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POLLINATION ECOLOGY OF *OREOCALLIS GRANDIFLORA* (PROTEACEAE) AT THE NORTHERN AND SOUTHERN ENDS OF ITS GEOGRAPHIC RANGE

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Abstract—Geographic variation in pollination ecology is poorly documented, if at all, in many plant-pollinator systems. Great insights could be gained into the abiotic and biotic factors which impact the evolution of floral properties and their potential to lead to speciation by doing so, as both can vary naturally over the geographic range of a plant species. We characterized the pollination ecology of the Andean tree *Oreocallis grandiflora* (Family: Proteaceae) at the northern and southern ends of its range in Ecuador and Peru in terms of flower morphology, nectar properties, pollinators and plant reproduction. We found significant divergence in the two populations in terms of style length and flower openness, nectar standing crop and secretion rate, and pollinator community. We did not find a significant difference in the length of the pollen presenter or in nectar sucrose concentration by weight (% Brix). The observed divergence in floral traits between the two study populations may be related to a combination of factors, including genetic drift and isolation by distance, distinctive suites of pollinators, or heterospecific pollen competition, which future studies should further investigate. This study demonstrates that pollination ecology can vary substantially across the geographic range of a species, with implications for delimiting species and subspecific taxa.

Keywords: Andes, biogeography, floral traits, hummingbirds, mammals, Proteaceae

INTRODUCTION

The study of pollination ecology has played an important role in our current understanding of co-evolution (Cook & Rasplus 2003) and speciation (Kay & Sargent 2009), and also provides important baseline information to inform practical ecosystem-level conservation efforts (Pauw 2007) in a time of pollinator declines (Biesmeijer et al. 2006). However, more basic information is needed to improve our understanding in all of these areas. For example, the lack of data on plant-pollinator interactions has been identified as one of the main obstacles to understanding how zoophilic pollination may act as a mechanism of speciation (Kay & Sargent 2009). Studies that document pollination ecology at different points along a single species' geographic range are rare, and could provide insights for future research into the role of pollination in driving floral isolation and even speciation, as well as how pollination mutualisms adapt to changing conditions.

Intraspecific variation in floral morphology and pollination ecology may arise through several mechanisms, primarily genetic drift, abiotic selection, and biotic selection driven by pollinators, herbivores, and competing plant species. Environmental factors such as temperature and precipitation are known to influence floral traits such as flower size (Sapir et al. 2002) and colour (Strauss & Whittall 2006). According to Stebbins' (1974) Most Effective Pollinator Principal (MEPP), selection should also favour floral traits that promote visitation by the most frequent and effective pollinator. The precise role of selection by pollinators in the speciation of Angiosperms is under debate, but many switches in pollinator syndrome have been documented in the literature. For example, the Neotropical genus *Costus* (Costaceae) has shifted from bee to hummingbird pollination multiple times (Kay & Schemske 2003), presumably because changes in the available pollinator community caused directional selection away from the original pollinator guild. Herbivores (Gómez et al. 2009a) and heterospecific pollen competition from other plant species in the community (Ashman & Arceo-Gómez 2013) may also exert selective pressure on specific floral traits such as flower number and pistil length. Conversely, in cases where the available pollinator community and environmental context are consistent and adequate levels of gene flow exist across a species range, pollination ecology and corresponding floral traits might be conserved due to stabilizing selection. At present, the lack of comparative studies between species and subpopulations limits our ability to distinguish between the frequency and likelihood of these alternative scenarios. Studies that document the pollination ecology of a species at different points in its geographic range will help to highlight possible

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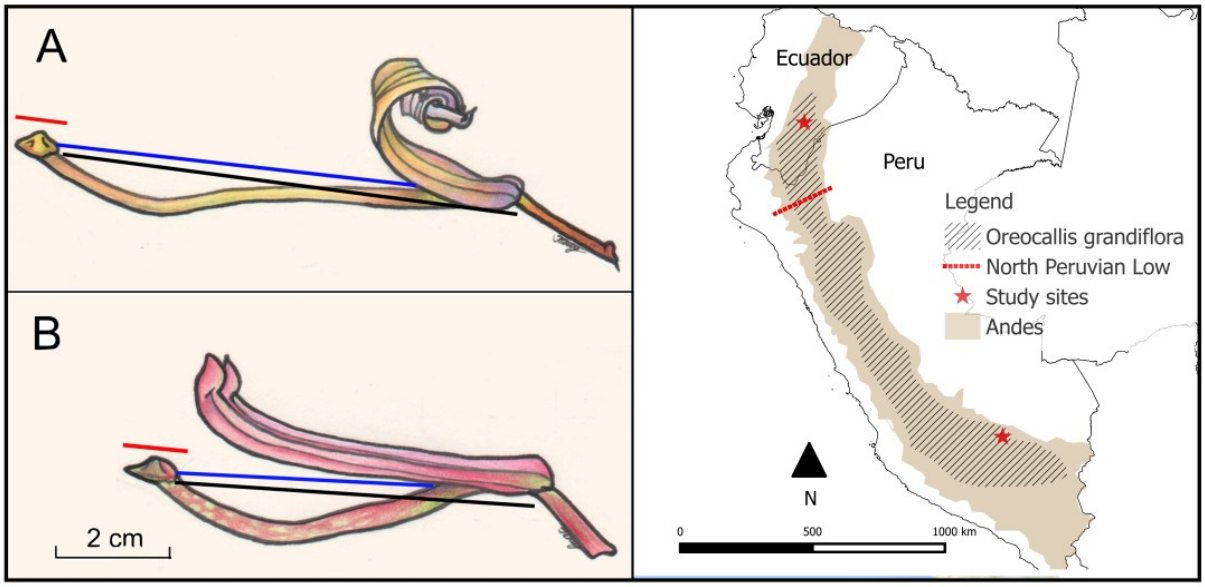

FIGURE 1. There is marked variation in floral morphology between Peruvian (A) populations at the southern range limit and Ecuadorian (B) populations at the northern range limits of *Oreocallis grandiflora*. Blue lines indicate the straight line distance between the tip of the pollen presenter and the point at which nectar accumulates (PED), back lines indicate the style length (SL), and red lines indicate stigma height (SH). On the map the red drops represent the study site locations in Ecuador and Peru, the dotted line represents an estimate of the range of *O. grandiflora*, and the double black line represents the location of the Northern Peruvian Low.

questions and study systems for future research into these mechanisms of divergence. In this study, we describe relevant floral traits and pollination ecology of the Andean tree *Oreocallis*.

The Andes are an important and under-studied centre of plant-pollinator system diversity (Kay & Sargent 2009) which is increasingly threatened by habitat conversion and climate change (Ocampo Peñuela & Pimm 2015). *Oreocallis grandiflora* is a widespread and abundant plant species whose range spans a distance of over 1,500 km along the Andes mountain range (Fig. 1). Based upon intraspecific variation in the colour and pubescence of *O. grandiflora* inflorescences, Sleumer (1954) defined two distinct species; *O. mucronata,* with white, glabrous inflorescences, and *O. grandiflora* with pubescent, pink-red inflorescences. This two-species conclusion was also reached by Weston & Crisp (1999). However, subsequent herbarium analysis suggested that the variation in pubescence was continuous and not associated with colour, and the two species were condensed into *O. grandiflora* (Prance 2008). Yet, neither of these more recent, herbarium-based studies appears to have considered the geographic distribution of this variation, suggesting that characterizing both the degree of variation that may exist between spatially distinct populations as well as any differences in their pollinator community may provide insight into the taxonomic status of this species.

The overarching goal of this study was to describe the pollination ecology of *O. grandiflora* from two research projects at either extreme of its geographic range and to discuss potential explanations for geographic variation in floral traits. To do so, we assess variation in floral morphology, nectar properties, and pollinator community between two populations in qualitatively similar evergreen montane forest habitat (Tovar et al. 2013) that are situated at the extremes of the species range along its north to south axis in the Andes, one in southern Ecuador and one in southern Peru.

MATERIALS AND METHODS

Study sites

Field work was conducted by two separate research teams over three years every July–November from 2012–2014 at the Wayqecha Biological Station (13°11'S, 71°35'W) in Manu National Park, Peru and from November–February from 2014–2015 at El Gullán Biological Station of the Universidad del Azuay in Ecuador (3°20'S, 79°10'W) at 3,000–3,400 m asl by the other team. At the time that most of the work was conducted, the respective Peruvian and Ecuadorian teams were unaware of each other. For this reason, some of the methods vary between the two sites, but the data are still compatible. The respective time frames correspond to the end of the dry season and the start of the rainy season in both habitats. The two sites are 1,378 km apart straight-line distance and located at the transition between the "evergreen montane forest" and "high elevation grasslands" biomes (Tovar et al. 2013). There exist several potential geographic barriers to gene flow for high-elevation plant species along the North-South axis of the Andes, including the North Peruvian Low (NPL), the lowest point in the Andes between Chile and Colombia (Fig. 1), which could disrupt gene flow and impact the degree of divergence in pollination ecology and floral traits.

Study species

The Andean firebush, *Oreocallis grandiflora* Lam. (Proteaceae), is a small tree up to 7 m in height that

produces terminal flowered raceme inflorescences of 10–50 long, paired flowers that open sequentially in groups of 2–20 at a time from the base of the inflorescences towards the top. Flowers have a tubular to cylindrical perianth that opens into 4 segments. Flowers are also bisexual and have a relatively large pollen presenter 0.35–0.45 mm long, which refers to any structure other than the anthers that distributes pollen, and in this case is a modification of the style and stigma (Prance et al. 2008). Fruits are woody follicles that dehisce to reveal winged, wind-dispersed seeds. Flowering and fruiting occur simultaneously year-round in both Ecuador and Peru. *O. grandiflora* is especially common in disturbed soils along its range in the Andes from southern Peru to Central Ecuador and has been reported from 1,200–3,800 m asl (Prance et al. 2008). Data on pollinators is scarce, with no information on geographic variation and only three published hummingbird species as visitors (Prance et al. 2008). The pollination ecology of Neotropical Proteaceae in general is poorly documented, but there are more reported cases of entomophily than ornithophily (Prance et al. 2008), and one possible case of chiropterophily (Fleming et al. 2009). There are also a few examples of mixed pollination systems in Neotropical Proteaceae (Devoto et al. 2006, Chalcoff et al. 2008). Proteaceae globally exhibit a wide range of pollinator communities with several reported cases of pollination by non-flying rodents (Rourke & Wiens 1977), bats (Daniel 1976), and birds and insects (Mast et al. 2012).

Flower colour and morphology

We visually assessed petal colour by photographing flowers against grid paper as belonging to either the "pink" or "white" morph. We studied flower morphology at both sites by randomly sampling two flowers ($N_{Ecuador}$ = 73, N_{Peru} = 94) from individual *O. grandiflora* trees. Flowers were photographed on a 1 cm × 1 cm grid background and the following measurements were extracted from photos using the program tpsDig version 2.16 (Rohlf 2010): style length (SL; the straight-line distance from the base of the corolla along the longest axis to the base of the stigma), stigma height (SH; the longest distance across the stigma (also the pollen presenter), and the minimum straight-line distance between the pollen presenter and the intersection of the petals and the style, where nectar accumulates (PED), and the angle of flower openness (AO; the smallest angle between the petals and the style) (Fig. 1).

Nectar properties

To quantify nectar properties, standing crop and sucrose concentrations were measured in 2 to 5 flowers that were sampled from randomly selected *O. grandiflora* trees within the study sites ($N_{Ecuador}$ = 90, N_{Peru} = 107) and nectar volume and sucrose concentration were measured using 50 µL microcapillary tubes (Sigma-Aldrich Co., St.Louis, Missouri, USA) and a handheld sucrose refractometer (Bellingham and Stanley Ltd, Basingstoke, UK). To measure daily patterns in nectar secretion, we randomly selected four trees and placed mesh bags on four flowers on each tree, two per inflorescence, to exclude pollinators. Nectar secretion was then measured every two hours (Peru) and three hours (Ecuador) from 6 AM until 6 PM from opening until flower dehiscence, 3 to 5 days. Nocturnal nectar secretion patterns were not quantified, because this data was originally recorded as part of research on diurnal hummingbird activity and nocturnal visitation was previously undocumented. Nectar was also extracted at the appropriate time interval before the first measurement to get an accurate reading at 6 AM. To measure nectar accumulation rates over 24 h, individual trees were randomly selected and one inflorescence on each tree bagged off from visitors. At 6 PM the evening prior to sampling all nectar was emptied from the flowers and after 24 h nectar volume was measured from the same flower.

Pollinator community

We documented the pollinator community and pollinator visitation rate of *O. grandiflora* in Peru and Ecuador by randomly selecting individuals for observation and then setting up digital camcorders (Sony Inc., New York, USA). At both sites, each plant was recorded for 2–6 h (depending on the available camera and the weather) in the morning and in the afternoon for a period of maximum period of five days. Videos were then reviewed manually and the time and identity of any floral visitors was recorded. Nocturnal pollination was opportunistically sampled at both sites using infrared-enabled trap cameras (EBSCO Inc., Birmingham, USA).

Reproduction of Oreocallis grandiflora

We used hand-pollination experiments to study how pollen source impacted fruit set, seed set, and mass. The Peruvian and Ecuadorian sites had slightly different protocols for the experiment. In Ecuador only fruit set was quantified, while in Peru fruit set was not quantified, but seed set and mass were. In Ecuador, 49 individual trees of *O. grandiflora* were randomly selected, and each flower on the same randomly selected inflorescence received one of the following hand-pollination treatments: Self-pollen (from the same inflorescence), natural self-pollination (a freshly opened flower isolated from visitation with a mesh bag), nearest-neighbour pollen, far pollen (pollen from plants > 1 km away), and a control treatment. Flowers were monitored monthly for three months and total fruit production was measured. In Peru, ten individuals of *O. grandiflora* of similar size and at least 20 m apart were selected. One inflorescence per tree was randomly selected to receive one of each of the following hand-pollination treatments in 5 freshly opened flowers: self-pollen, nearest-neighbour pollen, next-patch pollen, and far pollen. Nearest-neighbour pollen was collected from the nearest individual of *O. grandiflora*, next-patch pollen was collected from individuals 50–100 m away from the focal plant, and far pollen was collected from individuals 1 km away. The quantity of pollen to be applied was standardized as lying flat against a 1 cm × 1 cm square on grid paper. An applicator made out of hummingbird feathers was applied to the square and brushed against the stigma daily for four days after anthesis to simulate a hummingbird visit. After treatment, flowers were bagged. For both sets of experiments, fruit development was monitored monthly and collected once ripe. Fruit were dried in the sun until dehiscence and seeds were extracted and counted. Each seed was then measured along the longest axis and weighed to 0.000 g.

Statistical analyses

All statistics were conducted in R version 3.2.3 (R Core Team 2015). To assess variation between the populations in these parameters, we conducted a principal components analysis (PCA) and a linear discriminant analysis (LDA) on log-transformed morphological values using the package MASS (Venables & Ripley 2002). In order to determine which principal components to use in further comparative analysis, we used a broken-stick null model (Jackson 1993) with the package 'BiodiversityR' (Kindt & Coe 2005). We then conducted a two-tailed t-test using the package 'stats' (R Core Team 2015) to test whether significant principal component scores varied significantly between the Peruvian and Ecuadorian populations.

To determine the effects of site (Ecuador or Peru) on nectar standing crop we used two separate models to first analyse all the data for the presence or absence of nectar using a generalized linear mixed model (GLMM) with a binomial distribution using the package 'lme4' in R (Bates et al. 2015), then analysing only the log-transformed non-zero data using a general linear mixed model (GLMM) with a Gaussian error distribution using the package 'nlme' (Pinheiro et al. 2015). In both steps flowers were nested within plants if multiple flowers were sampled from the same individual, and time of day was included as a fixed effect. To determine the effects of site on nectar sucrose by weight concentration (% Brix) we used the square-root of Brix values as the dependent variable and site as fixed effect, with flower nested within plant as the random effect using a GLMM with a Gaussian error distribution with the package 'nlme' (Pinheiro et al. 2015). To determine the effects of site on 24 h nectar accumulation rates, we used the package 'stats' (R Core Team 2015) to conduct a nested ANOVA with log-transformed nectar volume as the dependent variable and site, flower, and date as the fixed effects and individual tree as the random effect. To determine the effect of time of day on nectar secretion rate we independently analysed the data from Ecuador and Peru, since they were collected at different sampling intervals. We used a GLMM with a Gaussian error distribution with square-root transformed nectar volume as the dependent variable, time of day as the fixed effect and flower nested within inflorescence within individual tree as the random effect using the package 'nlme' (Pinheiro et al. 2015). To compare daily nectar secretion patterns between the two sites while correcting for the differences in sampling intervals we summed nectar secretion between 6 AM and 12 PM and took the mean of sucrose concentrations recorded during that time. We then used a Gaussian GLMM with square-root transformed cumulative nectar secretion as the response variable and site as the fixed effect, with individual tree as the random effect, using the package 'nlme' (Pinheiro et al. 2015). To analyse the mean sucrose concentration (% Brix) over this 6 h period, we used the same analysis method but with % Brix as the response variable, country as the fixed effect, and tree as the random effect.

To compare the diurnal pollinator community between the sites we used a nonmetric multidimensional scaling (NMDS), with Bray-Curtis distance matrix to ordinate Ecuadorian and Peruvian plants in relation to the community of pollinators. One hr visitation rates per inflorescence were used as the quantitative link between plants and pollinators. Differences in the community of pollinators between Ecuadorian and Peruvian plants were tested with a non-parametric Manova (Anderson 2001) using the same distance matrix employed in the MNDS with the "vegan" package (Oksanen et al. 2016). We also calculated hourly visitation rates, Shannon's Diversity Index (H') and Pielou's Evenness (J') on a per-plant basis by pooling the observations for each plant, then taking the averages for all plants for each site and for the two sites pooled together using Excel. We compared visitation rates using a one-way ANOVA with the package 'stats' with square-root transformed visitation rate as the dependent variable, country as the fixed effect, and hours of observation per plant as the random effect. We analysed the effect of the "closed" treatment on fruit set in Peru using a Gaussian LMM with logit-transformed proportions of fruit set as the dependent variable, and treatment as the fixed effect and individual tree as the random effect using the package 'nlme' (Pinheiro et al. 2015).

We analysed the effect of the different hand-pollination treatments on seed set using a Gaussian LMM with seed set as the dependent variable and pollen treatment as the fixed effect. To test the effect of treatment on seed mass, we used a Gaussian LMM with square-root transformed seed mass as the dependent variable and pollen treatment as the predictor variable with the package 'nlme' (Pinheiro et al. 2015). In both tests inflorescence was nested within individual tree as the random effect. To analyse the Ecuadorian data, we used a binomial general linear mixed model (GLMM) in the package 'lme4' (Bates et al. 2015) with the presence or absence of fruit per hand-pollinated flower as the dependent variable, treatment as the fixed effect, and treatment nested within plant as the random effect.

RESULTS

Flower colour and morphology

Flowers from the Peruvian and Ecuadorian populations exhibited striking differences in colour and morphology (Fig. 1, Tab. 1). Peruvian individuals of *O. grandiflora* all presented obviously magenta flowers while Ecuadorian individuals presented white-green flowers. In general, Ecuadorian flower morphology was characterized by longer style length (SL) and the minimum straight-line distance between the pollen presenter and the intersection of the petals and the style (PED), and a wider angle of openness (AO), while Peruvian flowers were characterized by shorter SL and PED with a smaller AO (Tab. 1). This finding is corroborated by Principal Components Analysis (PCA), which showed distinct grouping of Ecuadorian and Peruvian flowers in the morphospace (Fig. 2). The first component (PC1) accounted for 62% of the variation, the second (PC2) for 23%, the third (PC3) for 13% and the fourth (PC4) for 1% (See Tab. 2). Only the first principal component had an eigenvalue greater than would be expected at random. The coefficients for contribution to PC1 were as follows: pollination efficiency distance was 0.60, stigma length was

TABLE 1. Summary of floral traits and pollination ecology of O. *grandiflora* in southern Ecuador and northern Peru. Mean values with standard errors for Ecuadorian and Peruvian populations of *O. grandiflora* and the combination of both. Asterix (*) indicates significant differences between the Ecuadorian and Peruvian populations < 0.05, ** indicates *P*-values < 0.01, *** indicates *P*-values less than 0.001. Numbers within parentheses represent sample sizes.

	Ecuador	Peru	Combined
Morphology			
Stigma length (mm)*	5.3 ± 0.4 (73)	3.3 ± 0.5 (94)	4.2 ± 0.1 (167)
Pollination efficiency distance (mm)*	4.6 ± 0.0 (73)	2.1 ± 0.0 (94)	3.2 ± 0.1 (167)
Angle of openness (°)*	25.0 ± 0.6 (73)	34.1 ± 0.6 (94)	29.0 ± 0.6 (167)
Stigma height (mm)	0.4 ± 0.6 (73)	0.4 ± 0.6 (94)	0.4 ± 0.0 (167)
Colour*	white-green	magenta	N/A
Nectar Properties			
Percent containing nectar***	94% (90)	37% (107)	63% (197)
Standing crop (μL)*	15.1 ± 1.5 (84)	10.9 ± 1.4 (39)	13.8 ± 1.2 (123)
Sucrose (%Brix)	27.8 ± 1.6 (84)	30.0 ± 3.0 (39)	28.5 ± 1.6 (123)
24-hr secretion (μL)**	31.7 ± 3.5 (36)	12.6 ± 1.0 (41)	21.5 ± 2.0 (77)
Pollination			
Visits per hour*	0.80 ± 0.19 (151)	0.64 ± 0.11 (295)	0.72 ± 0.11 (446)
Shannon's diversity index	0.31 ± 0.06 (17)	0.53 ± 0.09 (27)	0.44 ± 0.06 (44)
Pielou's evenness index	0.16 ± 0.03 (17)	0.30 ± 0.05 (27)	0.24 ± 0.04 (44)

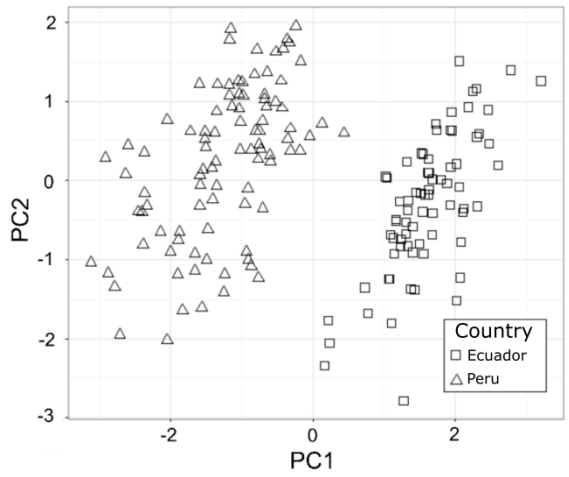

Figure 2. PCA results for a comparison of floral morphology in Peruvian (N = 94) and Ecuadorian (N = 73) populations of *Oreocallis grandiflora*. Squares represent Ecuadorian samples and triangles represent Peruvian samples.

0.60, angle of openness was 0.48, and stigma height was 0.21. A two-tailed student's t-test of PC1 values by site was significant (T = 28.05, DF = 165, P < 0.01). This grouping was also confirmed by a linear discriminant analysis (LDA), which had a 100% success rate at identifying specimens by country of collection.

Nectar properties

Flowers in Peru were found to be significantly more likely to be empty when randomly sampled than flowers in Ecuador, even when time of day was accounted for (Z = -4.5, DF = 193, P < 0.001). When nectar was present, Ecuadorian flowers had significantly more nectar than did Peruvian flowers (T = -2.25, DF = 41, P < 0.05, $R^2_{marginal}$ = 0.06, $R^2_{conditional}$ = 0.49). There was no significant difference in sucrose concentration by weight between Ecuadorian and Peruvian flowers (T = -0.60, DF = 41, P = 0.55, $R^2_{marginal}$ < 0.01, $R^2_{conditional}$ = 0.21) (Tab. 1). Ecuadorian flowers also had significantly higher 24-hr nectar accumulation rates than did Peruvian flowers (Tab. 1; $F_{1, 17}$ = 12.40, P < 0.001). Nectar secretion rates in Peru varied significantly by time of day between 6:00 am and 6:00 pm (T = -3.59, DF = 33, P < 0.00, $R^2_{marginal}$ = 0.41, $R^2_{conditional}$ = 0.61) with highest secretion rates at 6:00 am and then dropping off during the day (Fig. 3). Nectar secretion rates in Ecuador also varied significantly by time of day, with the secretion rate significantly higher in the afternoon (T = 4.94, DF = 141, P < 0.00, $R^2_{marginal}$ = 0.04, $R^2_{conditional}$ = 0.45) (Fig. 3). The mean cumulative nectar volume secreted between 6 am and noon in Ecuador was significantly greater in Ecuador (T = -3.25, DF = 32, P < 0.01, $R^2_{marginal}$ = 0.18, $R^2_{conditional}$ = 0.51).

Pollinator community

Pollinator visitation rates were significantly higher in Ecuador than in Peru (Tab. 3; $F_{1,41}$ = 4.52, DF = 41, P < 0.05). There were significant differences in community of diurnal pollinators between Peru and Ecuador plants (Fig. 4; $F_{1,39}$ = 4.86 , P < 0.001). *Aglaeactis cupripennis* was the most common visitor in both Peruvian and Ecuadorian plants (63% of visits in Peru, and 44% of visits in Ecuador), but other important hummingbird visitors were exclusive for each location, specifically *Boissoneaua matthewsii, Coeligena violifer, Heliangelus amethysticolis* in Peru and *Coeligena iris, Heliagelus viola, Lesbia nuna*, and *Lesbia victoria* and *Ramphomicron microhynchus* in Ecuador (Tab. 4). Both the Shannon's diversity and Pielou's evenness indices were greater in Peru (Tab. 3). Nocturnal trap cameras revealed the

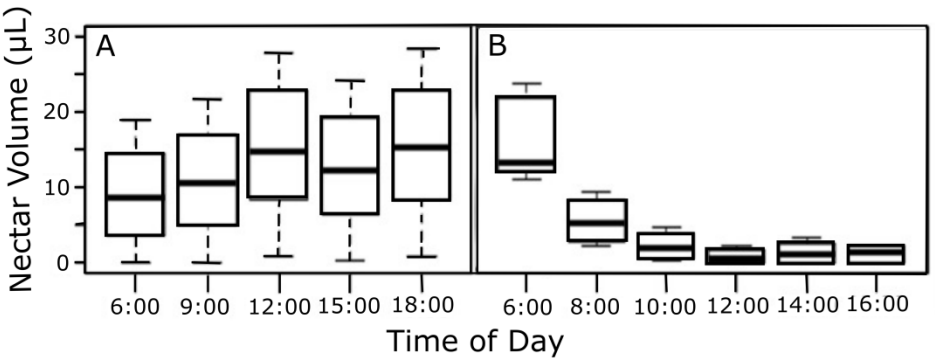

FIGURE 3. A comparison of nectar secretion rates of *Oreocallis grandiflora* in (A) Ecuador over 3-hr intervals (N = 16) and (B) Peru over 2-hr intervals (N = 16).

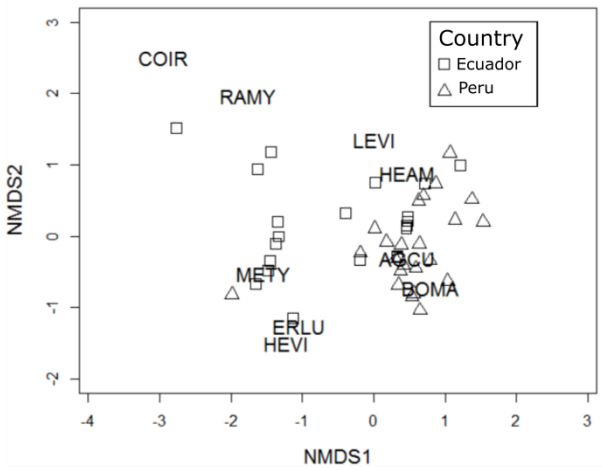

FIGURE 4. A comparison of hummingbird pollinator community of *Oreocallis grandiflora* in Ecuador (N = 175 hrs) versus Peru (N = 294 hrs). The analysis is based on a nonmetric multidimensional scaling ordination using visitation rates of the hummingbird species.

TABLE 2. Principal components analysis of floral morphology loading scores, standard deviation, and proportion of variance explained for each measured floral trait.

Measure	PC1*	PC2	PC3	PC4
Angle of openness	0.48	-0.20	0.85	0.72
Pollination efficiency distance	0.60	-0.13	-0.33	0.72
Stigma length	0.60	-0.05	-0.39	-0.70
Stigma height	0.21	0.97	0.11	0.04
Standard deviation	1.58	0.97	0.73	0.19
Proportion of variance	0.62	0.23	0.13	0.01

presence of bat and rodent visitors in Ecuador (Tab. 3) but no evidence of nocturnal visitation in Peru (N = 50 hours).

Reproduction of Oreocallis grandilfora

Results of the hand-pollination experiments in Ecuador showed no impact of pollen treatment on fruit set (χ^2 = 0.20, DF = 4, P > 0.5). Results of the hand-pollination experiments in Peru suggest that self-pollen results in lower seed set compared to the far treatment (T = -2.31, DF = 34, P < 0.05, $R^2_{marginal}$ = 0.08, $R^2_{conditional}$ = 0.18) but not compared to the nearest-neighbour or next-patch treatments. The self-pollen treatment also had significantly lower seed mass than the far treatment (T = -4.40, DF = 34, P < 0.01, $R^2_{marginal}$ = 0.18, $R^2_{conditional}$ = 0.62) as did the nearest-neighbour treatment (T = -2.30, DF = 34, P < 0.05) (Fig. 5).

DISCUSSION

Ecuadorian and Peruvian populations of *O. grandiflora* exhibited significant differences in a suite of characteristics

relevant to pollination. The Peruvian flowers were all magenta in colour while those in Ecuador were white with a green tinge (Fig. 1). We also observed significant separation in morphospace between the two populations, and style length (SL) and the minimum straight-line distance between the pollen presenter and the intersection of the petals and the style (PED) were most responsible for the variation between the populations. There was significantly higher nectar

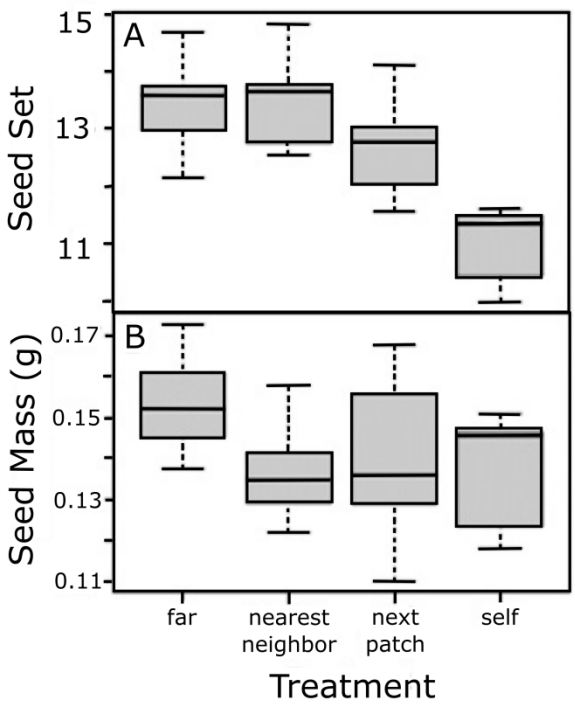

FIGURE 5. The effects of pollen source on (A) seed set and (B) seed mass in *Oreocallis grandiflora* from Peru. Sample sizes for the treatments were as follows: far (19), nearest neighbour (15), next patch (17), self-pollen (19). The Ecuadorian data was not included because no significant effects were found, though self-pollen and autogamous self-pollen treatments were successful at producing fruit as in Peru.

volume in Ecuador and greater 24 h and morning nectar secretion, and in Peru there were significantly more flowers with no nectar present. There was some variation in daily nectar secretion patterns (Fig. 3) though they are not directly comparable due to differences in sampling. Pollinator visitation rates were significantly higher in Ecuador than in Peru. The Peruvian diurnal pollinator community was both more diverse and more even than the Ecuadorian. Our hand-pollination experiments revealed that selfing was possible in both populations, and that in Peru pollen source may impact plant reproduction. In Ecuador however, hand-pollination experiments showed no effect of pollen source on fruit-set.

The two study populations are over 1,500 km apart following the species' geographic range in the Andes, and therefore variation in floral traits is expected. However, the extent of the variation that we observed, especially in terms of floral morphology, suggests that the *O. grandiflora* system may provide a good study system for future research into the evolutionary processes that shape floral morphology, nectar properties, and pollinator community. Our data is limited by the fact that we do not have replicate populations at each latitude, but the authors have opportunistically observed similar suites of floral characteristics, especially in terms of colour and style length, across populations at both latitudes (J. Hazlehurst and B. Tinoco, *personal communication*). Nonetheless, it cannot be known to what extent the observed differences in floral traits segregate geographically without standardized, repeated study populations at both latitudes. It is also possible, as has been found in other species of montane Angiosperms, that variation in floral characteristics and pollinator community follow a mosaic geographic pattern (Gómez et al. 2009*b*).

Our results suggest that this system is ripe for future research into the evolutionary mechanisms driving the observed variation in floral traits and nectar properties. Here, we discuss these relevant selective forces as areas of future research for which the *O. grandiflora* system would be suitable. Genetic drift is a possible source of variation in floral traits between the populations, however it is unlikely that genetic drift is the sole explanation for the divergence we observed because of the critical importance of floral morphology and pollination ecology on plant fitness (e.g. Murcia 1990, Armbruster 2014), but we recommend that future research on this system might include a genetic component that could better assess a potential role for drift. Both colour and flower size (correlated with style length) were divergent in our study populations and both traits are known to be subject to abiotic selection pressures in other systems. In terms of flower colour, increased anthocyanin pigmentation imbues flowers with increased heat and drought tolerance (Strauss & Whittall 2006), as does decreased flower size (Sapir et al. 2005).

Biotic selection pressures driven by differences in pollinator community have been demonstrated to significantly impact a suite of floral traits such as flower colour, style length, and nectar volume in other systems (e.g., Temeles et al. 2009, Whittall & Hodges 2007), including in *Embothrium coccineum,* an Andean Proteaceae which also shows distinct pollinator communities in different populations (Chalcoff et al. 2008). Many of the observed differences in diurnal pollinator community between our study populations represented substitution of one species by another morphologically similar member of the same genus outside the range of the original species and may therefore not represent significant shifts in selection pressure on floral traits. For example, *Coeligena violifer* in Peru is replaced by *C. iris* in Ecuador (Tab. 4). Additionally, a single species, *A. cupripennis*, was the dominant visitor in both populations, and it is possible that individuals move along the entire geographic range of *O. grandiflora*, thereby promoting pollen flow between the two populations. While it is known that the North Peruvian Low acts as a dispersal barrier to other high-elevation species of hummingbirds (Chaves et al. 2011), it is unknown if it also acts as a barrier to the hummingbird pollinators of *O. grandiflora*. Future research should determine if these differences in diurnal pollinator community are sufficient to produce differential selection pressures on relevant floral traits in *O. grandiflora* and the degree to which the NPL may or may not impact gene flow.

Several of the floral traits that varied between our study populations are known to be subject to pollinator-driven selection pressure. Flower colour is frequently selected for by pollinators (Fenster et al. 2004; Cronk & Ojeda 2008). For example, whitish flowers such as those found in the Ecuadorian population, are typically associated with mammal- or insect- pollination (Fenster et al. 2004), while pink-magenta flowers, such as those found in Peru, suggest ornithophily. Hummingbird pollinator bill length and shape has been shown to exert selection pressure on floral traits such as style length (Temeles et al. 2009). Style length (Stroo 2000) and nectar volume (Opler 1983), which also varied significantly between our study populations, both scale with pollinator body size and are therefore larger in mammal-pollinated plants; in the current study, both were larger in Ecuador. Overall, the Ecuadorian study population exhibited more floral traits associated with mammal pollination. Indeed, visitation of *O. grandiflora* by nocturnal rodents and bats has been observed in other studies in Ecuadorian populations (Cardenas 2016). In comparison, no nocturnal pollination was observed despite some effort in the Peruvian population.

TABLE 3. Observed and recorded visitors of *O. grandiflora* in Ecuador and Peru. Numbers represent the percentage of visits observed during this study, a line (-) represents circumstantial observation, "yes" indicates that the species is limited to either Ecuador or Peru, while "no" indicates it may occur at both.

Species	Ecuador	Peru	Range restricted?
Diurnal Visitors (Trochilidae)			
Aglaeactis cupripennis	45	63	no
Boissoneaua matthewsii	0	15	yes
Chalcostigma mulsant	-	0	yes
Chalcostigma ruficeps	0	-	yes
Coeligena violifer	0	3	yes
Coeligena iris	4	0	yes
Colibri coruscans	-	13	no
Heliangelus amethysticollis	0	1	yes
Heliangelus viola	9	0	yes
Lesbia nuna	3	-	no
Lesbia victoriae	4	0	yes
Metallura tyrianthina	34	4	no
Patagona gigas	-	0	no
Ramphomicron microhynchus	1	0	yes
Diurnal Visitors (Thraupidae)			
Diglossa brunneiventris	0	-	yes
Diglossa cyanea	-	-	no
Diglossa humeralis	-	0	yes
Diglossa mystacalis	0	-	yes
Nocturnal Visitors			
Anoura geoffroyi (Bat)	-	0	yes
Cricetidae spp. (Rodent)	-	0	yes

Future studies should systematically document nocturnal pollination in *O. grandiflora* across its range. In addition to pollinator selection, heterospecific pollen competition has also been shown to select for style length in other systems (Ashman & Arceo-Gómez 2013). A visual inspection of hummingbirds with similar or identical body sizes visiting *O. grandiflora* at the two sites revealed that in Peru *O. grandiflora* pollen was deposited on the gorget feathers, whereas in Ecuador it was deposited on the belly or chest, presumably due to the differences in style length between the plant populations (J. Hazlehurst, *personal observation*). Future research into the role of pollinator community, heterospecific pollen competition, and abiotic factors on selection for floral traits should consider using *O. grandiflora* as a study system given the potentially interesting variations we observed between our study populations.

In terms of plant reproduction, both the Ecuadorian and the Peruvian populations were capable of selfing, and autogamous selfing in particular was observed at both sites. There was no significant effect of pollen treatment on fruit set in the Ecuadorian population, however there was a significant positive effect of the far treatment on seed set and mass in Peru as compared to the self-pollen treatment. Selfing in other Proteaceae can result in poor pollen tube growth while outcrossing results in positive effects on fruit set (Fuss & Sedgley 1991). It is probable that *O. grandiflora* is protandrous, like many Proteaceae, and in natural conditions selfing is avoided when pollen is removed from the presenter by visiting pollinators before the stigma

becomes receptive. When pollen is not removed, the pollen presenter may act as a kind of "bet-hedging" strategy, wherein selfing is a last-ditch effort at reproduction should no outcrossed pollen be available.

Our findings suggest that, as recommended by Prance (2008), the two-species question raised by Sleumer (1954) for the *Oreocallis* genus should be considered using an analysis of living plants in the field. An informal inventory by the authors of online records of *O. grandiflora* specimens collected across the range of the species at the Missouri Botanical Garden suggests a change in flower colour from magenta to white as one moves from south to north (Appendix I), though there are scattered reports of southern white populations and northern magenta populations. It is important to note that these reports may not accurately represent geographic variation in flower colour, as colour can be subjective and the reports were collected opportunistically. In reality the transition may be clinal, abrupt, or a mosaic based on local abiotic and biotic variables. Indeed, other montane Angiosperms show a geographic mosaic pattern in floral traits as well as pollinator community (Gómez et al. 2009*b*). An analysis of pollinator community, nectar properties, style morphology, pubescence, colour, and genetics should be undertaken along the geographic range of *O. grandiflora* to aid in resolving the taxonomic status of this species.

Conclusion

We found that the pollination ecology of *Oreocallis grandiflora* fit generally within what has been reported in other members of the Proteaceae, though it is of note that autogamous selfing was possible. We found significant divergence between the Ecuadorian and Peruvian populations in terms of floral morphology, nectar volume, nectar secretion rates, and daily patterns of nectar production. Based on our observations, we suggest that the *O. grandiflora* system could be an ideal study system for further study on the abiotic and biotic factors that shape floral traits and pollination ecology, for example pollinator-driven selection and heterospecific pollen competition. While replication of study populations at both the northern and southern latitudes of *O. grandiflora*'s range are necessary to quantify if and how the variation in floral traits and pollinator community that we observed correlates with geography, the authors nonetheless recommend further testing of the *O. mucronata* species concept. This study is a clear example of how ecology can reveal important differences between plant populations not evident from herbarium specimens alone.

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APPENDICES

Additional supporting information may be found in the online version of this article:

APPENDIX I. Map of *O. grandiflora* specimen colour

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