymorphism *sensu stricto* (Brown & Clegg 1984; Jones & Reithel 2001). However, intraspecific variation in colour and other floral traits may also reflect multiple and often conflicting selection pressures, also involving antagonistic agents such as abiotic conditions (Strauss & Whittall 2006). Species growing in different types of habitats exhibit variation in morphological characters that is accounted for with differences in ecological conditions (Mamaev et al. 2004). However, there is neither a unified methodology nor a universal list of characters for revealing intraspecific variation, and the characters to be studied are selected depending on the purposes of each research.

The genus *Ophrys* comprises c. 250 species and has its centre of distribution in the Mediterranean region. Male hymenopterans pollinate members of the genus *Ophrys* by sexual deception; this represents a rare and remarkable strategy in angiosperms as it constitutes an example of extreme floral specialization (Vereecken et al. 2012). The flowers imitate olfactory, visual and tactile signals of receptive females to attract males and provoke them to land on the labellum (Stejskal et al. 2015). In the mating behaviour of hymenopterans, the most frequent pollinators in the genus *Ophrys* olfaction is the key sensory modality involved in male-female communication (Stejskal et al. 2015). It was shown, however, that some *Ophrys* species enhance pollinator attraction by means of colour signals (Spaethe et al. 2007) but also labellum-macula patterns (Stejskal et al. 2015).

Ophrys floral morphology varies substantially within species, the mechanisms maintaining this diversity are diverse, but inadequately studied. One possibility, partly documented within the genus, is that spatial variation in the pollinator fauna drives the evolution of spatially divergent floral ecotypes adapted to the local suite of pollinators. Another – until now neglected possibility – driver is that geographic variation in the abiotic environment and local climatic conditions favours different floral morphologies in different regions. As shown by Weber et al. (2020), both biotic and abiotic variables can explain a substantial amount of intraspecific variation in floral morphology even in very specialized interactions. Climatic conditions at the local scale, as an important driver of general floral variability, has so far been completely neglected in studies dealing with the floral variability of the genus *Ophrys* (Paušič et al. 2019), thus representing an important driver, causing local morphotypes that create confusion in the field within orchid-fanciers, especially splitters.

The present study was undertaken:

to study site-specific bioclimatic variable values in relation to floral morphological character values in order to evaluate, if the floral morphology and intraspecific character variability are in fact ecologically driven;

to define which floral characters of the *Ophrys* flower are subject to variability due to changes in the values of site-specific, individual environmental bioclimatic variables.

Materials and methods

Studied species

Early spider orchid, *Ophrys sphegodes* Mill. s. str., is a short-lived tuberous orchid of chalk, limestone and sandstone grassland (Hutchings 2010). It is a species of southern and central Europe, reaching its northern range limit in southern England. This species reaches southern range limits in south-eastern Europe (Croatia, Slovenia, Italy), often growing with other conspecific taxa, putative subspecies, making clear determination in the field quite challenging. It produces a rosette of leaves in September–October and flowers in the following year from mid-March till the end of May. Flower spikes are rarely more than 30 cm tall; a single inflorescence is produced per stem. Most plants bear two to five flowers that open successively. As reported, *O. sphegodes* is pollinated by males of the solitary bee *Andrena nigroaenea* (Schiestl et al. 1997). Early spider orchid is still widespread and abundant in some agriculturally extensive parts of Slovenia, despite the rapid decline of species rich, nutrient-poor grassland areas.

Sampled populations

We selected 12 *O. sphegodes* s. str. populations (Fig. 1), covering almost the entire distribution range in Slovenia (Fig. 1, Table 1). Only populations from central Slovenia are missing in the present study due to lack of data. The territory of Slovenia is climatically transitional, expressing clear precipitation and temperature gradients from east to west, making this area particularly suitable to study intraspecific floral variability in response to the local climatic conditions. In total, 253 flowers were morphologically evaluated in the present study.

Table 1. Sampled populations and number (N) of *Ophrys sphegodes* s. str. specimens used in this study. For distribution of the populations see Fig. 1.

Population	Location	Region	Latitude (°)	Longitude (°)	N
1	Kamnica	NE Slovenia	46.578	15.605	30
2	Krško	SE Slovenia	45.929	15.494	30
3	Trdobojci	NE Slovenia	46.304	15.926	11
4	Donačka gora	NE Slovenia	46.258	15.733	16
5	Mala Varnica	NE Slovenia	46.320	15.931	5
6	Podpeč	SW Slovenia	45.520	13.906	30
7	Kolomban	SW Slovenia	45.595	13.747	30
8	Dragonja	SW Slovenia	45.447	13.673	30
9	Hrastovje	SW Slovenia	45.513	13.905	40
10	Kubed	SW Slovenia	45.525	13.851	9
11	Gračišče	SW Slovenia	45.498	13.874	14
12	Podpeč 2	SW Slovenia	45.535	13.900	8

Analysed floral morphological characters

Specimens from each location are represented in a set of 26 measured macromorphological character (continuous variables only) values obtained from a single flower. 21 macromorphological character values were used for all the analyses, quantified with traditional morphometric methods. As suggested by Bateman & Rudall (2006) and Bateman & Denholm (1989), the representative flower was excised from one-third of the distance from the base to the apex of the spike, collected

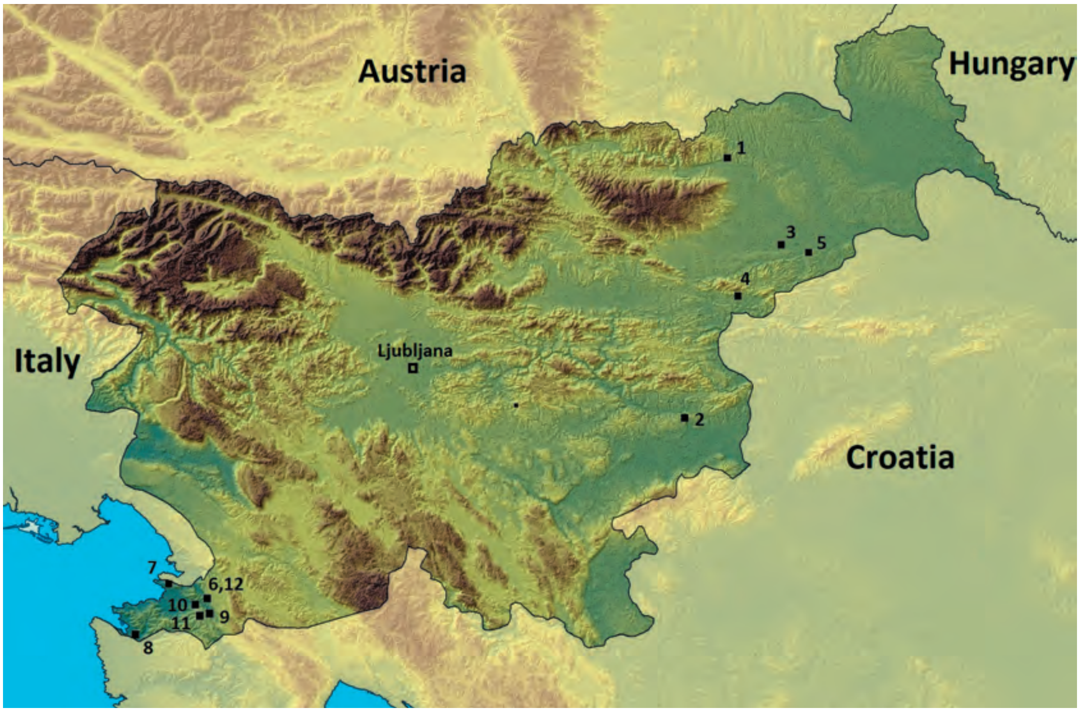
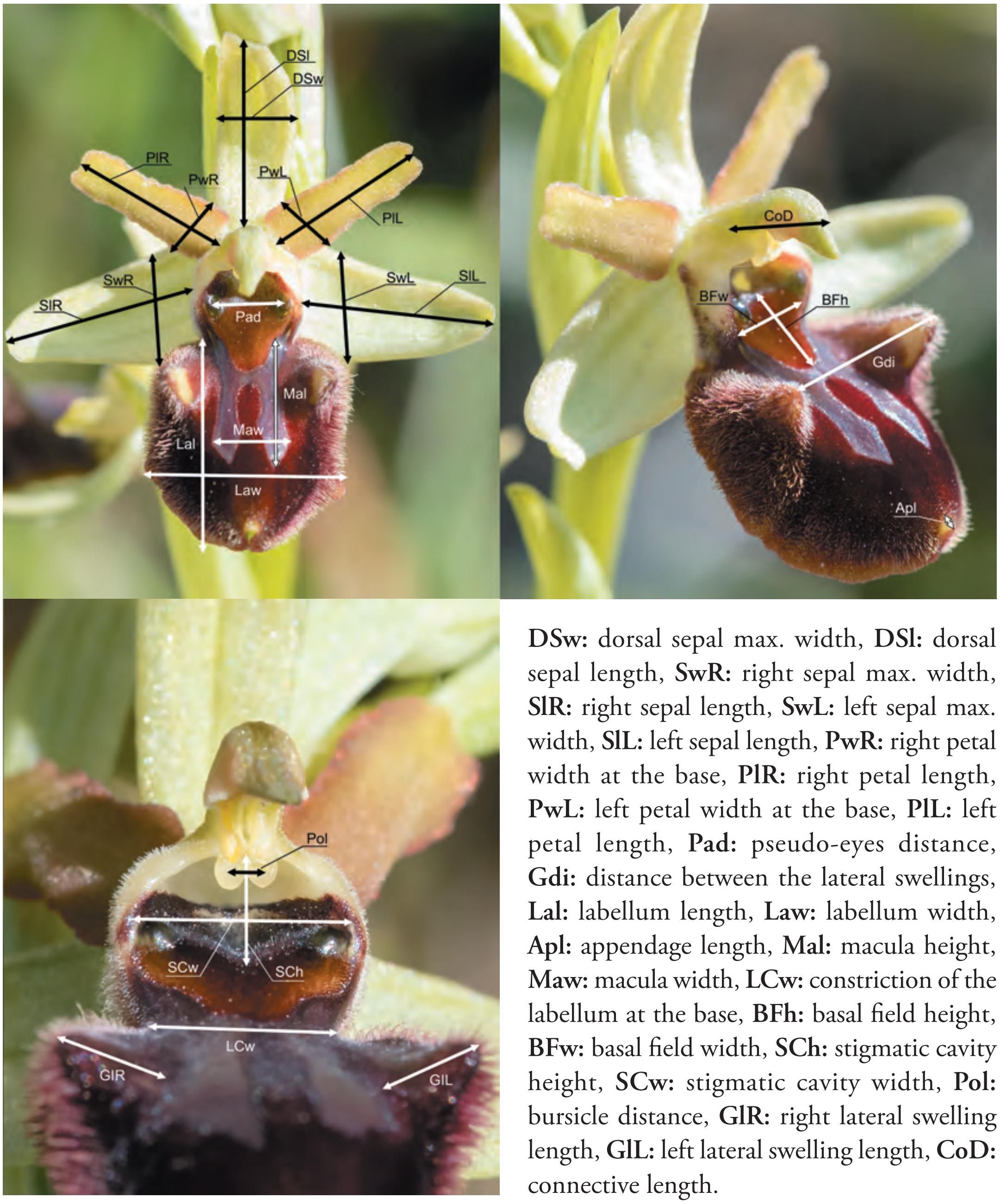


Figure 1. Distribution of sampling populations of *Ophrys sphegodes* s. str. in Slovenia. See Table 1 for population identities.



DSw: dorsal sepal max. width, **DSl:** dorsal sepal length, **SwR:** right sepal max. width, **SlR:** right sepal length, **SwL:** left sepal max. width, **SlL:** left sepal length, **PwR:** right petal width at the base, **PlR:** right petal length, **PwL:** left petal width at the base, **PlL:** left petal length, **Pad:** pseudo-eyes distance, **Gdi:** distance between the lateral swellings, **Lal:** labellum length, **Law:** labellum width, **Apl:** appendage length, **Mal:** macula height, **Maw:** macula width, **LCw:** constriction of the labellum at the base, **BFh:** basal field height, **BFw:** basal field width, **SCh:** stigmatic cavity height, **SCw:** stigmatic cavity width, **Pol:** bursicle distance, **GlR:** right lateral swelling length, **GlL:** left lateral swelling length, **CoD:** connective length.

Figure 2. Measured floral characters.

flowers were in the same phenophase. Only undamaged flowers were collected, all the collected flowers still contained pollinia. The 21 selected characters that were scored morphometrically describe the proportions of the floral elements: sepals and petals, labellum, stigmatic cavity and gynostemium.

Floral characters were measured under the binocular stereoscopic microscope Nikon SMZ 1000 (8–80×). NIS Elements D 4.2 software was used for the measurements. The floral character abbreviations are given in Fig. 2.

Table 2. A list of analysed bioclimatic variables. P: precipitation, T: temperature, CV: coefficient of variation.

Climatic variable	
BIO1	Annual mean T
BIO2	Mean diurnal T range
BIO3	Isothermality
BIO4	T seasonality (CV)
BIO5	Max. T of warmest month
BIO6	Min. T of coldest month
BIO7	T annual range
BIO8	Mean T of wettest quarter
BIO9	Mean T of driest quarter
BIO10	Mean T of warmest quarter
BIO11	Mean T of coldest quarter
BIO12	Annual P
BIO13	P of wettest month
BIO14	P of driest month
BIO15	P seasonality (CV)
BIO16	P of wettest quarter
BIO17	P of driest quarter
BIO18	P of warmest quarter
BIO19	P of coldest quarter
Sol_Rad	Annual solar radiation
Wind	Above ground wind speed

Morphometric analysis

Morphometric data for individual flowers were summarised on an Excel v14.3 spreadsheet. Shapiro-Wilk's test of normality was performed to test and ensure the normal distribution of data. Five floral characters were excluded from further analyses in order to prevent inbuilt positive correlation between the metric parameters. These are left-side oriented floral elements: left petal width at the base, left petal length, left sepal length, left sepal width and left lateral swelling length. These left-side floral elements express a higher degree of variance and standard deviation compared to the right-side ones and thus being less stable in their values among flowers of the same population (data not shown). For statistical analysis, SPSS was used (SPSS Inc. 2006).

Correlation of ecological variables with morphological variation

For each population (location centroid), we obtained latitudinal and longitudinal coordinates, including elevation data using ArcGIS 9.3 (ESRI 2010) base maps (coordinate system WGS84). Bioclimatic variables (for the 1950–2000 period; WorldClim database available at http://www.worldclim.org/) represented annual trends (e.g. mean annual temperature and annual precipitation), seasonality (e.g. annual range in temperature and precipitation) and extreme or limiting environmental factors (e.g. temperature of the coldest and warmest month and precipitation of the wet and dry quarters) (Table 2). Additionally, a geospatial bioclimatic database was developed using ArcGIS Spatial Analyst tools (ESRI 2010) by attributing all variables to the location points representing each population (Table 2).

Table 3. Mean character values (mm) for 12 analysed *O. sphegodes* s. str. populations.

Locality	**DSw**	**DSl**	**SwR**	**SlR**	**SwL**	**SlL**	**PwR**	**PlR**	**PwL**	**PlL**	**Pad**	**Gdi**	**Lal**	**Law**	**Apl**	**Mal**	**Maw**	**LCw**	**BFh**	**BFw**	**SCh**	**SCw**	**Pol**	**GlR**	**GlL**	**CoD**
Kamnica	5.022	11.438	5.743	11.860	5.513	11.795	3.019	8.111	3.001	7.979	3.333	6.532	10.082	9.726	0.405	6.674	3.306	4.419	1.908	3.921	1.887	3.817	0.725	1.656	1.573	3.098
Krško	4.205	10.411	5.063	10.632	5.094	10.664	3.376	7.224	3.348	7.136	3.422	6.135	9.156	8.554	0.377	6.189	3.576	4.506	1.694	4.004	1.752	3.514	0.674	1.282	1.248	3.414
Trdobojci	5.198	10.831	6.036	11.785	5.478	11.711	2.862	8.133	2.776	7.649	3.261	5.843	9.315	9.603	0.261	5.916	3.123	4.516	1.716	3.709	1.521	3.418	0.734	1.441	1.389	2.986
Donačka gora	5.538	10.506	6.197	10.687	6.069	10.404	4.017	7.427	4.041	7.361	3.626	6.446	9.547	9.703	0.565	6.551	3.568	4.376	1.942	4.317	1.902	4.112	0.746	1.901	1.557	2.895
Mala Varnica	5.211	12.722	5.536	13.320	5.474	12.932	3.316	9.354	3.396	9.222	3.431	5.992	10.171	10.458	0.512	6.261	3.348	5.154	1.926	3.838	1.642	3.736	0.924	1.288	1.366	2.532
Podpeč	5.187	11.811	6.006	12.582	6.163	12.591	3.508	8.680	3.512	8.631	3.359	7.091	10.502	10.456	0.278	7.348	4.159	3.561	2.032	4.082	1.649	3.971	0.888	1.381	1.527	3.292
Kolomban	5.373	11.673	6.136	11.229	5.987	11.205	3.644	7.515	3.779	7.563	3.313	6.290	9.710	9.955	0.351	6.492	3.696	3.156	1.755	3.781	1.886	3.754	0.793	1.346	1.383	3.361
Dragonja	4.638	11.157	5.590	11.731	5.394	11.867	3.305	8.171	3.321	8.137	3.311	6.254	10.032	9.986	0.411	6.499	3.857	3.419	1.926	3.83	1.624	3.842	0.793	1.405	1.452	3.577
Hrastovlje	5.133	10.858	5.629	11.393	5.736	11.114	3.425	7.971	3.413	7.989	3.396	6.552	10.209	9.866	0.387	6.799	4.116	3.094	1.939	3.901	1.638	3.906	0.794	1.408	1.374	3.429
Kubed	4.258	10.345	5.218	10.731	5.086	10.546	3.228	7.972	3.234	7.867	3.193	6.303	9.861	9.565	0.267	6.487	4.108	1.961	1.877	3.615	1.449	3.501	0.736	1.149	1.145	3.291
Gračišče	5.345	12.581	6.115	12.785	6.065	12.651	3.358	8.692	3.295	8.564	3.499	6.833	10.942	9.915	0.261	8.176	4.741	2.171	2.126	3.977	1.782	4.315	0.804	1.337	1.412	3.626
Podpeč 2	4.638	10.663	5.421	10.552	5.475	10.527	3.865	7.871	3.873	7.823	3.322	6.527	10.059	9.327	0.287	6.715	3.561	2.336	1.809	4.372	1.935	4.002	0.704	1.406	1.417	3.457

To reveal which site-specific bioclimatic variable values are statistically related to separate floral morphological character values, a Pearson product-moment correlation was performed between each of the 21 bioclimatic variables (for each population) and values of all the measured floral characters (population mean values).

In order to identify the predominant morphological characters in specific area for *O. sphegodes* s. str., the matrix of 21 morphological characters x 12 localities and 9 environmental data x 12 localities was used. Some bioclimatic parameters were dismissed in this part of analysis due to their inbuilt correlation. The ordination methods (RDA) and visualization of their results were carried out using the Canoco and CanoDraw programs (ter Braak & Šmilauer 2002).

Results

The sampled populations presented in this study exhibit clear variation in both mechanically inactive and active floral components, measured morphological character values (Table 3). Site-specific bioclimatic variable values are shown in Table 4.

Sampled populations are subject to changing gradients of the analysed bioclimatic parameter values (Table 4). According to local climatic conditions, sites of sampled population belong to three different climatic types. Five populations are located in the continental, NE part of Slovenia that is subject of humid, continental Euro-Siberian climate. These are Kamnica, Krško, Trdobojci, Donačka Gora and Mala Varnica (Fig. 1). Four populations are prone to transitional biogeographic, temperate, Euro-Siberian – submediterranean climate of the Karst Edge (SW Slovenia). These are Podpeč, Podpeč 2, Gračišče and Kubed. Three populations from SW Slovenia; Kolomban, Hrastovlje and Dragonja are located within humid, submediterranean climate type (Fig. 1).

In general, floral character values decrease with the increasing influence of the continental climate (Fig. 3), but it is not always the case. On average, five floral character values increase with the increasing influence of the continental climate, these being right sepal length, right petal length, right lateral swelling of the labellum, appendage length and the constriction of the labellum at the base. Six floral morphological characters exhibit statistically significant, strong relation with climatic conditions (analysed bioclimatic variable values) at the local scale. These are labellum length, proportions of the macula (height and width), constriction of the labellum at the base, connective (gynostemium) length and length of the lateral swellings of the labellum (Table 5).

RDA ordination plot in Fig. 3 shows clear division of the 12 analysed *O. sphegodes* s. str. populations, according to the measured morphological character values. These three categories correspondent clearly with the climatic conditions at the local scale representing morphotypes of the nominate form *O. sphegodes* s. str. (Fig. 3).

Looking at the analysed bioclimatic parameters, three distinct categories could again be interpreted (Fig. 4). Sampled populations should be grouped according to their morphological similarity (size and shape) which corresponds clearly to the climatic conditions at the local scale (Fig. 4).

Discussion

The striking morphological diversity of flowering plants has been long fascinating ecologists and evolutionary biologists (e.g. Darwin 1859). Most of the angiosperm flowers require assistance

Table 4. Bioclimatic variable values for each *O. sphegodes* s. str. population locality (polygon centroid).

Locality	Latitude	Longitude	BIO 1	BIO 2	BIO 3	BIO 4	BIO 5	BIO 6	BIO 7	BIO 8	BIO 9	BIO 10	BIO 11	BIO 12	BIO 13	BIO 14	BIO 15	BIO 16	BIO 17	BIO 18	BIO 19	Sol_ Rad	Wind
Kamnica	46.578056	15.605833	10.1	8.2	27.5	745.0	25.4	-4.3	29.7	20.0	0.4	20.0	-0.2	967	117	41	32	346	127	346	136	141755	1.825
Krško	45.929722	15.494167	11.8	8.2	27.6	744.9	27.4	-2.5	29.9	21.5	2.1	21.8	1.5	960	106	48	23	300	145	293	163	144678	1.616
Trdobojci	46.304333	15.926806	10.5	8.2	27.5	747.3	26.0	-3.7	29.8	20.5	0.8	20.5	0.2	1056	122	53	27	366	159	366	176	145057	1.950
Donačka gora	46.258908	15.733397	8.6	8.2	27.8	735.8	24.0	-5.5	29.5	18.2	-0.9	18.5	-1.5	1323	150	64	25	438	195	418	222	144262	2.092
Mala Varnica	46.320086	15.931169	10.7	8.2	27.5	747.8	26.2	-3.6	29.8	20.7	0.9	20.7	0.3	1011	117	51	27	351	155	351	169	144977	1.933
Podpeč	45.520000	13.906389	12.9	5.9	22.9	694.9	26.5	0.7	25.9	10.2	3.9	22.7	3.7	1081	125	70	18	362	214	243	239	147640	2.041
Kolomban	45.595000	13.747222	13.3	6.0	23.0	698.9	27.0	0.9	26.1	10.5	4.2	23.1	4.1	1525	170	100	16	495	308	358	348	149318	2.375
Dragonja	45.447222	13.673889	14.1	5.4	21.3	689.6	27.4	2.3	25.1	11.5	5.2	23.9	5.1	1022	118	66	18	341	203	246	231	150210	2.125
Hrastovje	45.513333	13.905833	13.2	5.9	22.7	695.6	26.9	1.0	25.9	10.5	4.2	23.0	4.1	1046	121	67	18	350	209	235	236	147726	1.958
Kubed	45.525097	13.851619	12.4	5.9	22.8	692.4	26.0	0.2	25.8	9.7	3.5	22.2	3.3	1331	150	85	17	435	277	311	315	148520	2.267
Gračišče	45.498842	13.874000	12.2	5.8	22.6	688.7	25.7	0.2	25.5	9.6	3.3	22.0	3.2	1293	148	83	17	426	263	297	302	148296	2.183
Podpeč 2	45.535450	13.900956	11.6	6.0	23.2	690.4	25.2	-0.7	25.8	8.9	2.6	21.3	2.5	1193	138	75	19	401	230	261	259	147702	2.300

Table 5. Statistically significant relations between measured floral characters and climatic variables. **Correlation is significant at the 0.01 level, *correlation is significant at the 0.05 level. P: precipitation, T: temperature., CV: coefficient of variation. Measured floral character abbreviations are given in Fig. 2.

Floral character	**r**	**Sig.**	**Variable**	**BIO description**
Lal	-.576*	0.0497	BIO7	T annual range
Mal	-.578*	0.0490	BIO4	T seasonality (CV)
Maw	-.740**	0.0060	BIO2	Mean diurnal T range
Maw	-.728**	0.0073	BIO3	Isothermality
Maw	-.766**	0.0036	BIO4	T seasonality (CV)
Maw	.673*	0.0163	BIO6	Min. T of coldest month
Maw	-.749**	0.0050	BIO7	T annual range
Maw	.627*	0.0291	BIO9	Mean T of driest quarter
Maw	.645*	0.0235	BIO11	Mean T of coldest quarter
Maw	.591*	0.0430	BIO14	P of driest month
Maw	-.751**	0.0048	BIO15	P seasonality (CV)
Maw	.621*	0.0310	BIO17	P of driest quarter
Maw	.637*	0.0260	BIO19	P of coldest quarter
Maw	.623*	0.0304	Sol_Rad	Annual solar radiation
LCw	.848**	0.0004	BIO2	Mean diurnal T range
LCw	.827**	0.0009	BIO3	Isothermality
LCw	.895**	0.0000	BIO4	T seasonality (CV)
LCw	-.708*	0.0100	BIO6	Min. T of coldest month
LCw	.868**	0.0002	BIO7	T annual range
LCw	-.641*	0.0245	BIO9	Mean T of driest quarter
LCw	-.676*	0.0159	BIO11	Mean T of coldest quarter
LCw	-.785**	0.0025	BIO14	P of driest month
LCw	.800**	0.0017	BIO15	P seasonality (CV)
LCw	-.814**	0.0012	BIO17	P of driest quarter
LCw	-.821**	0.0010	BIO19	P of coldest quarter
LCw	-.725**	0.0076	Sol_Rad	Annual solar radiation
LCw	-.718**	0.0085	Wind	Wind speed
CoD	.707*	0.0101	BIO1	Annual mean T
CoD	-.746**	0.0052	BIO2	Mean diurnal T range
CoD	-.746**	0.0053	BIO3	Isothermality
CoD	-.748**	0.0051	BIO4	T seasonality (CV)
CoD	.758**	0.0042	BIO6	Min. T of coldest month
CoD	-.743**	0.0056	BIO7	T annual range
CoD	.748**	0.0051	BIO9	Mean T of driest quarter
CoD	.687*	0.0135	BIO10	Mean T of warmest quarter
CoD	.759**	0.0042	BIO11	Mean T of coldest quarter
CoD	-.712**	0.0093	BIO15	P seasonality (CV)
CoD	-.681*	0.0147	BIO18	P of warmest quarter
GIR	-.662*	0.0189	BIO1	Annual mean T
GIR	-.665*	0.0183	BIO5	Max. T of warmest month
GIR	-.586*	0.0454	BIO6	Min. T of coldest month
GIR	-.624*	0.0299	BIO9	Mean T of driest quarter
GIR	-.677*	0.0156	BIO10	Mean T of warmest quarter
GIR	-.607*	0.0364	BIO11	Mean T of coldest quarter

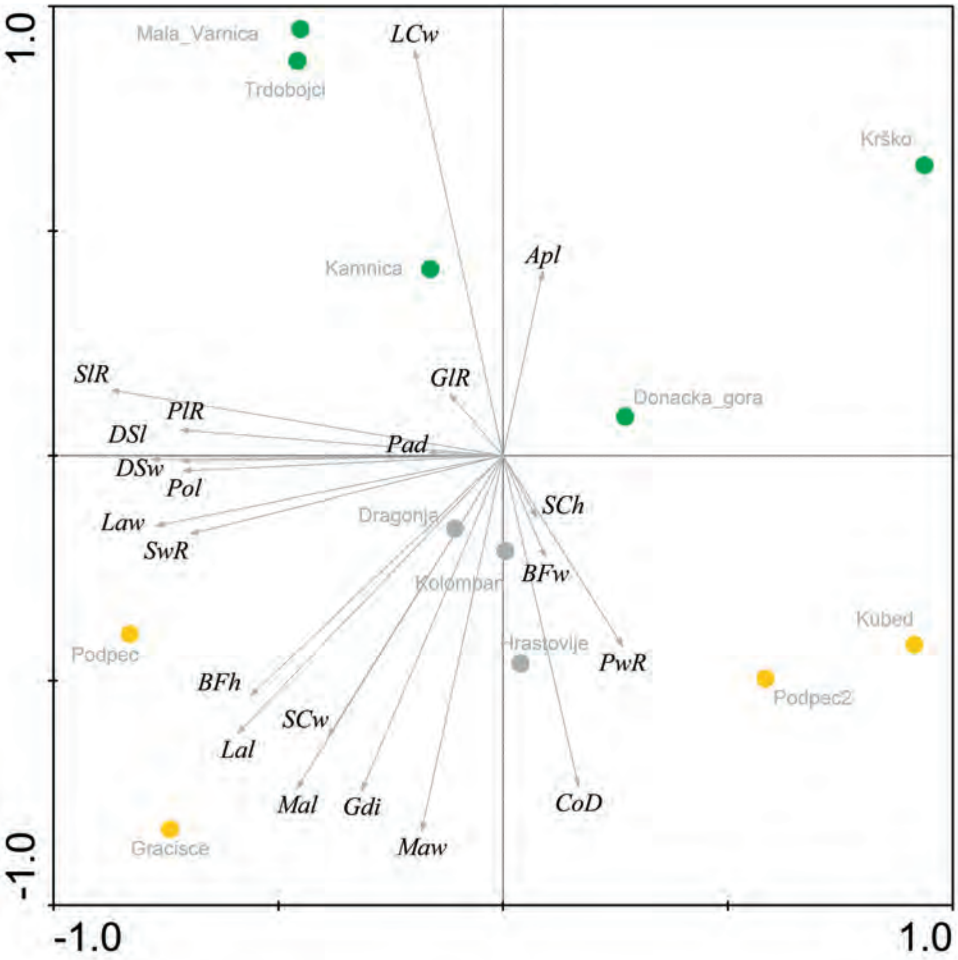


Figure 3. Redundancy analysis (RDA) of the 21 morphological character composition in 12 localities. Eigenvalues: Axis 1 = 0.366; Axis 2 = 0.313. Legend: Green – humid continental climate, Orange – transitional submediterranean – continental climate, Grey – submediterranean climate. Measured morphological character abbreviations are given in Fig. 2.

in transporting their pollen to the stigma of another flower of the same species. Pollination by insects (entomogamy) is the key strategy in the genus *Ophrys*. In fact, effective pollinators are very selective regarding the flowers visited (Paulus 2019). There is a strong intra-specific competition among *Ophrys* individuals for the attraction of their pollinators, which is due to the high learning and memorization abilities of naïve male hymenopterans that record the pheromone signatures of kin or of previously courted partner to avoid (further) copulation attempts (Baguette et al. 2020). Pollinators (also) act as selection factors attracted by signals emitted by the flower to increase its reproductive success above that of other individuals of the same species (Paulus 2019).

The labellum in the genus *Ophrys*, the largest of the three corolla elements is a very complex, three-dimensional structure, comprising several shape and size components. Only some of the lip structures, the so-called mechanically active components (Rakosy et al. 2017), are involved in effectively guiding pollinators towards the reproductive structures of the flowers (Gaskett 2012). Rakosy et al. (2017) hypothesize that these components (morphological characters), used by pollinators to functionally interact, will be under strong pollinator-mediated selection, whereas

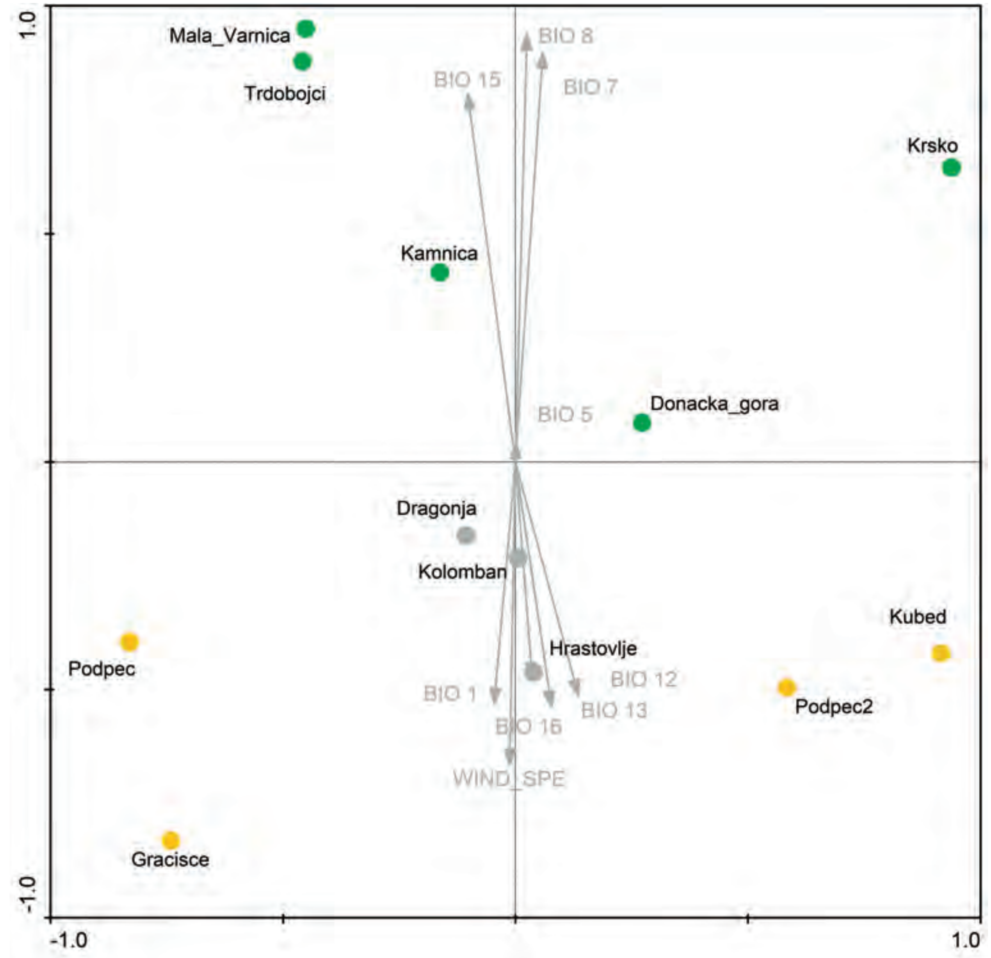


Figure 4. Redundancy analysis (RDA) of the 9 environmental data in 12 localities. Eigenvalues: Axis 1 = 0.366; Axis 2 = 0.313. Legend: Green – humid continental climate, Orange – transitional submediterranean–continental climate, Grey – submediterranean climate. Abbreviation of environmental data are explained in Table 2.

those, pollinators do not use for effective interaction, are more likely to be shaped by relaxed selection and other stochastic factors. In addition, Rakosy et al. (2017) define mechanically active components as those areas or points of the lip which pollinators use as fixed gripping or contact points during pollination, whereas mechanically inactive components relate to areas or points on which the pollinators move around freely or with which they do not come into use as fixed gripping or contact points during pollination, whereas mechanically inactive components relate to areas or points on which the pollinators move around freely or with which they do not come into contact during pollination.

The present study provides clear evidence that at least six floral morphological characters (traits) exhibit statistically significant, strong relation with climatic conditions at the local scale. These are labellum length, proportions of the macula (height and width), constriction of the labellum at the base, connective (gynostemium) length and length of the lateral swellings of the labellum.

Travelling away from coastal areas in NE direction, following the submediterranean–continental climate gradient, labellum length decreases. On average, specimens from coastal areas tend to express larger labellum compared to specimens belonging to Central European populations. The same is true for macula proportions. With the increasing influence of the continental climate, both proportions of the macula, width and height, decrease. Interestingly, the constriction of the labellum, narrowest part of the labellum just below the stigmatic cavity, becomes wider with the increasing influence of the continental climate. Constriction of the labellum presents an important morphological feature of the labellum since it offers a sturdy gripping point for the first pair of pollinator extremities. Karst Edge populations in particular express narrow constrictions of the labellum. These populations are exposed to constant bora and other above ground wind patterns. Narrow constriction of the labellum does ensure a stable position of the pollinator ensuring pollination success. The dominant wind patterns and their intensity, as already mentioned by Devey et al. (2009), do have an important role in affecting the dimensions of the active characters of the labellum. The present study shows for the first time that above ground wind speed in fact has a strong, statistically significant, negative relation with the constriction of the labellum. Connective length (gynostemium length) decreases with the increasing influence of the continental climate. Contrary to expectations, lateral swellings of the labellum increase with the increasing influence of the continental climate in this study. Surprisingly, no wind-related relation was determined between the lateral labellum swelling-length, although being an important feature, providing tight space on the central part of the labellum, keeping pollinator in the 'right' position during the pseudocopulation process.

It is clear that temperature and precipitation regime at the local scale significantly affects the dimensions, proportions of mechanically active components of the flower of the nominate form *O. sphegodes* s. str. These are morphological characters with which pollinators functionally interact during the pseudocopulation process. Contrary to expectations, mechanically inactive components of the flowers, such as proportions of calyx and lateral petals, do not show any correlation to precipitation and temperature regime at the local scale. Local climate affects floral morphology (and pollination success) in multiple ways. Still, the question of the relative contributions of climate and pollinators to affecting floral morphology is not currently well understood (Weber et al. 2020), but it is clear that climate directly explains variation in floral morphology at the intraspecific level.

Orchid–pollinator interactions are far more opportunistic than previously thought (Joffard et al. 2018). The question of the specialization of *Ophrys*–pollinator interactions is important, because it may affect the vulnerability of *Ophrys* populations to climate changes (Hutchings et al. 2018) and because it has major evolutionary consequences. The intraspecific variability of *Ophrys sphegodes* s. str. across the distribution range may attract different, closely related pollinators adopted to different climates and ensure successful pollination in regions with different native hymenopterans. This finding supports the idea on the importance of the ecological context, in which *Ophrys* pollination takes place, suggesting if their main pollinator species is locally absent, *Ophrys* plants may still be able to colonize or persist in the environment by co-opting alternative pollinator species (Baguette et al. 2020).

On the other hand, clear climatically-related intraspecific variability of the nominate form *O. sphegodes* has clear implication in *Ophrys* taxonomy. Especially in the North Adriatic region of Croatia and Italy, putative, morphologically poorly defined, conspecific taxa such as: *O. araneola*, *O. classica*, *O. incantata*, *O. liburnica* and other were described in the past. Subtle morphological differences should not be viewed as marking species boundaries without independent genetic and phenological and ecological evidence that they have become reproductively isolated (Bateman et al. 2010). Local climate related to geographical location, altitude, together with phenology, should be considered in an attempt to morphologically evaluate and delimitate any closely related *Ophrys* taxa, but also to understand the degree of morphological variability in the case of nominate, well known species such as *O. sphegodes* s. str. and also drivers causing it.

Acknowledgements

We thank Slovenian Environment Agency for permission needed to take flowers of *Ophrys sphegodes* s. str. from the habitat (ARSO nr. 35601-15/2019-4, 28.6.2019). Sincere thanks to M. Lipovšek, Ž. Cenc, J. Lango, I. Papež and M. Šenica for their help and support in the field.

References

Baguette M., Bertrand J.A.M., Stevens V.M. & Schatz B. (2020): Why are there so many bee-orchid species? Adaptive radiation by intraspecific competition for mnesic pollinators. – Biol. Rev. **95**: 1630–1663.

Bateman R.M. & Denholm I. (1989): Morphometric procedure, taxonomic objectivity and marsh-orchid systematics. – Watsonia **17**: 449–455.

Bateman R.M., Devey D.S., Malgrem S., Bradshaw E. & Rudall P.J. (2010): Conflicting species concepts underlie perennial taxonomic controversies in *Ophrys*. – Cah. Soc. Franç. Orchidophilie **8**: 87–101.

Bateman R.M. & Rudall P.J. (2006): Evolutionary and morphometric implications of morphological variation among flowers within an inflorescence: a case-study using European orchids. – Ann. Bot. **98**: 975–993.

Brown B.A. & Clegg M.T. (1984): Influence of flower color polymorphism on genetic transmission in a natural population of the common morning glory, *Ipomoea purpurea*. – Evolution **38**: 769–803.

Darwin C. (1859): On the origin of species by means of natural selection. – London: J. Murray.

Devey D.S., Bateman R.M., Fay M.F. & Hawkins J.A. (2009): Genetic structure and systematic relationships within the *Ophrys fuciflora* aggregate (Ochidaceae: Orchidinae): high diversity in Kent and a wind-induced discontinuity bisecting the Adriatic. – Ann. Bot. **104**: 483–495.

Dormont L., Joffard N. & Schatz B. (2019): Intraspecific variation in floral color and odor in orchids. – Int. J. Pl. Sci. **180**: 1036–1058.

ESRI (2010): ArcGIS Desktop: Release 9.3. – Redlands, CA: Environmental Systems Research Institute.

Fenster C.B., Armbruster W.S., Wilson P., Dudash M.R. & Thomson J.D. (2004): Pollination syndromes and floral specialization. – Annual Rev. Ecol. Syst. **35**: 375–403.

Gaskett A.C. (2012): Floral shape mimicry and variation in sexually deceptive orchids with a shared pollinator. – Biol. J. Linn. Soc. **106**: 469–481.

Hutchings M.J. (2010): The population biology of the early spider orchid *Ophrys sphegodes* Mill. III. Demography over three decades. – J. Ecol. **98**: 867–878.

Hutchings M.J., Robbirt K.M., Roberts D.L. & Davy A.J. (2018): Vulnerability of a specialized pollination mechanism to climate change revealed by a 356-year analysis. – Bot. J. Linn. Soc. **186**(4): 498–509.

Joffard, N., Massol F., Grenié M., Montgerald C. & Schatz B. (2018): Effect of pollination strategy, phylogeny and distribution on pollination niches of Euro-Mediterranean orchids. – J. Ecol. **107**(1): 1–13.

Jones K.N. & Reithel J.S. (2001): Pollinator-mediated selection on a flower color polymorphism in experimental populations of *Antirrhinum* (Scrophulariaceae). – Amer. J. Bot. **88**: 447–454.

Knudsen J.T., Eriksson R., Gershenzon J. & Stahl B. (2006): Diversity and distribution of floral scent. – Bot. Rev. **72**: 1–120.

Mamaev S.A., Knyazev M.S., Kulikov P.V. & Filippov E.G. (2004): Orkhidnye Urala. (Orchids in the Urals) – Yekaterinburg: Ural. Otd. Ross. Akad. Nauk.

Paulus H.F. (2019): Speciation, pattern recognition and the maximization of pollination: general questions and answers given by the reproductive biology of the orchid genus *Ophrys*. – J. Comp. Physiol. **205**: 285–300.

Paušič I., Lipovšek M., Jakely D., Pavlec N., Ivajnšič D. & Kaligarič M. (2019): Local climate and latitude affect flower form of *Ophrys fuciflora* (Orchidaceae): evidence for clinal variation. – Bot. Lett. **166**(4): 499–512.

Rakosy D., Cuervo M., Paulus H.F. & Ayasse M. (2017): Looks matter: changes in flower form affect pollination effectiveness in a sexually deceptive orchid. – J. Evol. Biol. **30**: 1978–1993.

Schiestl F.P., Ayasse M., Paulus H.F., Erdmann D. & Francke W. (1997): Variation of floral scent emission and postpollination changes in individual flowers of *Ophrys sphegodes* subsp. *sphegodes*. – J. Chem. Ecol. **23**: 2881–2895.

Spaethe J., Moser W. & Paulus H.F. (2007): Increase of pollinator attraction by means of a visual signal in the sexually deceptive orchid, *Ophrys heldreichii* (Orchidaceae). – Pl. Syst. Evol. **264**: 31–40.

SPSS Inc. (2006): SPSS Base 15.00 User's Guide. – Chicago, IL: SPSS.

Stejskal K., Streinzer M., Dyer A., Paulus H.M. & Spaethe J. (2015): Functional significance of labellum pattern variation in a sexually deceptive orchid (*Ophrys heldreichii*): evidence of individual signature learning effects. – PloS ONE **10**(11): 1–18.

Strauss S.Y. & Whittall J.B. (2006): Non-pollinator agents of selection on floral traits. – In: Harder L.D. & Barrett S.C.H. [eds]: Ecology and evolution of flowers: 120–138. – Oxford: Oxford University Press.

ter Braak C.J.F. & Šmilauer P. (2002): CANOCO reference manual and CanoDraw for Windows user's guide: Software for Canonical Community Ordination (version 4.5). – Wageningen: Biometris.

Valenta K., Nevo O., Martel C. & Chapman C.A. (2017): Plant attractants: integrating insights from seed dispersal and pollination ecology. – Evol. Ecol. **31**: 249–267.

Vereecken N.J., Wilson C.A., Hötling S., Schulz S., Banketov S.A. & Mardulyn P. (2012): Pre-adaptations and the evolution of pollination by sexual deception: Cope's rule of specialization revisited. – Proc. Roy. Soc. Biol. Sci. Ser. B **279**: 4786–4794.

Weber U.K., Scott L.N. & Espíndola A. (2020): Patterns of floral morphology in relation to climate and floral visitors. – Ann. Bot. **125**: 433–445.

Addresses of the authors:

Igor Paušič (corresponding author)
Danijel Ivajnšič
Nataša Pipenbaher
Marcela Bešter
University of Maribor
Faculty of Natural Sciences and Mathematics
Biology Department
Koroška cesta 160
2000 Maribor, Slovenia
E-mail: igor.pausic@um.si

Zeitschrift/Journal: Wulfenia

Jahr/Year: 2022

Band/Volume: 29

Autor(en)/Author(s): Pausic Igor, Ivajnsic Danijel, Pipenbaher Natasa, Bester Marcela

Artikel/Article: Intraspecific morphological variability in the genus Ophrys (Orchidaceae) is driven by changing environments 47-60