Brief Communication

Assessing floral trait variation in *Platanthera dilatata* (*Orchidaceae*) across an elevational gradient

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Abstract

Flower morphology often changes over altitude, although the patterns themselves can be variable, with flowers being either smaller or larger. Floral trait variation is often considered in the context of pollinator-mediated selection. However, other explanations, including underlying genetics and plasticity, resource availability and floral enemies have been proposed. Here, we assess 10 floral traits in *Platanthera dilatata* var. *dilatata* across an elevational gradient on Vancouver Island, British Columbia, Canada, to determine if floral traits vary with altitude. We find that floral traits are larger at the lowest elevation site. However, much of the floral trait variation appears to be driven by temperature, which is not necessarily correlated with the altitudinal gradient. Given the intrinsic link between climate and resource availability, we suggest that resource availability confers a local selection pressure on floral trait size that may be balanced at larger spatial scales by antagonistic pressure from shared pollinators. Direct investigations of the environmental and genetic factors driving floral trait variation are recommended.

1 Introduction

Floral diversity is often thought to be driven by pollinators [1], leading to many theories and studies in relation to the most effective pollinator principle [2] and its impacts on floral evolution. Numerous examples exist of pollinator-mediated speciation occurring among sympatric populations as a result of different pollinators selecting for diverging floral traits (e.g., [3, 4]) including floral size (e.g., [5–7]). However, several other hypotheses have been put forward to help explain interspecific floral size variation. For example, the 'escape hypothesis' suggests that many floral parts serve a protective function from herbivores, pollen and nectar thieves, and fungal infection in addition to their role as pollinator attractants [8]. The 'resource cost hypothesis' acknowledges heterogeneous resource availability and posits that flower size will increase to attract pollinators only if resources are sufficient [8]. The role of genetics and plasticity on development have also been suggested, as increased cell division and cell elongation have been observed contributing to floral size changes in some species [9]. One plant family that seems particularly amenable to floral variation studies is the Orchidaceae [1, 10, 11].

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Discover

Platanthera dilatata (Pursh) Lindl. *ex* Beck (Orchidaceae; white bog orchid) is native to North America [12]. The plants range in height (11–130 cm), have many white flowers in a single inflorescence, and the number of leaves at the base varies (typically 2–5) [13]. Three variants of *P. dilatata* are formally recognized and differentiated based on floral spur length—*P. dilatata* var. *leucostachys* (Lindl.) Luer has a spur longer than the labellum, *P. dilatata* var. *albiflora* (Cham.) Ledeberg. has a spur shorter than the labellum, and *P. dilatata* var. *dilatata* (Pursh) Lindl. *ex* Beck spurs are similar in length to the labellum [12] (Table 1). It has been suggested that variation in the length of the nectar-producing floral spurs drives, and responds to, pollinator efficacy as the length of the pollinator's proboscis/tongue must be compatible with the length of the spur [13]. Thus, variation in floral traits, such as spur length, could prevent gene flow among populations of *P. dilatata* by excluding certain pollinators, ultimately leading to reproductive isolation. Considerable variation has been observed among populations of *P. dilatata*, especially in spur and labellum lengths (see descriptions from [12, 14–17]) (Table 1).

The floral morphology of *P. dilatata* sensu lato has been assessed in previous studies in the continental USA [14], but not in Canada, which represents a substantial proportion of its range. Western Canada, in particular, is believed to exhibit great variation in floral form [17]. Assessments of population-level variation of the spur, and comparisons among other floral features of *P. dilatata* var. *dilatata* are sparse [15]. In addition, information on pollinators and important abiotic factors throughout the range of *P. dilatata* is limited. However, recent observations of *P. dilatata* show that a number of insect families are likely pollinators [18], that different habitats (e.g., bog versus fen) often result in different mycorrhizal associations [15], and that populations exhibiting morphological variation are often genetically differentiated but also contain hybrids [15]. These observations suggest a number of antagonistic processes may be at work on *P. dilatata*. For example, pollinators may provide a directional selection pressure while abiotic factors may confer balancing or disruptive selection pressure.

After observing considerable variation in *P. dilatata* plant height between low and high elevation sites (pers. obs.), we used floral morphometrics to investigate variation among populations of *P. dilatata* var. *dilatata*. Floral morphometrics has been successfully used to identify new plant species [19], and to better understand the evolutionary relationships among sympatric and closely related European *Platanthera* species [20]. We were particularly interested in understanding how variable floral traits are along the aforementioned elevational gradient, and if floral trait variation is a response to biotic or abiotic factors. This work provides an important stepping stone in teasing apart the factors contributing to the evolutionary mosaic of *P. dilatata* populations.

2 Methods

All plants were identified as *Platanthera dilatata* var. *dilatata* based on the spur being roughly equal in length to the labellum [17]. From here on we refer simply to *P. dilatata*. Table 2 provides full population details including the habitat type. Three flowers were sampled from five plants per population (N = 75) across an elevational gradient (> 1,000 m). Samples collected within the Strathcona Provincial Park boundary were collected in 2020 under a Letter of Authorization (98700-20/Strathcona). Fully expanded flowers were collected from the lower, middle, and upper portion of the inflorescence to capture any developmental variation [13]. Within a population, individual plants were at least 1 m apart to avoid sampling clones. Each flower was preserved in 70% ethanol until measurements could be made.

Table 1 Literature-derived labellum and spur lengths for variants of *Platanthera dilatata*

Species	Spur length (mm)	Labellum length (mm)	Literature source
P. dilatata var. *albiflora*	Up to 10.0	Longer than spur	Leur [14]
	2.0–7.0	6.0–10.0	Sheviak [17]
	2.5–3.7	3.9–5.0	Wallace [16]
P. dilatata var. *dilatata*	5.0–10.0	5.0–10.0	Leur [14]
	4.0–12.0	5.0–10.0	Sheviak [17]
	3.5–5.8	3.3–6.1	Wallace [16]
P. dilatata—no variant specified	6.5–12.0	5.25–9.5	Wallace and Bowles [15]
	2.0–12.9	3.3–8.17	Adhikari and Wallace [12]
P. dilatata var. *leucostachys*	10.0–20.0	5.0–13.3	Leur [14]
	8.0–20.0	4.0–11.0	Sheviak [17]
	7.0–10.6	4.8–7.1	Wallace [16]

Table 2 Site information for *Platanthera dilatata* samples used in this study.

Population	Altitude (m)	Latitude	Longitude	Habitat	Total plants	Total flowers
Ranger cabin (Rang)	1,229	49.70504	− 125.33553	Alpine meadow	5	15
Paradise meadows (Para)	1,071	49.73801	− 125.31448	Alpine meadow	5	15
Strathcona 800 (S800)	800	49.74280	− 125.21096	Roadside seepage	5	15
Strathcona 400 (S400)	400	49.74271	− 125.24601	Roadside seepage	5	15
Quadra Island (Quad)	88	50.17060	− 125.22848	Estuary	5	15
				Total	25	75

Three flowers were sampled from five plants per population ($N = 75$)

Flowers were dissected under 16X magnification (Wild M3B, Heerburgg, Switzerland), and 10 floral traits were measured with digital calipers (Neoteck 150 mm Digital Vernier Caliper, China; resolution 0.01 mm, accuracy to 0.03 mm) to the nearest 0.01 mm. Measured traits are listed in Table 3. These 10 traits were selected for two reasons: (1) preliminary assessments in which a subsample of flowers were measured twice showed that measurement replicates deviated from one another by < 5%; and (2) similar floral traits are commonly used in orchid morphometrics (e.g., [21, 22]). We did not include measurements of the gymnostemium, even though the distance between the anthers is often considered an important mechanism for reproductive isolation [23], because the percent deviation for these measurements was > 5% in preliminary assessments.

Climate variables for each population were obtained from ClimateBC v7.42 [24]. This application downscales gridded monthly climate data from PRISM [25] in order to provide scale-free seasonal and annual climate variables. Climate variables included measurements related to temperature, precipitation, radiation, degree days, evaporation, humidity, and moisture index. Supplementary Table 1 contains a full list of the seasonal and annual climate variables.

2.1 Data analysis

Analyses were performed in R 4.2.2 (R Core Team, 2021) and RStudio 2022.12.0.353 (Posit Team, 2022). Summary statistics (e.g., mean, standard deviation, standard error) were calculated for each floral trait. A mean value for the three flowers per plant was calculated to avoid non-independence in some analyses. The normality of morphometric measurements for each floral trait were assessed using a Shapiro–Wilk test [26]. The rstatix package [27] was used to perform one-way ANOVAs for parametric variables and Kruskal–Wallis [28] tests for nonparametric variables, with *post-hoc* Tukey [29] and Dunn [30] tests with Bonferroni correction [31], respectively. ANOVA results were visualized using ggplot2 [32]. Additionally, we performed a linear mixed model using all flower replicates per plant in the lme4 package [33]. Here, population was the fixed effect and plant was the random effect for each floral trait (response variable).

To visualize relationships among the morphological data, and reduce dimensionality, a principal component analysis (PCA) was performed using the packages vegan [34] and ellipse [35] in R. The number of principal components to be retained was assessed by screeplot and the influence of each trait on the PCA was determined by the PCA loadings. A

Table 3 Descriptions of floral traits measured, the range of values, and the mean measurement per trait (mm)

Trait	Description	Range (mm)	Mean (± SE)
Dorsal sepal length (dor.s.l)	Longest part of the dorsal sepal from perianth attachment to distal end	3.76–7.46	5.40 (±0.10)
Dorsal sepal width (dor.s.w)	Widest part of the dorsal sepal	2.24–3.98	3.08 (±0.04)
Lateral sepal length (lat.s.l)	Longest part of a lateral sepal from perianth attachment to distal end	4.29–8.18	6.55 (±0.11)
Lateral sepal width (lat.s.w)	Widest part of a lateral sepal	1.75–3.45	2.59 (±0.04)
Lateral petal length (lat.p.l)	Longest part of a lateral petal from perianth attachment to distal end	3.63–7.58	5.66 (±0.10)
Lateral petal width (lat.p.w)	Widest part of a lateral petal	1.74–4.08	2.64 (±0.06)
Labellum length (lab.l)	Longest part of the labellum from perianth attachment to distal end	2.37–9.97	7.10 (±0.18)
Labellum width (lab.w)	Widest part of the dilated portion of the labellum	1.95–6.82	2.98 (±0.10)
Spur length (spu.l)	Total length of the spur from perianth attachment to distal end	5.04–10.72	7.40 (±0.14)
Spur width (spu.w)	Width of the spur near the point of perianth attachment	0.47–1.46	0.93 (±0.02)

canonical correspondence analysis (CCA) was performed to assess the relationships among floral traits within populations and climate variables [36].

3 Results

Considerable variation in each floral trait was observed (Table 3). The majority of floral traits increased in size with decreasing altitude (Fig. 1). Dorsal sepal, lateral sepal, and spur width values were greater at the lowest altitude (Quadra Island) but these width values were also high at one of the mid-elevation sites (Strathcona 800) (Fig. 1). The greatest labellum lengths were observed at the second lowest altitude site, Strathcona 400 (Fig. 1). Using the mean from three flowers to avoid non-independence, the Shapiro–Wilk test of normality indicated that all floral traits except spur width were parametric. One-way ANOVA results of parametric floral traits showed a significant difference among populations for all floral traits ($P < 0.05$) except labellum width (Table 3; Fig. 1). Tukey test comparisons showed significant interactions for the following floral traits: dorsal sepal length (Rang-S400, $P < 0.01$; Rang-Quad, $P < 0.01$; Para-S400, $P < 0.05$; Para-Quad, $P < 0.05$; Fig. 1A), lateral petal length (Rang-S400, $P < 0.05$; Rang-Quad, $P < 0.05$; Para-Quad, $P < 0.05$; Fig. 1C), lateral petal width (Rang-Quad, $P < 0.01$; Para-Quad, $P < 0.05$; Fig. 1D), lateral sepal length (Rang-S400, $P < 0.01$; Rang-Quad, $P < 0.01$; Para-S400, $P < 0.01$; Para-Quad, $P < 0.01$; Fig. 1E), lateral sepal width (Para-Quad, $P < 0.05$; Fig. 1F), spur length (Rang-Quad, $P < 0.05$; Fig. 1G), and labellum length (Rang-S400, $P < 0.05$; Fig. 1I). Dorsal sepal width showed a significant difference among populations ($F = 3.36_{4, 20}$, $P = 0.02$) but the post hoc Tukey test comparisons showed no significant interactions (Fig. 1B). Spur width, which was nonparametric, was significantly different among populations according to the Kruskal–Wallis test (df = 4; $X^2 = 13.2$; $P = 0.01$; Table 4). A significant interaction was shown by the post hoc Dunn test (Para-Quad, $P < 0.05$; Fig. 1H). Results from the linear mixed effect models supported those obtained from the one-way ANOVAs (results not shown).

The first two PCA axes explained 77% of the variation among populations (Fig. 2A). The PCA loadings showed that lateral petal length and labellum width had the most influence (maximum and minimum, respectively) on PC1, while labellum width and labellum length had the most influence (maximum and minimum, respectively) on PC2 (Fig. 2B). All floral width traits had a positive influence on PC2 while all length traits had a negative influence (Fig. 2B). In ordination space, PC1 separated populations by elevation rather than habitat type, with higher elevation sites (e.g., Paradise Meadows and Ranger Cabin) being more similar to each other than to lower elevation sites (Fig. 2A). The lowest elevation population, Quadra Island (Quad), is influenced strongly by floral trait widths, whereas length of floral trait appears to influence the Strathcona 400 (S400) population (Fig. 2A).

The CCA biplot for annual climate variables shows that the temperature difference between the mean warmest and coolest months (TD—or continentality effect) has a strong influence on the lowest elevation site and width of floral traits (Fig. 3A, B). Mean annual temperature (MAT), mean warmest and mean coldest month temperatures (MWMT and MCMT, respectively) had a strong negative influence on the high elevation sites (Ranger Cabin and Paradise Meadows) (Fig. 3A). Maximum seasonal temperature variables (Tmax_wt = winter mean max. temp.; Tmax_at = autumn mean max. temp.; Tmax_sp = spring mean max. temp.; Tmax_sm = summer mean max. temp.) exhibit a similar influence on populations and floral trait widths (Fig. 3C, D). One mid-elevation site (Strathcona 800) experienced temperatures that were comparable to the lowest elevation site (Quadra Island) (Fig. 3).

4 Discussion

We assessed floral variation among plants of *P. dilatata* var. *dilatata* across an elevational gradient. Generally, floral traits were larger at low elevation sites, although some mid-elevation sites from roadside habitats displayed equally wide (i.e., spur, lateral sepal, dorsal sepal) or long (i.e., lateral sepal, labellum, dorsal sepal) floral traits (Fig. 1). The PCA separated populations according to elevation rather than habitat type, based on the fact that two well-separated mid-elevation populations were from the same habitat type (Fig. 2A; Table 2). The PCA, which explained 77% of the variation, showed that lateral petal length and labellum width were the most influential variables contributing to axes 1 and 2, respectively (Fig. 2). The CCA indicated that annual and seasonal climate variables associated with temperature explained much of the variation in floral traits, particularly the width of floral traits (Fig. 3).

Fig. 1 Box plots displaying ANOVA results with Tukey multiple comparison post hoc test significance (**A**–**G**, **I**) and Kruskal–Wallis results with Dunn multiple comparison post hoc test significance (**H**). Significance indicated by * > 0.05; ** > 0.01. The tenth floral trait, labellum width, is not displayed as ANOVA results were non-significant (refer to Table 4 for significance values)
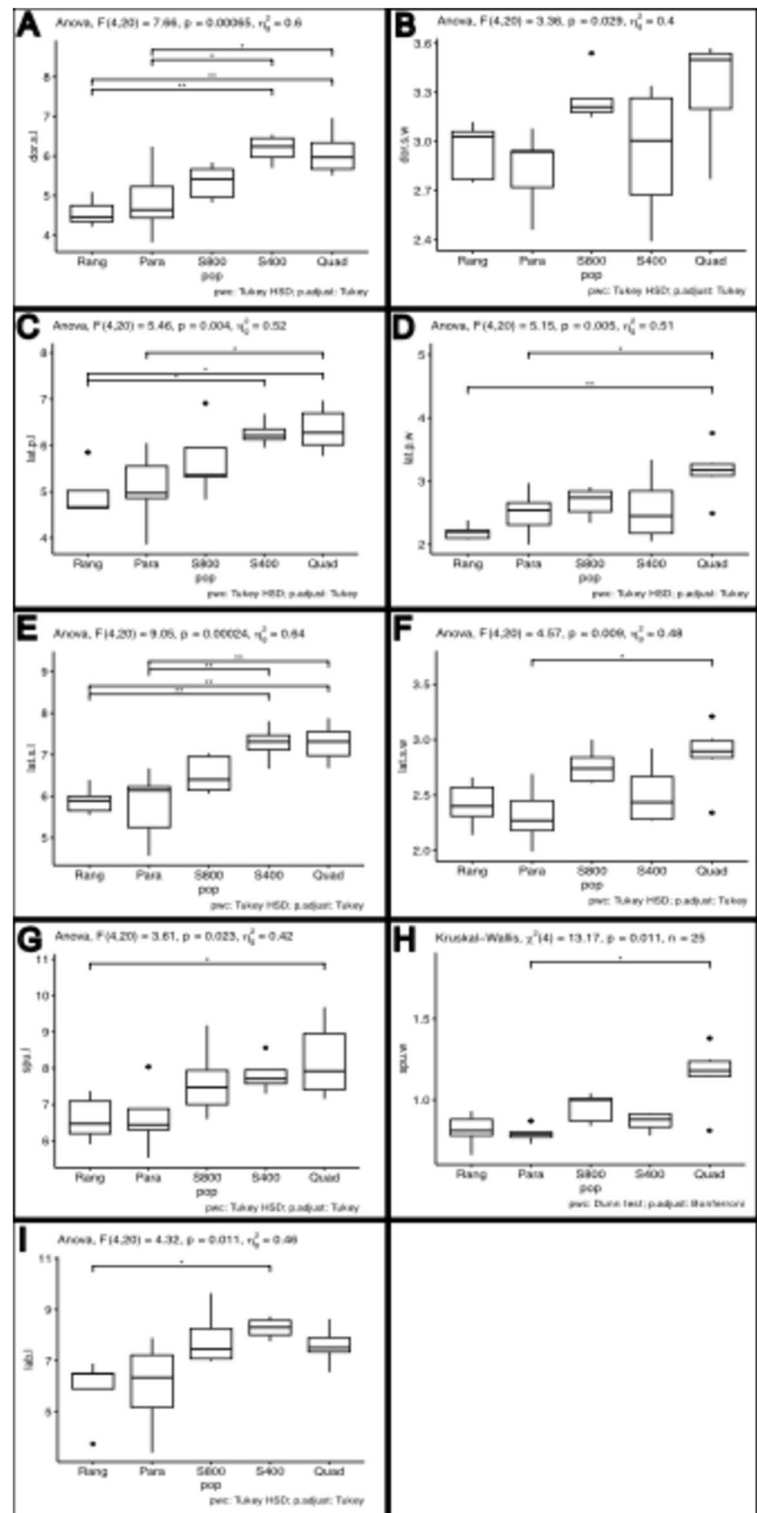

We observed considerable variation among floral traits (Fig. 1; Table 3). Our spur length range was within those previously recorded by [12, 14, 15, 17] (Table 1) but longer than the spur lengths recorded by [16]. The labellum lengths observed here were largely within the range of other recorded labellum lengths (Table 1) but we did observe some that were quite small by comparison (2.37 mm; Table 3). While floral traits appear quite variable across the range of *P. dilatata*, they do not appear to have changed over time (e.g., measures from 1975 are still comparable to measurements today),

Table 4 Results from one-way ANOVA and Kruskal–Wallis tests

Floral trait	Test	$F_{4, 20}$	$X^2(df)$	P-value
Dorsal sepal length	One-way ANOVA	7.66		0.00***
Dorsal sepal width	One-way ANOVA	3.36		0.02*
Lateral sepal length	One-way ANOVA	9.05		0.00***
Lateral sepal width	One-way ANOVA	4.60		0.00**
Lateral petal length	One-way ANOVA	5.46		0.00**
Lateral petal width	One-way ANOVA	0.74		0.00**
Labellum length	One-way ANOVA	4.32		0.01*
Labellum width	One-way ANOVA	0.74		0.58
Spur length	One-way ANOVA	3.61		0.02*
Spur width	Kruskal–Wallis		13.2(4)	0.01*

Significance levels: * 0.05; ** 0.01; *** 0.001

Fig. 2 **A** Principal component analysis (PCA) biplot depicting relationships among sites (representing an elevational gradient) and morphological flower traits (arrows). **B** PCA loadings for axes 1 and 2 of the biplot
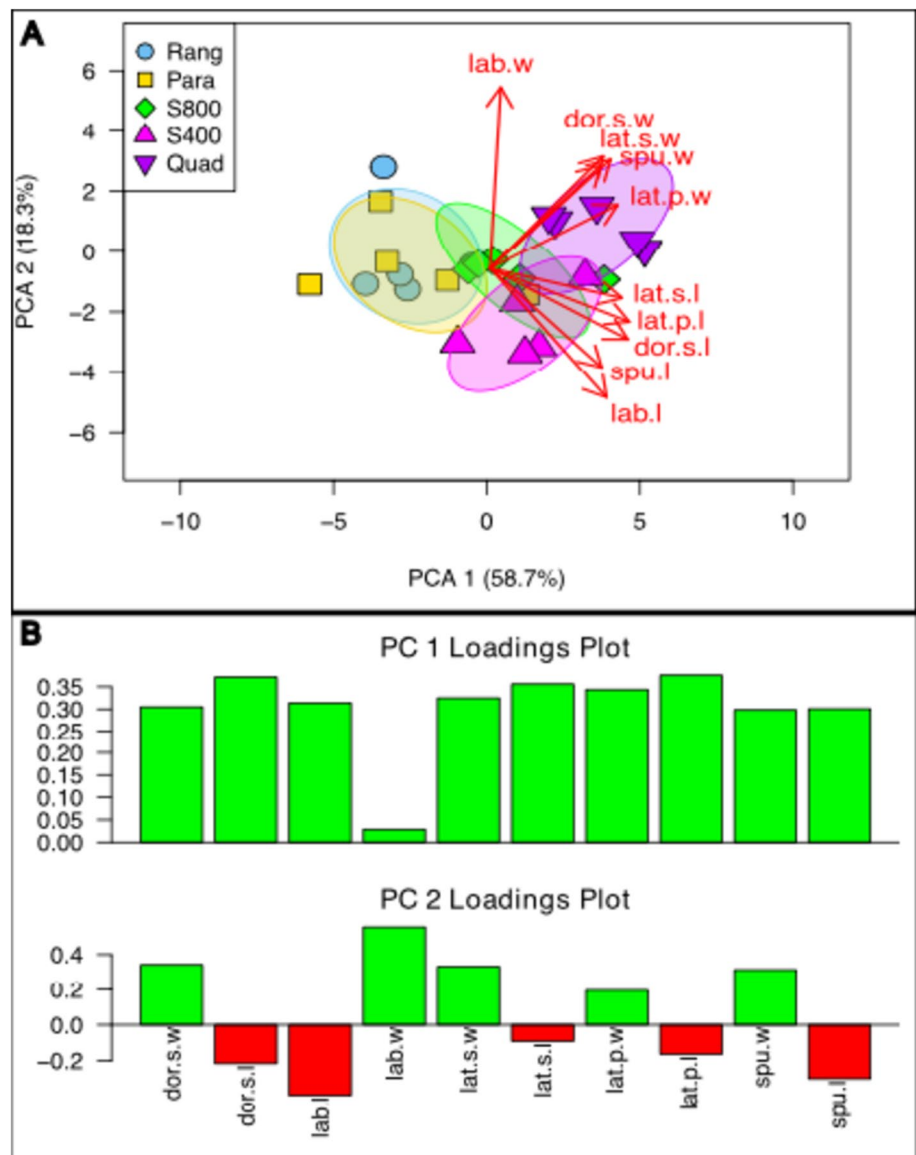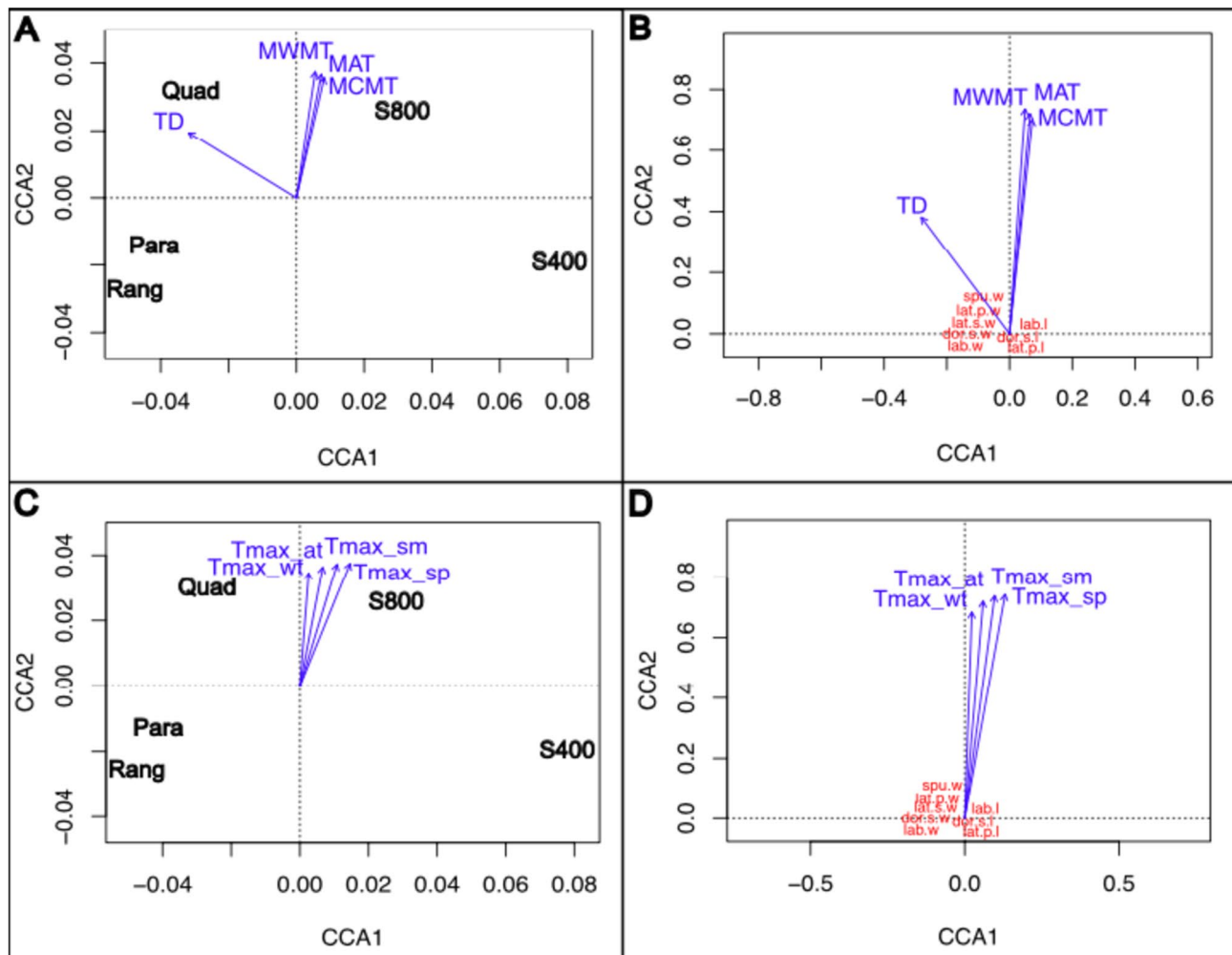
Fig. 3 Canonical correspondence analysis relationships among sites and annual climatic variables (**A**), morphological flower traits and annual climatic variables (**B**), sites and seasonal climatic variables (**C**), and morphological flower traits and seasonal climatic variables (**D**)

suggesting that different selection pressures operate at local scales (i.e., evolutionary mosaic) but an overall balancing selection pattern is maintained at larger spatial scales.

Altitudinal variation in floral traits has been observed in a number of different plant species [37–39], including orchids [40, 41]. Several explanations have been put forward to explain the changes, including: pollinator shifts [37], genetic variation [42], resource availability [43], and climate [37]. With respect to pollinators, recent studies of *P. dilatata* are revealing that the species likely has a number of pollinators in the Hymenoptera and Lepidoptera [15, 18]. In the context of this study area, previous work suggests that *Bombus flavifrons, B. melanopygus, B. mixtus, B. sitkensis*, and *B. vancouverensis* of the Hymenoptera, and *Argynnis hydaspe* (syn. *Speyeria hydaspe*), *Rhuemaptera sp.*, and a Pterophorid of the Lepidoptera are pollinators [18], at least at the higher elevation sites. Records from iNaturalist indicate that all of these insect species have been observed across the elevational gradient at some point in time but general information on *A. hydaspe* indicates that this species prefers higher altitude habitats [44]. The *Bombus* spp. are cited as having tongues in the 4.6–5.5 mm range [45, 46]), while *Argynnis* spp. may have proboscis lengths of 12–14.6 mm [47]. We were unable to find specific length observations for *Rheumaptera* or Pterophoridae except general descriptions of 'well-developed' [48]. Given the observed tongue lengths for many *Bombus* spp. relative to the spur lengths observed for the lower altitude sites, this genus may not be a particularly effective pollinator at these sites. Typically, the length of the tongue or proboscis must be compatible in order for the pollinator to obtain its nectar reward [13, 49, 50]. However, nectar production in *P. chlorantha* can reach a point where the spur overflows [51]. Thus, ample nectar production, coupled with wider and longer spurs at lower elevations, may eliminate incompatibility among pollinators and spur lengths that might otherwise prevent gene

flow between populations at different altitudes. Nectar production in *P. dilatata* appears unstudied so further work on correlations between spur length/width on nectar production and reproductive success would be valuable.

Research on *P. dilatata* populations in Alaska identified two broad categories of floral trait variation—large versus small flowers [15]. These morphotypes were found to be genetically distinct, although admixed individuals were identified in each population [15]. The prevalence of admixture suggests that pollinators and their efficacy were not negatively impacted by changes in floral size. While a genetic basis to floral size variation in *P. dilatata* has been identified, it is unclear how much of the phenotypic variation observed is due to plasticity from GxE interactions. The influence of herbivores and nectivores may also have a direct link as genetically distinct populations and morphotypes may exhibit differential palatability and susceptibility to floral enemies [8]. In this context, selection pressure from floral enemies (i.e., disruptive or directional—maintaining different genotypes) may provide an antagonistic pressure to pollinators (i.e., stabilizing— mixing genotypes). References to nectivores and herbivores that target flowers in the Orchidaceae are not common (e.g., [52]); most studies report damage to leaves [13, 53]. Direct investigations of floral enemies and common garden experiments could provide considerable insight into the ecological and genetic processes at work.

Another explanation for floral trait variation over elevation is the availability of resources [8] although, it is often difficult to disentangle the link between resource availability (i.e., water, nutrients) and climate [54]. Studies show that populations at higher altitudes typically experience shorter growing seasons [55] and greater seasonal fluctuation in resources [56], resulting in smaller floral traits and plant sizes [43]. We observe an interaction among annual and seasonal temperature variables with floral trait sizes, particularly the width of floral traits (Fig. 3). One of the mid-elevation sites (Strathcona 800), experienced annual and seasonal temperatures that were comparable to the lowest elevation site (Quadra Island). This may be due to slight differences in aspect and/or distance to the road, which may reduce albedo and contribute to slightly warmer temperatures. Warmer temperatures have been shown to have mixed effects on flowering traits across a broad range of species, with some species producing fewer [57] or more [58], or larger [59] or smaller [60] flowers in response. Further, temperature is known to impact numerous plant physiological responses [61] and the availability of resources both directly (e.g., evaporation of water) and indirectly (e.g., cycling of nutrients by microbial communities) [54]. Interactions among climate and resources could lead to changes in gene expression and phytohormone production [61] resulting in changes to cell division and anisotropy (e.g., differential growth) in floral meristems. Anisotropy is known to be the main driver of spur length diversity in several species [9, 62]. It would be valuable to design studies that systematically assess the effects of environmental or climatic variables correlated with altitude on the development of orchid flowers.

5 Conclusion

Our study reveals that floral traits of *P. dilatata* var. *dilatata* typically increase in size at lower elevations. Evaluations of climate variables along this elevational gradient suggest that temperature has the greatest influence, although temperature is not necessarily correlated with altitude in this study. Thus, the 'resource cost hypothesis' appears to be the best explanation for floral trait variation in *P. dilatata* given the strong interactions among climate and resources. This study provides an important basis for future work investigating the ecological and genetic mechanisms behind floral trait variation, how such variation contributes to or diminishes local adaptation, and what consequences this may have on pollinator-mediated selection moving forward.

Declarations

Ethics and consent to participate   Plant samples collected from within the Strathcona Provincial Park boundary were collected in 2020 under a Letter of Authorization (98700-20/Strathcona). Samples collected outside of the Strathcona Provincial Park boundary were on Crown Land and did not require a permit.

Competing interests   The authors declare no competing interests.

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