ACADEMIA BIOLOGY

Research Article
Published: 2025-08-13
https://doi.org/10.20935/AcadBiol7815

Variation in *Anthyllis vulneraria* L. populations in adjacent regions across acidic and basic soils in Val Piora

Ermelinda Gjeta[1], Diellëza Lajçi[2], Avni Hajdari[2], Xhesi Shabanllari[3], Reinhard Bachofen[4,*], Jakob Schneller[5,*]

Academic Editors: Kevin Cianfaglione, Eric Lewallen

Abstract

Populations of *Anthyllis vulneraria* ssp. *vulneraria* and *A. vulneraria* ssp. *vallesiaca* are found in close vicinity in Val Piora, where the geological situation changes abruptly between basic and acidic substrates. At three sites with populations of *A. vulneraria* s. lato, one in basic soil, one in acidic soil, and one in a mixed area, three samples of a total of sixty plants were collected, and flower morphology and physiological activity was determined. Of each population, the number of plants using Braun-Blanquet squares was determined. The frequency and indication values showed that the two subspecies are adapted to different pH values. *A. v.* ssp. *vallesiaca* and *A. v.* ssp. *vulneraria* are clearly well separated by the red striped keel, the flag dimension, and flower size. In areas where the two species overlap, introgression occurs. The hybrid population consisted of yellow hybrid plants with red keel, yellow plants, and white plants with a red keel. The hybrids are present at a pH value between the ones of the two subspecies. The rapid increase in chlorophyll fluorescence also clearly showed that the two subspecies differed in their kinetics. The values of the hybrid plants (yellow with red keel) were between those of the populations of the two subspecies.

Keywords: *Anthyllis vulneraria ssp. vallesiaca, A. ssp. vulneraria, hybrids; floral morphology, phytosociology, fast chlorophyll fluorescence*

Citation: Gjeta E, Lajçi D, Hajdari A, Shabanllari X, Bachofen R, Schneller J. Variation in *Anthyllis vulneraria* L. populations in adjacent regions across acidic and basic soils in Val Piora. *Academia Biology* 2025;3. https://doi.org/10.20935/AcadBiol7815

1. Introduction

Anthyllis vulneraria L. is a highly variable, complex, and heterogeneous species that is found in very different habitats of Europe and North Africa. Approximately 45 subspecies have been described for Europe [1], and six have been described for Switzerland [2]. Our study addresses the subspecies *vallesiaca* (Beck) Guyot, which is found on acidic substrates, and subspecies *vulneraria* L., which is found on basic substrates, as well as their hybrids. The geology of the Piora Valley is specified by abrupt changes in the chemical conditions of the underlying rock [3]. The northern and southern parts are characterized mainly by Gneiss, which is responsible for the acidic conditions. The central part contains a deep dolomitic band, which leads to basic soil conditions. Such changes are often found within quite short distances. In the contact zone between the two substrates, hybrids of the two subspecies were found. The occurrence of intraspecific hybrids in *A. vulneraria* in general, has been mentioned before, e.g., by Jalas [4], Puidet [5], and Rola [6]. However, natural hybrids are, according to experiments by Couderc [7], quite rare. The aim of thiss to describe the natural variability and differences within and between the populations of the two subsp. *vallesiaca* and *vulneraria*, as well as between populations containing hybrids. The investigation is based on morphological, phytosociological, and physiological features among the three distinct populations, with one growing within the dolomitic band, one in the acidic area, and one in the mixed zone.

2. Materials and methods

2.1. Sampling

Three sites of populations of *Anthyllis vulneraria* s. lato were chosen in the Val Piora region (**Figure 1**). Population 1 was situated near Scopello, 46°32′48″ N/8°42′11″ E, at an altitude of 1915 m and within the dolomitic area; population 2 was located near Piano Bello, 46°32′57″ N/8°43′00″ E, at 1960 m within the border of the acidic and basic areas; and population 3 was located near Piano Corona, 46°32′52″ N/8°44′08″ E, at 2185 m within the acidic soil.

At every site, two plots of 1 m² were selected for the evaluation of local plant diversity. The plants in the plots were identified and classified following the phytosociological method of Braun-Blanquet [8]. Within an area of approximately 10 m around each plot, 30 flowering plants of *Anthyllis* were randomly collected.

[1]Flora and Fauna Research Centre, Museum of Natural Sciences, Tirana, Albania.
[2]Department of Biology, Faculty of Mathematics and Natural Sciences, University of Prishtina, Prishtina, Kosovo.
[3]Department of Biology, Natural Sciences Faculty, Tirana University, Tirana, Albania.
[4]Department of Plant- and Microbial Biology, University of Zürich, Zürich, Switzerland.
[5]Department of Systematic and Evolutionary Botany, University of Zürich, Zürich, Switzerland.
*email: reinhard.bachofen@uzh.ch; jakob.schneller@systbot.uzh.ch

In the laboratory, the number of flowers, the color and length of the calyx, and the flag for each collected individual were determined (**Table 1**). Leaves of the same plants were used on site for the measurement of fast chlorophyll fluorescence kinetics as a measure of physiological plant activity under field conditions.

2.2. Floristic investigation

The floristic investigations are based on frequency values after Braun-Blanquet's method [8] and the environmental indication R values are from Landolt et al. [9]. The identified frequency (Freq.) of the species is given following the cover abundance scale of Braun-Blanquet and slightly changed to the following values suggested by McNellie [10]: 0: species missing; 1: rare; 2: up to 10%; 3: 10% to 25%; 4: 25% to 50%; 5: 50% to 75%; and 6: 75% to 100%. The R value implies the pH reaction on a scale from 1 (species associated with very acidic soil) to 9 (species associated with strongly alkaline soil). To evaluate the mean values of R of the different plots, the frequency of the species was multiplied by the indication value R. The mean value of R was evaluated by dividing the sum of the Freq x R value of each plant by the sum of Freq (**Table 2**).

2.3. pH measurement

To determine the pH of the soil samples at the three sites, 20 g of soil, combined from 3 different sites, was strongly mixed with 40 ml of deionized water. The pH was measured after sedimentation of the soil particles after approximately one hour using a MultimeterSG23 (Mettler Toledo, Greifensee, Switzerland).

2.4. Chlorophyll fluorescence

A rapid increase in chlorophyll fluorescence after a strong light pulse is a rapid and noninvasive tool for screening photosynthetic activity in the field. The shape of the kinetics offers information on the state of the electron transport chain and the enzymatic reactions beyond it and may be used as a marker of plant vi- tality. Chlorophyll fluorescence was analyzed using the portable fluorometer Pocket PEA (Hansatech, King's Lynn, UK) [11]. The detached leaves were fixed in commercial leaf clips and kept in the dark for 20 min prior to measurement to bring the electron acceptors of photosystem II into a fully oxidized state [12–15].

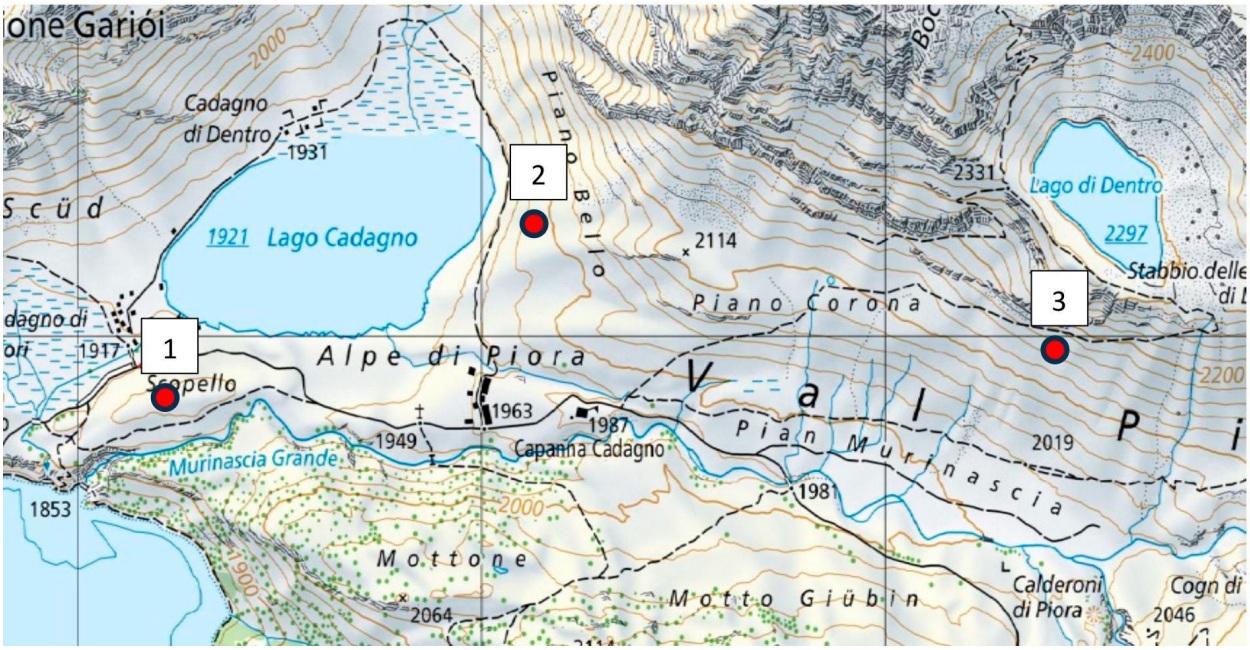

Figure 1 • Map of Val Piora with sampling sites (1, 2, 3) of the three populations, net distance 1 km. (swisstopo, 2022).

Table 1 • Floristic parameters of *A. vulneraria* (mean values and ± sd of 30 individuals).

	Population 1	**Population 2**	**Population 3**
Flower and keel color	Yellow/Yellow	Yellow/Yellow; Yellow/Red; White/Red	White/Red
Presence of red keel	-	+	+
Flower length (mm)	16.97 ± 1.13 [a]	16.67 ± 1.55 [a]	17.63 ± 1.35 [b]
Calyx length (mm)	10.45 ± 1.17 [a]	10.75 ± 1.31 [a]	10.75 ± 1.05 [a]
Flag length (mm)	6.52 ± 0.97 [a]	6.12 ± 0.99 [a]	6.83 ± 1.20 [b]
No. of flowers per inflorescence	19.07 ± 6.57 [a]	18.23 ± 6.71 [a]	18.70 ± 6.25 [a]

Letters [a] and [b] below the mean values and sd indicate significant differences ($p < 0.05$).

Table 2 • Floristic composition at the sampled sites and soil pH.

		Population 1				Population 2				Population 3			
		Plot 1		Plot 2		Plot 1		Plot 2		Plot 1		Plot 2	
Plant species	**R**	**Freq.**	**Freq. × R**	**Freq.**	**Freq. × R**	**Freq.**	**Freq. × R**	**Freq.**	**Freq. × R**	**Freq.**	**Freq. × R**	**Freq.**	**Freq. × R**
Anthyllis vulneraria	3	4	12	4	12	3	9	3	9	3	9	1	3
Achillea millefolium	3	3	9	-	-	-	-	-	-	1	3	-	-
Agrostis rupestris	2	-	-	-	-	-	-	-	-	-	-	3	6
Anthoxanthum odoratum	3	-	-	1	3	-	-	-	-	2	6	1	3
Astragalus penduliflorus	3	-	-	-	-	-	-	-	-	-	-	2	6
Bartsia alpina	3	-	-	-	-	-	-	-	-	1	3	-	-
Campanula barbata	2	-	-	-	-	-	-	-	-	1	2	-	-
Campanula scheuchzeri	3	2	6	1	3	3	9	3	9	2	6	-	-
Carex ferruginea	4	-	-	-	-	3	12	3	12	2	8	1	4
Carlina acaulis	3	-	-	-	-	1	3			2	6	1	3
Centaurea nervosa	2	-	-	-	-	-	-	-	-	3	6	1	2
Chaerophyllum hirsutum	3	-	-	-	-	3	9	3	9	1	3	1	3
Coeloglossum viride	2	-	-	-	-	1	2			1	2		
Daphne striata	4	-	-	-	-	-	-	-	-	1	4	-	-
Euphrasia rostkoviana	3	1	3	1	3	-	-	-	-	1	3	-	-
Festuca rubra (R-varia agg.)	2	1	2	2	4	3	6	-	-	2	4	4	8
Galium pumilum (anisophyllum)	3			3	9			-	-	1	3	1	3
Gentiana punctata	2	-	-	-	-	-	-	-	-	1	2	3	6
Geranium sylvaticum	3	-	-	-	-	-	-	2	6	2	6		
Geum montanum	2	-	-	-	-	1	2	-	-	1	2	1	2
Gymnadenia conopsea	4	-	-	-	-	-	-	-	-	1	4	1	4
Helianthemum alpestris	5	-	-	-	-	-	-	-	-	1	5	2	10
Hieracium pilosella	3	-	-	-	-	-	-	-	-	1	3	-	-
Homogynea alpina	3	-	-	-	-	1	3	-	-	-	-	-	-
Laserpitium halleri	2	-	-	-	-	-	-	-	-	2	4	-	-
Laserpitium latifolium	4	-	-	-	-	-	-	-	-	-	-	1	4
Leontodon hirsutum (R-helveticus)	2	-	-	-	-	3	6	4	8	3	6	-	-
Leontodon hispidus	3	3	9	4	12	-	-	-	-	-	-	-	-
Leucanthemum adustum	4	-	-	-	-	-	-	-	-	1	4	-	-
Lotus alpinus	3	2	6	-	-	-	-	-	-	2	6	-	-
Lotus corniculatus	3	-	-	4	12	-	-	-	-	-	-	-	-

Table 2 • *Cont.*

		Population 1				Population 2				Population 3			
		Plot 1		Plot 2		Plot 1		Plot 2		Plot 1		Plot 2	
Plant species	**R**	**Freq.**	**Freq. × R**	**Freq.**	**Freq. × R**	**Freq.**	**Freq. × R**	**Freq.**	**Freq. × R**	**Freq.**	**Freq. × R**	**Freq.**	**Freq. × R**
Luzula sylvatica	2	-	-	1	2	-	-	-	-	-	-	-	-
Nardus stricta	2	-	-	-	-	2	4	2	4	2	4	-	-
Paradisea liliastrum	3	-	-	-	-	-	-	-	-	-	-	1	3
Parnassia palustris	4	-	-	-	-	-	-	1	4	-	-	-	-
Phleum alpinum	3	1	3	-	-	-	-	-	-	1	3	-	-
Phyteuma scheuchzeri	3			-	-	-	-	-	-	1	3	-	-
Poa alpina	3	-	-	-	-	-	-	2	6			-	-
Polygala alpestris	4	-	-	-	-	1	4	-	-			-	-
Polygonum viviparum	3	-	-	-	-			-	-	1	3	1	3
Potentilla alpina (R-grandiflora)	2	-	-	-	-	2	4	-	-	1		1	2
Potentilla erecta	1	-	-	-	-	1	1	-	-	1	1	-	-
Prunella vulgaris	3	-	-	-	-	1	3	-	-	-	-	-	-
Pulsatilla apiifolia	2	-	-	-	-	-	-	-	-	-	-	3	6
Rhinanthus glacialis	4	1	4	-	-	2	8	4	16	2	8	1	4
Rumex scutatus	3	-	-	1	3	1	3	-	-	-	-	-	-
Scabiosa lucida	4	-	-	-	-	-	-	1	4	-	-	-	-
Selaginella selaginoides	3	-	-	-	-	1	3	-	-	-	-	-	-
Silene cucubalus (R-vulgaris)	3	-	-	1	3	1	3	-	-	-	-	-	-
Silene nutans	3	1	3	1	3	-	-	-	-	-	-	-	-
Soldanella alpina	3	-	-	-	-	1	3	-	-	-	-	-	-
Thesium alpinum	3	-	-	1	3	-	-	-	-	1	3	-	-
Thymus serpyllum	4	-	-	-	-	-	-	1	4	-	-	-	-
Thymus vulgaris (R-polytrichus)	4	-	-	-	-	1	4			-	-	-	-
Trifolium pratense (R-nivale)	3	3	9	2	6	1	3	2	6	1	3	1	3
Tussilago farfara	4	1	4	2	8	-	-	-	-	-	-	-	-
Vaccinium uliginosum	1	-	-	-	-	2	2	-	-	-	-	-	-
Σ		23	70	29	86	39	106	31	97	50	138	32	88
Mean of R value			3.04		2.97		2.71		3.13		2.76		2.75
Sum of plants		12		15		23		13		34		21	
Soil pH		6.42				6.09				5.55			

The excitation intensity was 3500 μmol photons m^{-2} s^{-1} with red light at 650 nm for 3 s. From the fluorescence induction signal with high temporal resolution from 10 μs to 3 s, the instrument determined the initial (F_o) and maximum (F_m) fluorescence and the variable fluorescence (F_v) at specified time intervals and calculated specific parameters such as the potential quantum yield of PS II (F_v/F_m) and the performance index (PI). Further calculations and visualization of the experimental data were performed after the data was transferred to Excel. Fast chlorophyll fluorescence has been widely used to describe the effects of environmental stress on plants [16–18]. For statistical analyses, the software MaxStat (3.6) was used. All data points presented are based on the measurements of 30 leaves from each plot.

2.5. Plant morphometry and statistical analyses

In the laboratory, the following morphometric parameters were determined: the color of the flowers, the size of the calyx, the length of the flag, and the number of flowers per inflorescence (**Table 1**). Descriptive statistical analyses and graphical creation were carried out using Microsoft Excel 2013. To evaluate variations in morphological and chlorophyll fluorescence parameters across different localities, a *t* test in Excel 2013 and an analysis of variance (ANOVA) with Tukey's post hoc test were used. An ANOVA was performed using PAST software, version 4.13, for Windows.

2.6. Discriminant analysis

Discriminant analyses were performed using PAST software, version 4.13 [19], to assess whether photosynthetic parameters could distinguish between the two subspecies (*vulneraria* and *vallesiaca*) and their hybrid. Photosynthetic parameters, including Fv/Fm, PI, and fluorescence induction values measured at points O, J, I, and P, were used as independent variables, while the subspecies (*vulneraria* and *vallesiaca*) and their hybrid served as the grouping variable.

3. Results

3.1. Characteristics of flowers of *A. vulneraria*

The two subsp. *vulneraria* and *vallesiaca* differed only concerning the characteristics of the flower, as illustrated in **Figure 2** and **Table 1,** while the hybrid shows a combination of the two subspecies.

3.2. Morphometric measurements

Table 1 summarizes the results of the measurements of the selected characteristics. The mean values for each population are presented, derived from 60 samples per population, except for the flowers of the inflorescences, where 20 per population were measured. The 60 samples from population 2 exhibited three different phenotypes, namely yellow, 16; white, 21; and hybrid, 38. The statistical analyses did not reveal significant differences between population 1 and population 2 for any of the tested morphological parameters. Neither population (1 or 2) exhibited significant differences within population 3 regarding calyx length or the number of flowers per inflorescence. However, there were significant differences in flower length and flag length within population 3 (**Table 1**).

3.3. Biodiversity, presence, and frequency of accompanying plants at three sampling sites

The floristic quantification was based on the methods of Braun-Blanquet [8] and Landolt et al. [9]. In the three plots selected for the different *Anthyllis* populations, 57 plant species were recorded (**Table 2**). The number of species varied among the populations and even among the plots. The richest population with plant species was population 3, with 34 recorded species in plot 1 and 21 in plot 2, followed by population 2, with 23 species in plots 1 and 13 in plot 2. The population with the fewest species was population 1, with 12 species in plot 1 and 15 species in plot 2 (**Table 2**).

3.4. Soil pH and indicator values of three populations

Additionally, **Table 2** shows the frequencies of each species per plot, as well as the reaction value number R and soil pH. The soil pH differed among the populations: it was 6.42 for the first population, 6.09 for the second population, and 5.55 for the third population. The pH values clearly differed within the three plots and followed the changes in the reaction number R between the plots.

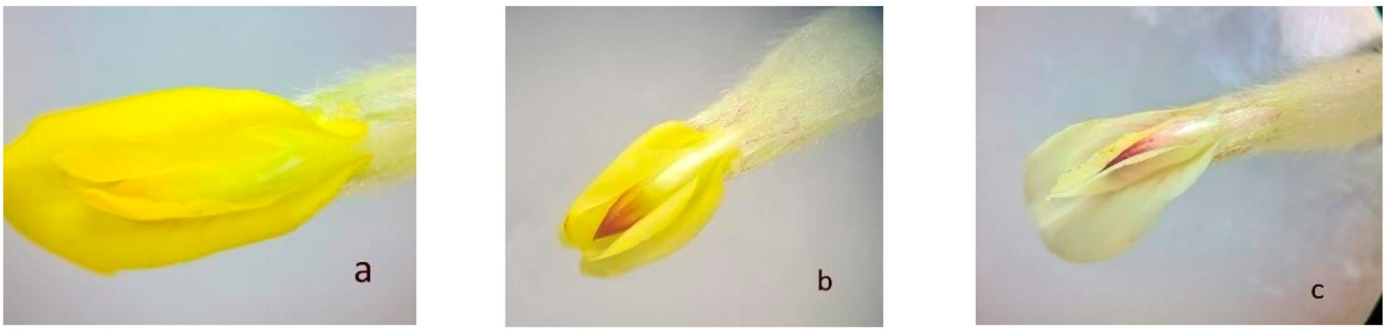

Figure 2 • Description of the three phenotypes of *A. vulneraria*. (**a**) Population 1: *A. vulneraria* subsp. vulneraria (keel yellow), (**b**) Population 2: Hybrid yellow (keel red) and (**c**) Population 3: *A. vulneraria* subsp. *vallesiaca* (keel red).

3.5. Fluorescence measurements

Fast chlorophyll fluorescence, the so-called OJIP test [14], was selected as a measure of plant physiological activity. All the *Anthyllis vulneraria* samples from the three sites showed the typical multistep induction kinetics of fast fluorescence increase observed in the higher plants with two distinct steps J and I between start O and maximum P (**Figure 3**). The maximum Fm is reached after approximately 0.3 s, and the J and I steps are present at 0.003 and 0.03 s, respectively. Step O > J indicates the reduction in acceptor QA, step J > I the reduction in QB, and step I > P the reduction in the electron carriers between photosystem 2 and photosystem 1 and beyond.

To eliminate differences in fluorescence intensity between the leaves due to variable chlorophyll content and other factors determining the absolute fluorescence intensity, the data were normalized by setting $F_o = 0$ and $F_{max} = 1$. The increase in the fluorescence of leaves from 30 different yellow *A. vulneraria* plants sampled from an area of approximately 100 m2 resulted in a broad band of single traces (**Figure 3**). Although the plants had grown within short distances, the environmental micro-conditions differed considerably. Even broader distributions were found for populations 2 and 3. The greatest differences between the single traces are observed for F_J after 0.003 s.

For a statistical evaluation, several typical parameters were examined, and they are presented in **Table 3**. The quantum efficiencies of PSII (Fv/Fm), which has been widely used in plant physiology research in recent decades, were close to the maximum observed for healthy plants for all three populations. A significant difference ($p < 0.05$) between populations 1, 2a, 2b, and 3 was observed, while population 2c did not differ from populations 1 to 2b or from population 3. A similar distribution was found for the more variable PIs, which cover more parameters beyond the primary processes in photosystem II. Fj, the relative position of shoulder J at 3 ms, fits well with the parameters above; in this case, population 2c is combined with population 3 and is separated from populations 1 to 2b.

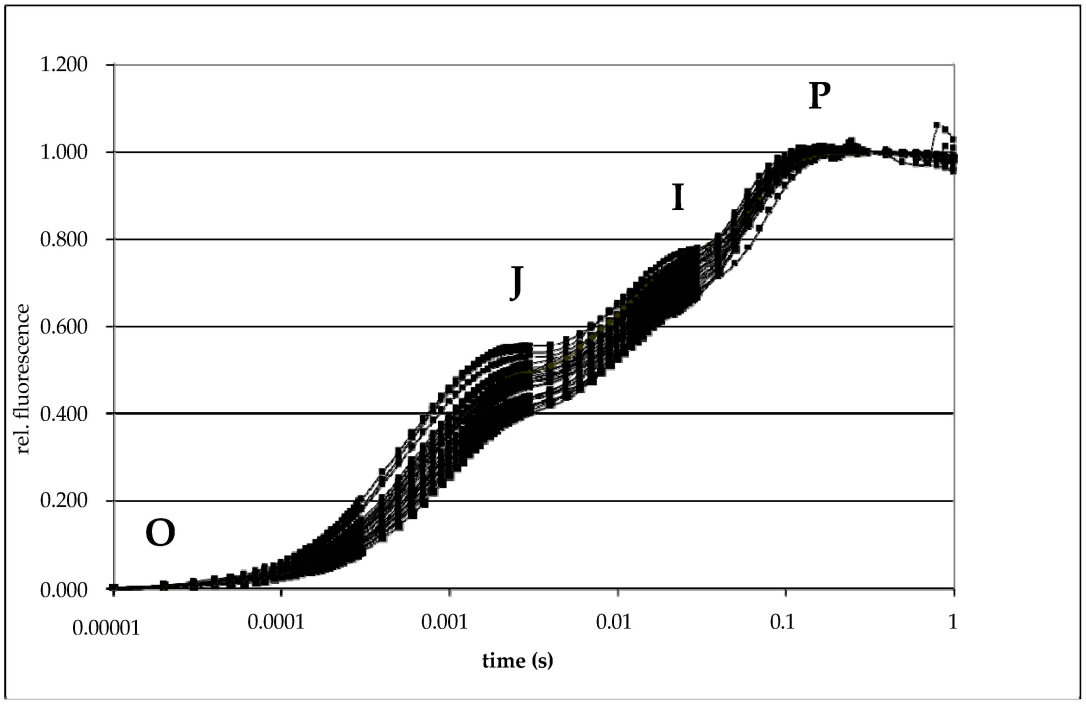

Figure 3 • Fluorescence induction scans from 10 µs to 3 s of 30 different yellow *A. vulneraria*. individual leaves from population 1 after normalization ($F_o = 0$, F_{max} at 300 ms = 1). The J and I steps are at 0.003 and 0.03 s, respectively. Step O > J indicates the reduction in acceptor QA, step J > I the reduction in QB, and step I > P the reduction in the electron carriers between photosystem 2 and photosystem 1 and beyond.

Table 3 • Maximum quantum efficiency of photosystem II (Fv/Fm), Fj, and the photosynthetic performance index (PI) among different populations of *A. vulneraria*.

Plot	1	2a	2b	2c	3
Flowers	yellow	yellow	yellow/red	white/red	white
Fv/Fm	0.82 ± 0.02 [a]	0.83 ± 0.02 [a]	0.83 ± 0.03 [a]	0.81 ± 0.04 [ab]	0.80 ± 0.04 [b]
Fj	0.45 ± 0.05 [a]	0.47 ± 0.03 [a]	0.47 ± 0.05 [a]	0.54 ± 0.12 [b]	0.59 ± 0.08 [b]
PI	4.44 ±1.71 [a]	3.84 ± 0.93 [a]	4.40 ± 1.54 [a]	3.10 ± 1.31 [ab]	2.10 ± 1.26 [b]

Letters [a] and [b] between different groups indicate significant differences among the populations, according to Tukey's test.

The mean values for each population are derived from the average of 30 samples. According to Tukey's test, different letters (a and b) between different groups indicate significant differences among the populations presented in **Figure 4**. The mean values of the fluorescence induction scans from 10 ms to 3 s of populations 1, 2, and 3 after normalization (F_o = 0, F_{max} at 300 ms = 1) (average of 30 samples) show that population 3 clearly separated from populations 1 and 2, as expected from the data in **Table 3**.

3.6. Discriminant analyses

Discriminant analysis (DA) was used to evaluate the differences between the two subspecies (*vulneraria* and *vallesiaca*), as well as their hybrids. The populations were used as grouping variables to classify or discriminate based on the analyzed photosynthetic parameters (Fv/Fm, PI, and fluorescence induction values measured at points O, J, I, and P) as independent variables. The first discriminant function (F1) explained 83% of the total variance, while F2 explained 17% of the total variance, as illustrated in **Figure 5**. The DA scatter plot supports the results obtained from the ANOVA. The analyzed individuals of subsp. *vulneraria* were separated from the individuals of subsp. *vallesiaca*. However, a few individuals of subsp. *vallesiaca* showed similarities and were grouped with samples of subsp. *vulneraria*. The hybrid individuals were distributed among individuals of both subspecies, with more similarities to subsp. *vulneraria*. The scatter plot indicated high variability among individuals within all three populations.

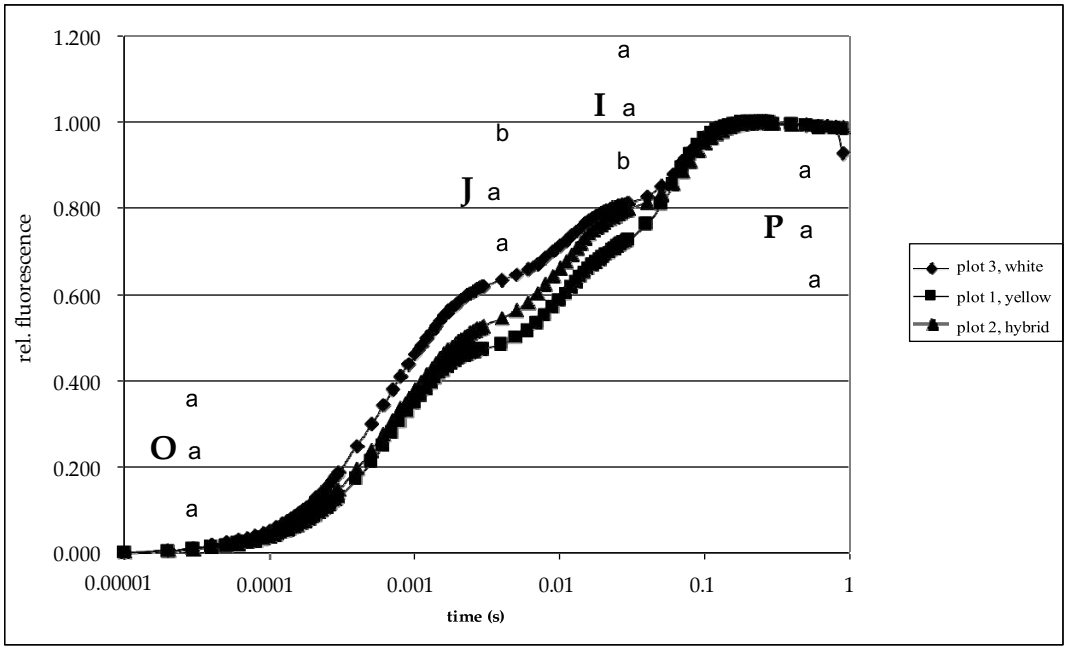

Figure 4 • Mean values (30 measurements per population) of fluorescence induction scans from 10 µs to 3 s after normalization (F_o = 0, F_{max} at 300 ms = 1). Letters indicate significant differences ($p < 0.05$).

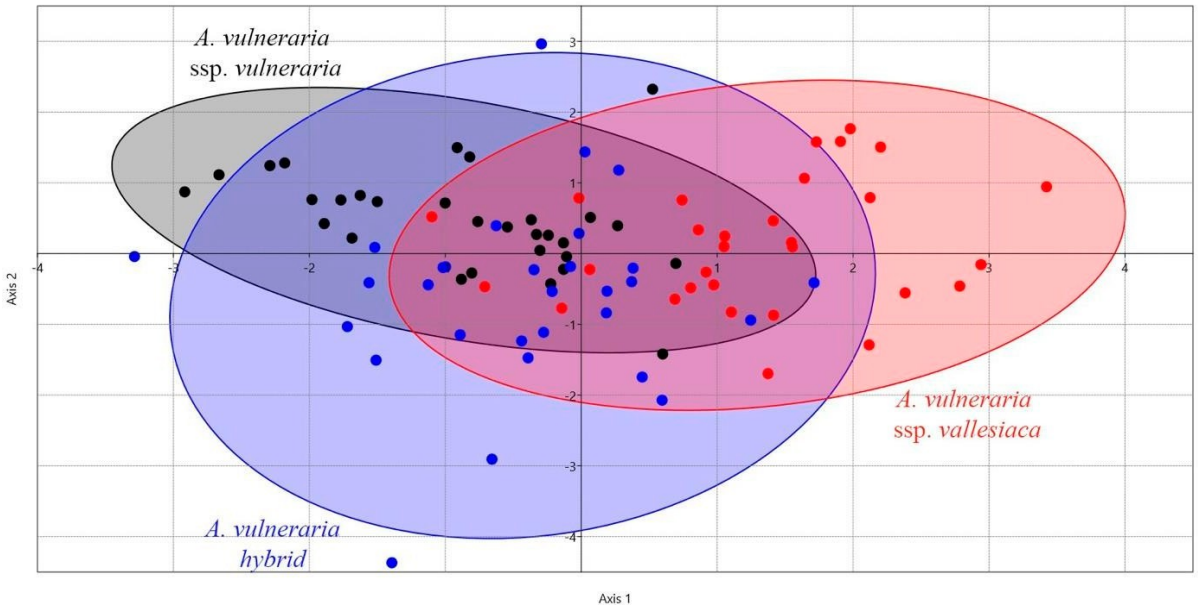

Figure 5 • Scatter plot of discriminate analyses. Axis 1: morphometric measurements, axis 2: relative fluorescence.

4. Discussion

In reviewing numerous publications on *Anthyllis vulneraria* s.l., we have noticed that only a limited number of them address the occurrence of intraspecific hybrids (e.g., [6]). Our study revealed that hybrids between subspecies *vulneraria* and subspecies *vallesiaca* occur under special conditions, such as those found in the Val Piora. Here, we observe abrupt changes in the geological conditions and, consequently, in the pH of the soil. These changes enable the two subspecies to meet and hybridize within quite small patches at the border of the geologically different substrata. The analysis of floristic characteristics and pH measurements show that the different subspecies are adapted to different pH values [2, 20]. *Anthyllis vulneraria* subsp. *vulneraria* is found on soil with a relatively high pH (influenced by dolomitic or limestone substrates), while *A.v. vallesiaca* grows at a relatively low pH influenced by an acidic, hardly buffered (gneissic, granitic) substate. In Val Piora, the distribution area of the two subspecies overlaps, and introgression occurs.

Morphologically, the flower color and the presence or absence of a red stripe on the hypanthium differ between the two subspecies. Hybrids are characterized by the presence of a red stripe on the hypanthium and yellow flowers. The number of flowers and the size of the calyx did not differ between the two subspecies (plots 1 and 3) and the mixed population (plot 2). In contrast, the flag size and flower length of the plants in plot 3 were significantly different from those of the plants in plots 1 and 2.

A similar separation as for the morphological data was also found for the photosynthetic parameters; populations 1, 2a, and 2b were clearly different from population 3. In contrast, F_j in population 2c does not differ from that in population 3. For the other parameters, population 2c did not differ from populations 1 to 2b or from population 3.

Fast chlorophyll fluorescence indicates the potential for distinguishing between individuals of *Anthyllis* subspecies and assessing the vitality of individual plants within a population. It has been widely used to evaluate the vitality of single plants within a population. Local environmental differences result in variable kinetics of the fluorescence signal, providing evidence for local environmental variations within short distances [17, 21]. In Norway spruce, various ecotypes have been distinguished [22], and tree diversity has been described in boreal forests [16].

Acknowledgments

We thank Reto Strasser for the use of the PEA fluorometer and for many fruitful discussions and the Alpine Biological Center Piora (http://www.cadagno.ch [cited 2020 Jul 10]) for their kind hospitality. Furthermore, we thank Verena van Fleet for thoroughly editing the text.

Funding

This research was sponsored by Sylvia and Reinhard Bachofen-Stiftung (https://www.srbachofenstiftung.ch/, accessed on 2025 Jul 2) and the Department of Plant and Microbial Biology of the University of Zürich.

Author contributions

Conceptualization, R.B. and J.S.; methodology, E.G., D.L., A.H., X.S., R.B. and J.S.; validation, E.G., D.L., A.H., X.S., R.B. and J.S.; formal analysis, E.G., D.L., A.H., X.S., R.B. and J.S.; investigation, E.G., D.L., A.H., X.S., R.B. and J.S.; data curation, E.G., D.L., A.H., X.S., R.B. and J.S.; writing—original draft preparation, J.S., R.B., A.H. and E.G.; writing—review and editing, J.S., R.B., A.H., and E.G.; visualization, J.S., R.B., A.H. and E.G.; funding acquisition, R.B. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

Data availability statement

The data supporting the findings of this publication can be made available upon request.

Institutional review board statement

No endangered plants were involved; furthermore, the Center for Alpine Biology, Piora, has permission for environmental and ecological experiments in the Piora valley. *Anthyllis* species were identified by Jakob Schneller, Ermelinda Gjeta, and Avni Hajdari. The specimen of the two subspecies and hybrids were deposited at the herbarium at the Department of Systematic and Evolutionary Botany of the University of Zürich.

Additional information

Received: 2025-03-24

Accepted: 2025-07-11

Published: 2025-08-13

Academia Biology papers should be cited as *Academia Biology 2025*, ISSN 2837-4010, https://doi.org/10.20935/AcadBiol7815. The journal's official abbreviation is *Acad. Biol.*

Publisher's note

Academia.edu Journals stays neutral with regard to jurisdictional claims in published maps and institutional affiliations. All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright

© 2025 copyright by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

References

1. "*Anthyllis* L." 2020 plants of the world online. board of trustees of the royal botanic gardens, kew. 2017 [cited 2020 Jul 10]. Available from: https://powo.science.kew.org

2. *Anthyllis vulneraria*. [cited 2020 Jul 10]. Available from: https://www.infoflora.ch

3. Peduzzi R, Wildi W. Piora–Lago di Cadagno–Ritom: a guide to nature and environment. Bellinzona, Switzerland: Centro Biologia Alpina; 2016.

4. Jalas J. Rassentaxonomische probleme im Bereich des *Anthyllis vulneraria* L.: komplexes in Belgien. Bull Jard Bot État Brux. 1957;27:405–16. doi: 10.2307/3667073

5. Puidet E, Liira J, Paal J, Pärtel M, Pihu S. Morphological variation in eight taxa of *Anthyllis vulneraria* s. lato (Fabaceae). Ann Bot Fennici. 2005;42:293–304.

6. Rola K. A morphometric study on *Anthyllis vulner- aria* (Fabaceae) from Poland and its taxonomic impli- cations. Kaja Rola Biologia. 2012;67(2):296–309. doi: 10.2478/s11756-012-0013-1

7. Couderc H. Etude expérimentale de la reproduction de *Anthyllis vulneraria* L. Bull Soc Bot Fr. 2014;118:359–73. doi: 10.1080/00378941.1971.10838912

8. Braun-Blanquet J. Plant sociology. Fuller GD, Conrad HS, translators. New York (NY): McGraw-Hill; 1932.

9. Landolt E, Bäumler A, Hegg O, Klötzli F, Lämmler W, Nobis M, et al. Flora indicativa. Bern: Haupt Verlag Bern; 2010.

10. McNellie MJ, Dorrough J, Oliver I. Species abundance distributions should underpin ordinal cover-abundance transformations. Appl Veg Sci. 2019;22:361–72. doi: 10.1111/avsc.12437

11. Hansatech operations manual. 2006 [cited 2020 Jul 10]. Available from: https://www.hansatech-instruments.com/continuous-excitation-chlorophyll-fluorescence/

12. Kalaji HM, Jajoo A, Oukarroum A, Zivcak M, Samborska IA, Cetner MD, et al. Chlorophyll a fluorescence as a tool to monitor physiological status of plants under stress conditions. Acta Physiol Plant. 2016;38:102. doi: 10.1007/s11738-016-2113-y

13. Klisz M, Chakraborty D, Cvjetkovic B, Grabner M, Lintunen A, Mayer K, et al. Chapter 12: Functional traits of boreal species and adaptation to local conditions. In Boreal forests in the face of climate change. Berlin/Heidelberg: Springer; 2023. p. 323–55.

14. Kalaji HM, Schansker G, Brestic M, Bussotti F, Calatayud A, Ferroni L, et al. Frequently asked questions about in vivo chlorophyll fluorescence, the sequel. Photosynth Res. 2017;132:13–66. doi: 10.1007/s11120-016-0318-y

15. Strasser BJ, Strasser RJ. Measuring fast fluorescence transients to address environmental questions: the JIP-test. In: Mathis P, editor. Photosynthesis: from light to bio- sphere. Proceedings of the 10th International Photosynthe- sis Congress; 1995 Aug 22; Montpellier, France. Dordrecht: Kluwer Academic Publishers; 1995. p. 977–80.

16. Strasser RJ, Srivastava A, Tsimili-Michael M. Analysis of the chlorophyll a fluorescence transient. In: Papageorgiu G, Govindjee C, editors. Chlorophyll a fluorescence. A signature of photosynthesis. Advances in photosynthesis and respiration. Vol. 19. Dordrecht: Springer; 2004. p. 321–62.

17. Pollastrini M, Nogales AG, Benavides R, Bonal D, Finer L, Fotelli M, et al. Tree diversity affects chlorophyll a fluorescence and other leaf traits of tree species in a boreal forest. Tree Physiol. 2017;37:199–208. doi: 10.1093/treephys/tpw132

18. Rexha K, Bego K, Zyruku D, Shuka L, Husi R, Schneller J, et al. Alpine ecology, plant biodiversity and photosynthetic performance of marker plants in a nitrogen gradi- ent induced by *Alnus* bushes. BMC Ecol. 2020;20:23. doi: 10.1186/s12898-020-00292-9

19. Zivcak M, Brestic M, Olsovska K, Slamka P. Performance index as a sensitive indicator of water stress in Triticum aestivum. Plant Soil Environ. 2008;54:133. doi: 10.17221/392-PSE

20. Hammer Ø, Harper DAT, Ryan PD. PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron. 2001;4(1):9.

21. Sadriu Hoti A, Hasaj E, Ibrahimi L, Heqimi S, Schneller J, Bachofen R. Drought stress and the photosynthetic activity of peat moss measured by fast fluorescence in alpine peatlands. Trends Photobiol Photochem. 2023;21:41–54. doi: 10.31300/TPCPB.21.2022.41-54

22. Tomaskova I, Pastierovic F, Krejzkova A, Cepl J, Hradecky J. Norway spruce ecotypes distinguished by chlorophyll a fluorescence kinetics. Acta Physiol Plantarum. 2021;43:24. doi: 10.1007/s11738-020-03190-1