Plant morphological variability at the distribution edges: the case of Dryas octopetala (Rosaceae) in northern Greece

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Abstract: Plant populations at their distribution limits may often deviate morphologically from those at the centre of their range (core populations). A similar pattern was observed in Dryas octopetala (Rosaceae), a circumpolar, arctic-alpine species distributed in northern and central Europe, reaching south to northern Greece. The three southernmost populations of Europe, located in Greece, were sampled and specific leaf morphometric traits were measured and analysed using canonical variates analysis (CVA) and hierarchical cluster analysis. CVA revealed that the three samples of D. octopetala did not form one group but were significantly discriminated. Despite the rather similar climatic conditions of Mts Falakron and Orvilos, the sample of Mt Falakron was grouped with that of Mt Tzena, based on leaf morphology. These findings contradict findings from other studies on the ways with which plants react and adapt on areas of harsh climatic conditions. Micro-site conditions or a potentially different post-glacial origin could possibly explain the pattern observed in this study.

Key words: canonical variates analysis, CVA, distribution edges, Dryas octopetala, hierarchical cluster analysis, morphometrics, Greece, Rosaceae, variability

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Introduction

Species, as biological organisms, are morphologically variable to a greater or lesser extent. Although there are plant species that are characterized by a restricted morphological variability, especially those that are range restricted or self-pollinated, others can be greatly differentiated throughout their distributional range (e.g. Christensen 1992; Marcysia 2012; Forsman & Wennersten 2015; Tsiftsis 2016; Zarei & al. 2019).

It is well known that, especially in species with wide distributions, populations occurring at the edges of their range may often deviate morphologically from the more central populations and they also present great morphological variability (Antonovics 1976; Garcia-Ramos & Kirkpatrick 1997; Jonas & Geber 1999). However, specific morphological traits may also be influenced by several environmental factors, e.g. climate, soil (Mal & Lovett-Doust 2005; Peppe & al. 2011; Blinova 2012). Moreover, post-glacial migration and natural selection of specific morphotypes could differentiate fragmented and isolated populations (Hatziskakis & al. 2011; Soubani & al. 2015). Plants that exhibit large intraspecific variation (e.g. in physiology, morphology, phenology) constitute ideal...
cases for studying local or regional adaptations (Gra -
148 tani 2014). Until now, sev-
149 eral studies have been pub-
lished exploring the range of
150 morphological variability of
151 species or referring to plant
discrimination. Such studies use a variety of plant traits,
mostly depending on the
taxonomic group (family or
genus) to which the species
concerned belongs. In some
studies, flower morphology
has been used to explore
the morphological variability
of different populations
(e.g. Medrano & al. 2006;
da Cruz & al. 2011; Tsiftsis
2016). Leaf morphology has
also been widely used as a
taxonomic tool by botanists,
mostly for separating taxa at
the specific or even subspe-
cific level (e.g. Viscosi & al.
2009; de Oliveira & al. 2018).

Leaf morphology is widely considered as a group
of trait variables from which biogeographical informa-
tion and species evolution can be deduced (Klingenberg
2010; Dkhar & Pareek 2014; Marcysiak 2014; Tian &
al. 2016; Lai & al. 2018). This is because leaves are es-
sential parts of the adaptation of a species to a given eco-
nological niche, and such information can be expressed
through a number of leaf morphological traits. A pre-
vious study demonstrated that both leaf size and leaf
shape of Dryas octopetala L. (Rosaceae) follow a bio-
geographical pattern (Marcysiak 2014). Specifically, it
was found that D. octopetala populations
are clustered into several distinct groups,
in which the leaf morphological traits (e.g.
leaf area, number of teeth, maximal width
of the apical tooth) are mainly influenced
by climate, throughout Europe. Based on
these findings, Marcysiak (2014) consid-
ered D. octopetala as an ideal species for
exploring geographical patterns. Moreo-
over, the study of widespread species at
their southernmost distribution limits is
interesting because it can provide valuable
information about their ecology (Schuler
2004).

Dryas octopetala (Fig. 1) is a circumpo-
lar, arctic-alpine plant species growing in
areas where climatic conditions are harsh.
In the north of its distributional range, it
can be found even in low-altitude areas,
whereas at its southern distribution lim-
its it exclusively occurs high on mountains (Tutin & al.
1968; Strid 1986). It is a woody, long-lived dwarf shrub
(life expectancy over 500 years; de Witte & al. 2012) and
constitutes the ground vegetation cover in the arctic and
alpine tundra ecosystems. The populations of the species
currently extend from Eurasian and American arctic tun-
dra to the temperate mountains of Europe, including the
Balkan Peninsula (Hultén & Fries, 1986).

In her study, Marcysiak (2014) sampled material of
Dryas octopetala from numerous populations, almost
throughout the European range of the species, although
not from northern Greece, its southernmost distribution

Fig. 1. Dryas octopetala in flower. – Greece, Eastern Macedonia, near summit of Mt Falakron,
26 June 2006, photograph by S. Tsiftsis.

Fig. 2. Map of the studied populations of Dryas octopetala.
limit in Europe. In Greece, *D. octopetala* is considered a remnant species, which probably migrated there during the last glacial age, now forming three distinct and isolated populations in the northern part of the country (Schuler & Tsiripidis 2009). The fact that the Greek populations of *D. octopetala* have been fragmented and isolated for a long time (maybe for several centuries or even millennia) makes them an ideal case to study their morphological differentiation or divergence. Based on this background, the aim of the present study is to examine whether there are leaf morphological differences between individuals from the three Greek populations, and if any differentiation could be explained by the biogeographical patterns identified by Marcysiak (2014).

**Material and methods**

We collected leaf material from the three known *Dryas octopetala* populations in Greece, namely Mt Tzena (41°09’N, 22°14’E), Mt Orvilos (41°22’N, 23°37’E) and Mt Falakron (41°17’N, 24°05’E) (Fig. 2). Collection of leaves from individuals of all populations took place in July of 2018. We sampled 35, 22 and 37 individuals, respectively, from each population. A total of 10 leaves was collected from each individual and stored in portable cooler within plastic bags. Leaves were taken from the same height and from the outer part or the plant canopy. The fresh leaf material was scanned and images were processed in black and white mode before morphometric analysis, using GIMP v2.9 software. Following Marcysiak (2014), we measured the following leaf morphometric variables (leaf morphological traits): leaf area (LA), leaf perimeter (LP), leaf circularity (LC), leaf length (LL), leaf width (LW), number of lobes (NL), maximum width of apical tooth (AW), length from midrib to leaf periphery (BL) and leaf base width (BW) (Fig. 3). Leaf circularity, or else called shape factor, was calculated according to the following formula:

\[
\text{Leaf circularity} = \frac{(4 \times \pi \times LA)}{LP^2} \quad \text{(de Heredia & al. 2009)},
\]

where

- \( \pi \): Archimedes’ constant (\( \pi = 3.14159 \))
- LA: leaf area and
- LP: leaf perimeter

Leaf circularity values close to 1 (a circle) indicate more circular leaves compared to values close to zero, which indicate non-circular (more elongated) leaves. All measurements were performed with the freely available software ImageJ (Rueden & al. 2017) and are reported in millimetres (mm). Leaf variables were expressed on an individual plant basis by calculating the average value from 10 leaves per plant.

To study the multivariate patterns of size variation of the measured *Dryas octopetala* individuals, a Canonical Variates Analysis (CVA) (Mardia & al. 1979) was performed, having the sampled population as the categorical variable. CVA is a widely used technique for assessing and displaying variation among pre-defined groups relative to the variation within the groups. The method successively extracts axes (Canonical Variates, CVs), which are a linear combination of the original variables and orthogonal to all others, having however the greatest ratio of among-group to within-group variance. Therefore, plots of the first few CVs are optimal displays of differences among groups and are scaled relative to the pooled estimate of within-group variation (Webster & Sheets 2010). A cross-validation using the leave-one-out procedure (jackknife test) was performed to estimate the expected actual error rates in classifying *D. octopetala* individuals in the respective groups.

Fig. 3. Way of measuring leaf morphological traits of *Dryas octopetala*; LL: leaf length; LW: leaf width; AW: maximum width of apical tooth; BL: length from midrib to leaf periphery; BW: leaf base width.
The morphometric relationships among the three Dryas octopetala populations were further illustrated by a hierarchical cluster analysis using the unweighted pair group method with an arithmetic mean (UPGMA). Cluster analysis was based on the Mahalanobis distances between the means of the samples, as these were calculated by the canonical variate analysis. To test for statistical differentiation between the samples of the three populations, the leaf morphometric data were firstly checked for normality using the Shapiro-Wilk normality test, because this test outperforms others (e.g. Kolmogorov-Smirnov), especially when the sample size is small. After checking for normality, and because not all variables were normally distributed, the differences between the samples of the three populations were tested using Wilk’s lambda statistic, calculated using a nonparametric comparison of multivariate samples. The Mann-Whitney U test was used to test for statistical differences among samples of the three populations in each of the nine leaf morphometric variables. Finally, Pearson correlation coefficients between the nine leaf morphological traits and the bioclimatic/climatic variables (obtained from the WorldClim database; Fick & Hijmans 2017) of the three populations were calculated.

All analyses were performed in R version 3.5.2 (R Foundation for Statistical Computing) using the Morpho (Schlager & al. 2019) and npmv (Burchett & Ellis 2017) packages.

Results

The result of the canonical variates analysis is graphically presented in Fig. 4. The first CV axis accumulated 56.2% of the variance and was highly correlated with leaf circularity (LC) and negatively correlated with maximum width of the apical tooth (AW), whereas the second axis accounted for the remaining 43.8% of the total variance and was found to be highly – albeit negatively – correlated with AW and positively with LC (Table 1). Leaf area (LA) was found to be the least significant variable, followed by the number of lobes (NL).

Based on the nine leaf morphometric variables, CVA discriminated the three sampled populations, because they overlap only to a rather small extent. Furthermore, Wilk’s lambda statistic demonstrated that the samples of the three populations were significantly discriminated (p < 0.001), whereas the correct classifications for the whole dataset was 70.21%.

The number of correctly classified individuals in the three studied populations is shown in Table 2. The overall percentage of correctly classified individuals was rather high, ranging from 63.63% to 77.14%. The lowest percentage of correctly classified individuals was calculated for the population of Mt Orvilos, whereas the highest was for the population of Mt Tzena. Moreover, it is worth mentioning that eight out of the 12 not correctly classified individuals of Mt Falakron were assigned to the

Table 1. Canonical discriminant coefficients showing the contribution of the nine leaf morphological traits to the two canonical variates (CV) analysis axes.

| Trait code | Leaf morphological trait | CV1  | CV2  |
|------------|--------------------------|------|------|
| LA         | leaf area                | -0.0070 | -0.0078 |
| LP         | leaf perimeter           | -0.1325 | 0.1550 |
| LC         | leaf circularity         | 12.8621 | 5.1089 |
| LL         | leaf length              | 0.5362 | -0.3151 |
| LW         | leaf width               | -1.0468 | -0.1260 |
| NL         | number of lobes          | -0.1305 | 0.0958 |
| AW         | maximum width of apical tooth | -1.6355 | -5.5467 |
| BL         | length from midrib to leaf periphery | 0.9387 | 1.0515 |
| BW         | leaf base width          | 0.5195 | -0.1045 |
% variance contribution | 56.2 | 43.8 |
cumulative variance contribution | 56.2 | 100 |
population of Mt Tzena, demonstrating their rather close morphological relationship.

The UPGMA cluster based on Mahalanobis distances among the pairs of the three sampled populations is shown in Fig. 5. As can be seen, the population of Mt Tzena was grouped together with that of Mt Falakron, whereas that of Mt Orvilos was separate. The greater morphological affinity of the *Dryas octopetala* individuals from Mt Tzena and Falakron, compared to those from Mt Orvilos, confirmed the greater overlap of these two populations on the CVA graph and the results presented on Table 2 for the correctly classified individuals.

Finally, the differences in the measured leaf traits of the samples of the studied populations are shown in Fig. 6, whereas the results of the Mann-Whitney U test for their statistical differences are presented in Table 3. Specifically, individuals from Mt Falakron are characterized by larger leaves (in terms of area, perimeter, width, length and number of lobes) compared to those from the other two populations, whereas those from Mt Tzena are the smallest.

The pair-wise differences of the populations of Mt Falakron and Tzena were statistically significant for all the leaf traits, except for AW and BW. Contrary to the comparison of these two mountains, in which most differences were statistically significant, the comparison between the other pairs of mountains revealed more non-significant pair-wise differences. Specifically, the comparison between Mt Falakron and Orvilos revealed non-significant differences for LA, LC, LL, BL and BW, whereas the comparison between Mt Orvilos and Tzena revealed non-significant differences for LL, LW, NL, BL and BW.

The Pearson correlation coefficients between the morphological traits and the bioclimatic/climatic variables are shown in Table 4. As can be seen, the effect of both bioclimatic and climatic factors on the morphological traits is in general significant, either positively or negatively. Annual precipitation (bio12) and solar radiation during the summer period (May–September) were factors that significantly affected only one trait, the maximum width of the apical tooth (AW). The number of lobes (NL) and leaf base width (BW) were the least affected morphological traits,

| Population | Falakron | Orvilos | Tzena | Total | % correctly classified |
|------------|----------|---------|-------|-------|-----------------------|
| Falakron   | 25       | 4       | 8     | 37    | 67.56                 |
| Orvilos    | 5        | 14      | 3     | 22    | 63.63                 |
| Tzena      | 4        | 4       | 27    | 35    | 77.14                 |
| Total      | 34       | 22      | 38    | 94    |                       |

Table 3. Results of the Mann-Whitney U test comparison among all pairs of mountains. The mountain name in a cell indicates the mountain having a statistically higher value in the specific leaf morphological trait. For trait codes see Table 1; *** p < 0.001; ** p < 0.01; * p < 0.05; ns = non significant.

| Population pair | LA | LP | LC | LL | LW | NL | AW | BL | BW |
|-----------------|----|----|----|----|----|----|----|----|----|
| Orvilos – Tzena | Orvilos* | Orvilos** | Tzena** | ns | ns | Orvilos*** | ns | ns |
| Orvilos – Falakron | ns | Falakron* | ns | ns | Falakron* | Falakron* | Orvilos*** | ns | ns |
| Tzena – Falakron | Falakron*** | Falakron*** | Tzena*** | Falakron** | Falakron*** | Falakron* | ns | Falakron* | ns |
Table 4. Pearson correlation coefficients between bioclimatic (bio) and climatic (srad, prec, tmin) variables with leaf morphological trait variables. For trait codes see Table 1; for bioclimatic/climatic variable codes see Appendix; ** p<0.01; * p<0.05. Values for bioclimatic/climatic variables were downloaded from the WorldClim database (Fick & Hijmans 2017).

|          | LA  | LP  | LC  | LL  | LW  | NL  | AW  | BL  | BW  |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| bio1     | -0.28** | -0.34** | 0.39** | -0.21* | -0.24* | -0.07 | -0.50** | -0.14 | -0.09 |
| bio2     | 0.44** | 0.50** | -0.47** | 0.29** | 0.38** | 0.19 | 0.29** | 0.27** | 0.19 |
| bio3     | 0.37** | 0.43** | -0.44** | 0.26* | 0.32** | 0.13 | 0.42** | 0.21* | 0.14 |
| bio4     | -0.43** | -0.50** | 0.47** | -0.29** | -0.38** | -0.18 | -0.30** | -0.26* | -0.18 |
| bio5     | -0.22* | -0.28** | 0.34** | -0.18 | -0.19 | -0.03 | -0.53** | -0.10 | -0.06 |
| bio6     | -0.33** | -0.40** | 0.42** | -0.24* | -0.29** | -0.10 | -0.46** | -0.18 | -0.12 |
| bio7     | 0.40** | 0.43** | -0.30** | 0.23* | 0.36** | 0.25* | -0.21* | 0.29** | 0.21* |
| bio8     | 0.45** | 0.51** | -0.47** | 0.29** | 0.39** | 0.20 | 0.26* | 0.28** | 0.19 |
| bio9     | -0.37** | -0.44** | 0.44** | -0.26* | -0.32** | -0.13 | -0.41** | -0.21* | -0.14 |
| bio10    | -0.32* | -0.38** | 0.41** | -0.23* | -0.28** | -0.09 | -0.47** | -0.17 | -0.11 |
| bio11    | -0.24* | -0.29** | 0.36** | -0.19 | -0.20* | -0.04 | -0.52** | -0.11 | -0.07 |
| bio12    | 0.00 | -0.03 | 0.15 | -0.04 | 0.01 | 0.09 | -0.55** | 0.05 | 0.05 |
| bio13    | 0.47** | 0.53** | -0.46** | 0.30** | 0.42** | 0.23* | 0.13 | 0.30** | 0.22* |
| bio14    | -0.43** | -0.50** | 0.47** | -0.29** | -0.38** | -0.18 | -0.32** | -0.26* | -0.18 |
| bio15    | 0.45** | 0.51** | -0.47** | 0.29** | 0.39** | 0.19 | 0.26** | 0.27** | 0.19 |
| bio16    | 0.46** | 0.52** | -0.47** | 0.29** | 0.40** | 0.21* | 0.22* | 0.29** | 0.21* |
| bio17    | -0.44** | -0.51** | 0.47** | -0.29** | -0.39** | -0.19 | -0.28** | -0.27** | -0.19 |
| bio18    | 0.27** | 0.33** | -0.38** | 0.21* | 0.23* | 0.06 | 0.51** | 0.14 | 0.08 |
| bio19    | 0.47** | 0.53** | -0.46** | 0.30** | 0.42** | 0.23* | 0.13 | 0.30** | 0.22* |
| srad2    | 0.36** | 0.43** | -0.44** | 0.26* | 0.32** | 0.12 | 0.42** | 0.20* | 0.14 |
| srad3    | -0.43** | -0.47** | 0.35** | -0.25* | -0.39** | -0.25* | 0.13 | -0.30** | -0.22* |
| srad4    | -0.43** | -0.49** | 0.47** | -0.27** | -0.37** | -0.17 | -0.32** | -0.26* | -0.18 |
| srad5    | -0.01 | -0.04 | 0.16 | -0.05 | 0.01 | 0.09 | -0.55** | 0.04 | 0.05 |
| srad6    | -0.06 | -0.09 | 0.20* | -0.08 | -0.04 | 0.06 | -0.56** | 0.01 | 0.02 |
| srad7    | 0.11 | 0.09 | 0.04 | 0.02 | 0.10 | 0.14 | -0.51** | 0.12 | 0.10 |
| srad8    | 0.10 | 0.08 | 0.05 | 0.02 | 0.10 | 0.14 | -0.52** | 0.11 | 0.10 |
| srad9    | -0.12 | -0.17 | 0.26* | -0.12 | -0.10 | 0.03 | -0.56** | -0.03 | -0.01 |
| srad10   | 0.46** | 0.52** | -0.42** | 0.29** | 0.41** | 0.25* | 0.02 | 0.31** | 0.23* |
| srad11   | 0.28** | 0.29** | -0.15 | 0.14 | 0.26* | 0.22* | -0.38** | 0.22* | 0.17 |
| srad12   | 0.40** | 0.43** | -0.30** | 0.23* | 0.36** | 0.25* | -0.21* | 0.29** | 0.21* |
| prec6    | 0.44** | 0.51** | -0.47** | 0.29** | 0.39** | 0.19 | 0.28** | 0.27** | 0.19 |
| prec7    | 0.41** | 0.48** | -0.46** | 0.28** | 0.36** | 0.16 | 0.36** | 0.24* | 0.17 |
| prec8    | 0.46** | 0.52** | -0.47** | 0.30** | 0.40** | 0.21* | 0.22* | 0.29** | 0.21* |
| prec9    | -0.36** | -0.43** | 0.44** | -0.25* | -0.31** | -0.12 | -0.43** | -0.20 | -0.14 |
| tmin2    | -0.27** | -0.33** | 0.38** | -0.21* | -0.23* | -0.06 | -0.51** | -0.14 | -0.08 |
| tmin3    | -0.36** | -0.42** | 0.44** | -0.25* | -0.31** | -0.12 | -0.43** | -0.20 | -0.13 |
| tmin4    | -0.34** | -0.40** | 0.43** | -0.24* | -0.29** | -0.11 | -0.45** | -0.19 | -0.12 |
| tmin5    | -0.21* | -0.21* | 0.06 | -0.09 | -0.19 | -0.19 | -0.45** | -0.18 | -0.14 |
| tmin6    | -0.28** | -0.35** | 0.39** | -0.21* | -0.24* | -0.07 | -0.50** | -0.14 | -0.09 |
| tmin7    | -0.27** | -0.33** | 0.38** | -0.21* | -0.23* | -0.06 | -0.51** | -0.14 | -0.08 |
| tmin8    | -0.24* | -0.29** | 0.35** | -0.19 | -0.20 | -0.04 | -0.52** | -0.11 | -0.07 |
as they were affected only by specific bioclimatic and climatic factors (i.e. bio7, bio13, bio16, bio19, specific monthly values of solar radiation and August precipitation), but even in these cases the calculated correlation coefficients were low (< |0.25|). Moreover, harsh climatic conditions, as expressed by the minimum monthly temperatures, negatively affect leaf size (leaf area, perimeter, length and width) and maximum width of the apical tooth, whereas only leaf circularity was positively affected.

Discussion

The results of the present study confirmed that morphological traits may vary and differ when studying populations at the edges of the distribution of a species (Jonas & Geber 1999; Tsiftsis 2016). Despite the fact that the samples of the three studied populations overlapped to some extent in the CVA graph, they proved to be statistically discriminated. This suggested that either ecological factors (e.g. climatic, soil/geological) or natural selection and a potentially different post-glacial history might have shaped this morphological differentiation.

*Dryas octopetala* exclusively occurs in sites with limestone, the Greek populations being no exception (Strid 1986; Schuler & Tsiripidis 2009; Marcysiak 2014). On-site examination showed that *D. octopetala* occurs in Greece above 1900 m in rocky micro-sites of northern or northwestern exposure (see Appendix), where competition with other plant groups (e.g. grasses) is greatly reduced or does not exist, on all three mountains.

Apart from soil/geological conditions and stress due to competition with other plants, climatic conditions have also been found to affect plant morphologi-
cal traits, including leaf size (Sultan 2000; Peppe & al. 2011). Despite the general worldwide trend of decreasing leaf size with latitude and elevation (Wright & al. 2017), studies that contradict these patterns are not rare in the literature. Specifically, Royer & al. (2008) found that Acer rubrum L. tends to have larger leaves toward colder climates, whereas Quercus kelloggii Newb. has smaller leaves. Based on the results of Marcysiak (2014), Dryas octopetala does not follow the general worldwide trend in its European range. Specifically, Marcysiak (2014) found that leaf size (expressed by LA, LL and BW) was positively correlated with the minimum temperatures during the first part of the growing season (April–June), demonstrating that it increased towards southern regions. Moreover, she found that AW was positively correlated with rainfall during August and September, as well as with the mean temperature of the wettest quarter.

In the present study, we found that leaf size (in terms of LA, LL, LW and LP) is negatively correlated with the minimum temperatures, whereas it is positively correlated with August precipitation but negatively with September precipitation. Moreover, we found that individuals from Mt Falakron had larger leaves compared to individuals from the other two mountain ranges. This finding contradicts the pattern described by Marcysiak (2014), because Mt Tzena has milder climatic conditions (based on data downloaded from the WorldClim database [Fick & Hijmans 2017]; see Appendix), whereas Mt Orvilos has harsher conditions compared to the others. Moreover, under harsh climate conditions, plant leaves tend to have larger lobes and more teeth, and this constitutes an adaptation for increased carbon uptake at the beginning of the growing season (Royer & Wilf 2006; Peppe & al. 2011). However, neither Marcysiak (2014) nor we found such a correlation. Specifically, Marcysiak (2014) did not find any significant correlation among the factors “number of teeth” and “monthly minimum temperatures”, whereas, in our study, the maximum number of lobes (equivalent to the number of teeth sensu Marcysiak 2014) was recorded in the individuals from Mt Falakron.

Besides the differences between our findings and those of other studies, another interesting pattern was the clustering of the Mt Falakron population with the Mt Tzena population, where leaf morphology differentiation was not consistent with the biogeographical patterns of post-glacial origin. During the last glacial period, in the late Quaternary era, D. octopetala, as many other alpines, migrated toward southern Europe including the Balkan Peninsula (Birks 2008). This upward-downward movement has taken place more than once and caused population fragmentation and isolation after the end of the last glacial episode (Allen & al. 2015). Hence, genetic analyses should be conducted to enable the attribution of the observed differences in morphological traits among the three D. octopetala populations in Greece either to microclimate or to biogeographical patterns of post-glacial origin.

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References

Allen G. A., Marr K. L., McCormick L. J. & Hebda R. J. 2015: Geographical origins, migration patterns and refugia of Sibbaldia procumbens, an arctic-alpine plant with a fragmented range. – J. Biogeogr. 42: 1665–1676.

Antonovics J. 1976: The nature of limits to natural selection. – Ann. Missouri Bot. Gard. 63: 224–247.

Birks H. H. 2008: The Late-Quaternary history of arctic and alpine plants. – Pl. Ecol. Diversity 1: 135–146.

Blinova I. V. 2012: Intra- and interspecific morphological variation of some European terrestrial orchids along a latitudinal gradient. – Russ. J. Ecol. 43: 111–116.

Burchett W. & Ellis A. 2017: npmv: nonparametric comparison of multivariate samples. R package version 2.4.0. – Published at https://CRAN.R-project.org/package=npmv

Christensen K. I. 1992: Revision of Crataegus sect. Crataegus and nothoc. Crataegeinae (Rosaceae-Maloi-deae) in the Old World. – Syst. Bot. Monogr. 35: 1–199.

da Cruz D. T., Selbach-Schneidelbach A., Lambert S. M., Ribeiro P. L. & Borba E. L. 2011: Genetic and morphological variability in Cattleya elongata Barb. Rodr. (Orchidaceae), endemic to the campo rupestre vegetation in northeastern Brazil. – Pl. Syst. Evol. 294: 87–98.
de Heredia U. L., Valbuena-Carabaña M., Córdoba M. & Gil L. 2009: Variation components in leaf morphology of recruits of two hybridising oaks [Q. petrea (Matt.) Liebl. and Q. pyrenaica Willd.] at small spatial scale. – Eur. J. Forest Res. 128: 543–554.

d de Oliveira M. I. U., Reboças D. A., Leite K. R. B., de Oliveira R. P. & Funch L. S. 2018: Can leaf morphology and anatomy contribute to species delimitation? A case in the Campomanesia xanthocarpa complex (Myrtaceae). – Flora 249: 111–123.

d de Witte L. C., Armbustre G. F. J., Gielly L., Taberlet P. & Stöcklin J. 2012: AFLP markers reveal high clonal diversity and extreme longevity in four arctic-alpine species. – Molec. Ecol. 21: 1081–1097.

Dkhar J. & Pareek A. 2014: What determines a leaf’s shape? – EvoDevo 5: 47.

Fick S. E. & Hijmans R. J. 2017: WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. – Int. J. Climatol. 37: 4302–4315.

Forsman A. & Wennersten L. 2015: Inter-individual variation promotes ecological success of populations and species: evidence from experimental and comparative studies. – Ecography 39: 630–648.

Garcia-Ramos G. & Kirkpatrick M. 1997: Genetic models of adaptation and gene flow in peripheral populations. – Evolution 51: 21–28.

Gratani L. 2014: Plant phenotypic plasticity in response to environmental factors. – Advances Bot. 2014: 208747.

Hatziskakis S., Tsiripidis I. & Papageorgiou A. C. 2012: Calculated characters of leaves are independent on environmental conditions in Salix herbacea (Salicaceae) and Betula nana (Betulaceae). – Acta Soc. Bot. Poloniae 81: 153–158.

Marcysiak K. 2014: Geographical differentiation of Dryas octopetala in Europe based on morphological features. – Dendrobiology 72: 113–123.

Mardia K. V., Kent J. T. & Bibby J. M. 1979: Multivariate analysis. – London: Academic Press.

Medrano M., Castellanos M. C. & Herrera C. M. 2006: Comparative floral and vegetative differentiation between two European Aquilegia taxa along a narrow contact zone. – Pl. Syst. Evol. 262: 209–224.

Peppe D. J., Royer D. L., Cariglino B., Oliver S. Y., Newman S., Leight E., Correa E., Currano E. D., Erickson J. M., Hinojosa L., Hoganson J. W., Iglesias A., Jaramillo C. A., Johnson K. R., Jordan G. J., Kraft N. J. B., Loveland E. C., Lusk C. H., Niinemets U., Peñuelas J., Rapson G., Wing S. L. & Wright I. J. 2011: Sensitivity of leaf size and shape to climate: global patterns and paleoclimatic applications. – New Phytol. 190: 724–739.

Royer D. L., McElwain J. C., Adams J. M. & Wilf P. 2008: Sensitivity of leaf size and shape to climate within Acer rubrum and Quercus kelloggii. – New Phytol. 179: 808–817.

Rueden C. T., Schindelin J., Hiner M. C., DeZonia B. E., Walter A. E., Arena E. T. & Eliceiri K. W. 2017: ImageJ2: ImageJ for the next generation of scientific image data. – BMC Bioinf. 18: 529.

Schröder J., Jefferis G. & Ian D. 2019: Morpho: Calculations and visualizations related to Geometric Morphometrics. R package version 2.7. – Published at https://github.com/zarquon42b/Morpho.

Schuler A. 2004: Charakterarten mitteleuropäischer Pflanzengesellschaften an ihrer Verbreitungsgrenze in Griechenland. Eine Studie zum ökologischen und syntaxonomischen Verhalten am Arealrand. – Bot. Chron. (Patras) 17: 1–168.

Schuler A. & Tsiripidis I. 2009: Dryas octopetala L. Tpoortó (VU) [Vulnerable]. – Pp. 382–383 in: Phitos D., Konstantinidis Th. & Kamari G. (ed.), The Red Data book of rare and threatened plants of Greece A – D [in Greek]. – Patras: Hellenic Botanical Society.

Skrede I., Eidesen P. B., Portela R. P. & Brochman C. 2006: Refugia, differentiation and postglacial migration in arctic-alpine Eurasia, exemplified by the mountain avens (Dryas octopetala L.). – Molec. Ecol. 15: 1827–1840.

Soubani E., Hedrén M. & Widén B. 2015: Genetic and morphological differentiation across a contact zone between two postglacial immigration lineages of Helianthemum nummularium (Cistaceae) in southern Scandinavia. – Pl. Syst. Evol. 301: 1499–1508.

Strid A. (ed.) 1986: Mountain flora of Greece I. – Cambridge: Cambridge University Press.

Sultan S. E. 2000: Phenotypic plasticity for plant development, function and life history. – Trends Pl. Sci. 5: 537–542.

Tian M., Yu G.-R., He N.-P. & Hou J.-H. 2016: Leaf morphological and anatomical traits from tropical to temperate coniferous forests: mechanisms and influencing factors. – Sci. Rep. 6: 19703.
Tsiftsis S. 2016: Morphological variability of Himantoglossum s.s. (Orchidaceae) in Greece. – Phytotaxa 245: 17–30.

Tutin T. G., Heywood V. H., Burges N. A., Moore D. M., Valentine D. H., Walters S. M. & Webb D. A. (ed.) 1968: Flora europaea 2. Rosaceae to Umbelliferae. – Cambridge: Cambridge University Press.

Viscosi V., Lepais O., Gerber S. & Fortini P. 2009: Leaf morphological analyses in four European oak species (Quercus) and their hybrids: a comparison of traditional and geometric morphometric method. – Pl. Biosyst. 143: 564–574.

Webster M. & Sheets D. H. 2010: A practical introduction to landmark-based geometric morphometrics. – The Paleontological Society Papers 16: 163–188.

Wright I. J., Dong N., Maire V., Prentice I. C., Westoby M., Díaz S., Gallagher R. V., Jacobs B. F., Kooyman R., Law E. A., Leishman M. R., Niinemets Ü., Reich P. B., Sack L., Villar R., Wang H. & Wilf P. 2017: Global climatic patterns of leaf size. – Science 357: 917–921.

Zarei A., Erfani-Moghadam J. & Jalilian H. 2019: Assessment of variability within and among four Pyrus species using multivariate analysis. – Flora 250: 27–36.
## Appendix

Site characteristics of the three studied *Dryas octopetala* populations. Values for bioclimatic/climatic variables were downloaded from the WorldClim database (Fick & Hijmans 2017).

| Geological substrate | Altitude | Aspect  |
|----------------------|----------|---------|
| Falakron limestones  | 1950–2050 m | northwest |
| Orvilos limestones   | 1950–2150 m | north   |
| Tzena limestones     | 1900 m    | north   |

Values for bioclimatic variables (temperature): bio1: annual mean temperature; bio2: mean diurnal range; bio3: isothermality; bio4: temperature seasonality; bio5: max temperature of warmest month; bio6: min temperature of coldest month; bio7: temperature annual range; bio8: mean temperature of wettest quarter; bio9: mean temperature of driest quarter; bio10: mean temperature of warmest quarter; bio11: mean temperature of coldest quarter.

| Site    | bio1  | bio2  | bio3  | bio4  | bio5  | bio6  | bio7  | bio8  | bio9  | bio10 | bio11 |
|---------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Falakron| 4.04  | 9.32  | 34.76 | 607.45| 16.30 | -10.50| 26.80 | 6.38  | 9.00  | 11.40 | -3.13 |
| Orvilos | 3.30  | 9.23  | 35.08 | 611.89| 15.30 | -11.00| 26.30 | 5.70  | 8.30  | 10.70 | -3.88 |
| Tzena   | 5.09  | 8.79  | 33.30 | 638.27| 17.20 | -9.20 | 26.40 | 3.62  | 12.67 | 12.92 | -2.37 |

Values for bioclimatic variables (precipitation): bio12: annual precipitation; bio13: precipitation of wettest month; bio14: precipitation of driest month; bio15: precipitation seasonality; bio16: precipitation of wettest quarter; bio17: precipitation of driest quarter; bio18: precipitation of warmest quarter; bio19: precipitation of coldest quarter.

| Site   | bio12 | bio13 | bio14 | bio15 | bio16 | bio17 | bio18 | bio19 |
|--------|-------|-------|-------|-------|-------|-------|-------|-------|
| Falakron| 724   | 82    | 37    | 22.54 | 227   | 126   | 167   | 191   |
| Orvilos| 711   | 80    | 38    | 20.96 | 224   | 130   | 170   | 184   |
| Tzena  | 725   | 78    | 46    | 15.76 | 218   | 146   | 163   | 177   |

Climatic variable: solar radiation (srad), monthly values (2–12 = February–December).

| Site    | srad2 | srad3 | srad4 | srad5 | srad6 | srad7 | srad8 | srad9 | srad10 | srad11 | srad12 |
|---------|-------|-------|-------|-------|-------|-------|-------|-------|--------|--------|--------|
| Falakron| 8872  | 12227 | 16114 | 18917 | 21380 | 22315 | 20085 | 15463 | 10963  | 6968   | 5203   |
| Orvilos | 8904  | 12333 | 16140 | 18578 | 21159 | 21928 | 19476 | 15281 | 10796  | 6890   | 5139   |
| Tzena   | 8734  | 12330 | 16372 | 18951 | 21428 | 22262 | 20008 | 15539 | 10726  | 6931   | 5152   |

Climatic variable: precipitation (prec), monthly values (6–9 = June–September).

| Site   | prec6 | prec7 | prec8 | prec9 |
|--------|-------|-------|-------|-------|
| Falakron| 74    | 48    | 45    | 33    |
| Orvilos| 71    | 48    | 44    | 32    |
| Tzena  | 58    | 45    | 42    | 37    |

Climatic variable: minimum temperature (tmin), monthly values (2–8 = February–August).

| Site    | tmin2 | tmin3 | tmin4 | tmin5 | tmin6 | tmin7 | tmin8 |
|---------|-------|-------|-------|-------|-------|-------|-------|
| Falakron| -8.20 | -6.70 | -3.30 | 1.00  | 4.40  | 6.30  | 6.50  |
| Orvilos | -8.80 | -7.10 | -3.80 | 5.00  | 3.80  | 5.70  | 5.90  |
| Tzena   | -7.40 | -5.20 | -1.90 | 2.30  | 5.30  | 7.10  | 7.10  |