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REPRODUCTIVE PERFORMANCE OF *PIPTANTHUS NEPALENSIS* ALONG AN ELEVATION GRADIENT IN CENTRAL NEPAL

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ABSTRACT

Assessing altitudinal variation in reproductive outputs of a plant species provides important insights on the reproductive adaptation of plants towards the stressful environmental conditions. However, such studies are yet meagre, particularly with reference to Nepalese plants. In this study, we assessed the variation in reproductive performance of *Piptanthus nepalensis* along the elevation gradient in central Nepal. We considered ovule production, seed production per fruit, seed set proportion, ovule/seed abortion proportion, and individual seed mass as the various proxies of reproductive performance of *P. nepalensis* and estimated those traits at six sites from 3225 m to 3610 m asl within Gaurishankar Conservation Area, Dolakha, Nepal. We tested if the various reproductive performance traits of *P. nepalensis* were correlated with elevation. We also tested if an individual seed mass of *P. nepalensis* was correlated with an intrinsic factor, seed number per fruit. The result revealed that most of the studied reproductive performance traits were not related with the elevation. Moreover, individual seed mass also did not show correlation with seed number per fruit. The almost independent relationship of important reproductive outputs of *P. nepalensis* with elevation and seed number per fruit thus suggest that this plant species could endure wide ranges of abiotic and intrinsic stresses for achieving optimal reproductive performance and survivorship.

Keywords: Abiotic variables, allometric traits, elevation gradient, reproductive performance, survivorship.

INTRODUCTION

The elevational gradients of the mountain regions of Nepal provides excellent natural avenues to investigate plant responses to environmental changes because with the small change in elevation, most of the abiotic factors such as temperature, atmospheric pressure, rainfall, light intensity, humidity, and concentration of atmospheric gaseous change rapidly (Hovenden and Brodribb, 2000; Körner, 2007). Variation in these abiotic forces affects plants' performance both directly by influencing plant growth, physiology, morphology, pollination, reproduction and indirectly by modifying the length of growing season (Cordell *et al.*, 1998; Hovenden and Brodribb, 2000; Hultine and Marshall, 2000). In general, with increasing elevation partial pressure of all atmospheric gases, overall atmospheric pressure, concentration of oxygen and carbon dioxide, and air temperature decrease while solar radiation, particularly short wavelength UV-A (315-400 nm) and UV-B (280-315 nm) radiation increases (Diffey, 1991; Friend and Woodward, 1990; Rozema *et al.*, 1997, 2002). In the humid mountains such as temperate and subalpine mountains of the Nepalese

Himalayas, the growing season decreases sharply with the increase in elevation (Körner, 2007; Pato and Ramón Obeso, 2012). Moreover, the elevation gradient serves the best proxy to evaluate real time/site plants' performance under predicted global climate change because most of the abiotic variables varying with elevation are expected to vary with the global warming (Montesinos-Navarro *et al.*, 2011).

The variation in abiotic variables either related with increasing elevation or by global warming may impose different stress levels on plants (Beniston, 2003; Byars *et al.*, 2007). To cope with the stressful environment at higher elevation/accelerated climate change, plants either develop different adaptative strategies such as local adaptation by modifying morphological/physiological traits or developing innovative reproductive contrivances or exhibit altitudinal/latitudinal range shift (Ackerly *et al.*, 2002; Bresson *et al.*, 2011; Hirano *et al.*, 2017; Paudel *et al.*, 2019; Wang and Gao, 2004). Although, plants' responses particularly variation in distribution range, growth, morphology, and physiology to increasing elevation and predicted global warming have been widely studied both in the global and Nepalese context (Bastida *et al.*, 2015; Cordell *et al.*, 1998; Hulshof *et al.*, 2013; Hultine and Marshall, 2000; Kiełtyk, 2018; Körner, 2003; Li *et al.*, 2008; Pato and Ramón Obeso, 2012; Paudel *et al.*, 2019; Takahashi and Matsuki, 2017), to date studies on variation in reproductive performance of plants along the elevation gradient are meagre. Moreover, most previous studies are primarily focused to explore the responses of either tree species or herbaceous plants to elevation gradient while studies on shrubs are very limited.

Studies suggest that various extrinsic environmental conditions and intrinsic reproductive and life history constrains affect the reproductive outputs in plants (Guo *et al.*, 2010, 2012; Moles *et al.*, 2004; Primack, 1987). Change in elevation gradient generates the changes in most of the various environmental conditions, and thus study on the effect of elevation serves as the best proxy for assessing the impacts of extrinsic environmental conditions on the reproductive outputs of a plant species (Guo *et al.*, 2012; Moles *et al.*, 2004). Likewise, various intrinsic factors such as plant size and flowering phenology affect the reproductive output in plants. In particular, the individual seed mass is greatly affected by the fruit size and seed number per fruit. In some instances, the impact of extrinsic factors and intrinsic factors covary and thus the direct impacts of extrinsic factors are confounded (Primack, 1987). Thus, the impacts of both extrinsic and intrinsic factors are to be assessed while studying the variation in the reproductive outputs of a plant species at its various distribution ranges.

The Himalayan endemic Laburnum (*Pipanthus nepalensis*) is an almost evergreen shrub distributed at altitudes of 1600- 4000 m asl. Moreover, our observation indicates that despite the wide difference in distribution range, this plant species flowers at the same time across its lower to higher distribution range. Thus, this plant species serves as the model system to explore how stressful environmental conditions associated with increasing elevation and recent climate change influence on the reproductive performance of Himalayan shrubs. In this study, we addressed how the variation in extrinsic factors (abiotic factors associated with the increasing elevation gradient), and intrinsic factors affect the reproductive output of *P. nepalensis*. Here, we specifically tested (i) What is the natural level of seed set in *P. nepalensis* across different elevations, i.e., how does seed production per fruit in *P. nepalensis* vary across different elevations? (ii) How does seed set proportion in *P. nepalensis* vary across different elevations? (iii) How does ovule abortion proportion in *P. nepalensis* vary across different elevations? (iv) How does individual seed mass of *P. nepalensis* vary across different elevations? (v) Does the individual seed mass of *P. nepalensis* depend upon the seed production per fruit, i.e., is there a correlation between seed production per fruit and individual seed mass? The study on reproductive performance of plants along the elevation gradient provides valuable insights not only on how the changes in abiotic variables affect the reproductive fitness of plant but also how plants respond to environmental stress imposed by rapid change in climatic condition. Moreover, as the sub-alpine and alpine environments of the Nepalese Himalayas are more susceptible to predicted climate change and anthropogenic disturbances (Beniston, 2003; Byars *et al.*, 2007), the findings of this study would be instrumental in devising conservation strategies and mitigation measures against the impact of climate change on high mountainous Nepalese shrubs and herbs.

MATERIALS AND METHODS

Study species

Pipanthus nepalensis (Hook.) Sweet, a member of the shrubby genus belongs to the family Fabaceae. Of the two members of the genus, *P. nepalensis* has wider distribution and is distributed in Nepal, Bhutan, China, India, and Burma while another species of the genus, *P. tomentosus* is endemic to China. The study species, *P. nepalensis* is a medium sized shrub that exhibits variation in morphology, particularly in the arrangement of hairs on the leaves and other parts (Fig.1). It is distributed between the elevations of 1600- 4000 m asl and grows primarily through temperate to subalpine regions in coniferous forests, Montane grasslands, thickets and forest margins, and meadows (Shu, 2010). Like morphology, the habit of this Himalayan shrub is also variable; it is evergreen in mid sheltered sites while in other sites it shows semi-evergreen nature. Flowering occurs from Mid-April to late July. The flowers are large and bright yellow. Fruiting occurs from late June to early September. Fruits get completely matured towards the end of October. The detailed taxonomic description of the study species is found elsewhere such as Flora of China (Shu, 2010).

Figure 1: *Piptanthus nepalensis* at its natural habitat. A- A flowering twig, B-A fruiting individual

Study sites

The study was conducted along an elevation gradient in Gaurishankar Conservation area, Dolakha, Nepal (Fig. 2). The lowest elevation point of the study site is located close to Thangding and lies at an elevation of 3225 m asl while the uppermost point of the study site is close to Beding and lies at an elevation of 3610 m asl. The vegetation of the study site mainly comprises *Rhododendron-Abies* Forest. The site has a subalpine climate and cool weather throughout the year, and heavy snowfall from November to March. The geographical details and elevation of the study site is presented in Table 1.

Table 1: Geographic coordinates and elevation of study sites

S.N.	Latitude	Longitude	Elevation (m asl)
1	27.90361	86.31972	3225
2	27.90222	86.32861	3323
3	27.90306	86.33389	3373
4	27.90361	86.34472	3438
5	27.90528	86.3525	3532
6	27.90722	86.36028	3610

Figure 2: Map of the study sites

Assessment of reproductive outputs

We considered ovule number per flower, seed production per fruit (seed number per capsule), seed set success proportion, ovule/seed abortion proportion, and individual seed mass as the proxy measures of reproductive outputs of *P. nepalensis* along the different elevation gradient. We randomly selected six sampling plots along a vertical transect from 3225 m to 3610 m asl. We tried to establish the adjacent sampling plot at an elevation gap of *ca* 100m, but due to the unavailability of study species at the gap of every 100m, we randomly established six sampling plots in between the lowest elevation zone and uppermost elevation zone at which the study species was spotted in the study site. The details on the elevation gap of the six sampling plots is provided in Table 1. At each sampling plot, 40 fruits from the available cluster of the study species were randomly collected. Later from each fruit, the number of seeds per fruit and number of unfertilized ovules/aborted seeds were counted. Then seed set success proportion and ovule abortion proportion were estimated. The seed set success proportion was estimated as the ratio of the seed set success to total ovule number per flower while the ovule/ seed abortion proportion was estimated as the ratio of aborted ovules/seeds to the total number of ovules per flower. The seed mass of each seed from each of the fruit was calculated using a digital balance with three decimal point precision. We conducted the field sampling in late April 2023 to observe flowering phenology of the study species at different elevation points along the vertical transect while data on reproductive outputs were collected in the early September 2023.

Statistical Analyses

We first tested the data normality using Shapiro-Wilk test. Since, the data were not normally distributed, we performed square root transformation for all the response variables. We then performed generalized linear regression models (GLMs) to assess the variation in reproductive output of *P. nepalensis* along the elevation gradient. For each of the regression model, elevation was considered as a predictor while number of ovules per flower, seed number per fruit, number of aborted seed/ovules, seed set success proportion, seed abortion proportion, and individual seed mass were considered as the response variables. Likewise, the relationship between seed production per fruit and individual seed mass was also analyzed with a generalized linear regression model with seed production per fruit as an independent variable and individual seed mass as the dependent variable. All the regression analyses were performed in R 4.3.1 (R Core Team, 2023).

RESULTS

We found a significant negative correlation between elevation and number of ovules per flower, i.e., with the increase of elevation, the number of ovules per flower decreases. Likewise, number of aborted seeds/ovules per fruit also showed significant negative correlation with the elevation. For the rest of the studied variables, the relation was not significant, i.e., seed number per fruit, seed set proportion, seed/ovule abortion proportion, and individual seed mass did not change significantly with elevation (Fig. 3). Likewise, there was no correlation between individual seed mass and seed production per fruit (number of seeds per fruit).

DISCUSSION

Previous studies on other plant species reveal that the variation in reproductive outputs, particularly seed quantity and seed mass are the indicators of survivorship and fecundity of a plant species (Guo *et al.*, 2010; Moles *et al.*, 2004, 2005). The variation in the seed trait of a species across different ecological zones thus indicate the level of survivorship and fecundity of a plant species at a particular site/population (Moles *et al.*, 2005). Many previous findings suggest that most of the reproductive outputs are intricately related with both extrinsic factors such as variation in elevation and intrinsic factors such as plant height, resource allocation etc. (Guo *et al.*, 2010; Moles *et al.*, 2004, 2005). The finding from the previous studies suggest that the variations in reproductive outputs of plants are not highly consistent with increasing elevation as well as increasing seed production per fruit, however, in general there is a negative correlation between elevation/intrinsic factors and seed mass as well as other reproductive outputs (Baker, 1972; Bu *et al.*, 2007). In addition, few previous studies suggest a positive correlation between elevation and seed mass in congeneric lowland and alpine species (Pluess *et al.*, 2005), across species (Alexander *et al.*, 2009), and within species (Ayana and Bekele, 2000; Blionis and Vokou, 2002). These findings argue that with the increase of elevation, the photosynthetic rate and growing season decrease and thus the conditions become unfavourable for the enhanced reproductive outputs such as for the seed development and seed provisioning (Baker, 1972; Bu *et al.*, 2007). Likewise, several previous findings suggest the positive correlation between seed mass and plant size (Moles *et al.*, 2004; Moles and Westoby, 2004; Queenborough *et al.*, 2009). The increase in seed mass with increasing elevation indicates an adaptive response of a plant species towards competitive interactions among seedlings and dispersal requirements and/or plastic response of plant allometric traits towards local environment (Grubb *et al.*, 2005; Guo *et al.*, 2010; Moles and Westoby, 2004). Therefore, we anticipate negative correlation between seed number/seed mass/ seed set success proportion and elevation/seed number per fruit. However, contrary to the previous results and our current hypothesis, the current findings reveal that most of the important reproductive outputs in *P. nepalensis* are neither affected by the variation in elevation gradient nor by the seed production per capsule. This suggests that the current study sites though occur across different elevations and apparently serve as the different populations could be the single population of *P. nepalensis*. It further suggests that the current study sites could be the most optimal ecological zones of *P. nepalensis,* and thus the reproductive outputs are neither affected by elevation gradient nor by the allometric intrinsic factors of plants. Moreover, this finding consistent with the findings of Pluess *et al.* (2005) may indicate the little or no role of *in-situ* natural selection on reproductive performance of *P. nepalensis*. It further implies that shorter growing season or cooler temperature at the higher elevation has little or no effect on the reproductive performance of *P. nepalensis*, at least in the study sites and other similar ecological zones across the Nepalese mountains. However, our current finding is based on limited data of a single season, and also does not include other potential extrinsic factors such as various parameters of soil, biotic interactions (plant-pollinator interaction and potential variation in reproductive contrivances along the elevation gradient), and competition among the co-existing species. The study also does not fully include all the potential intrinsic factors such as plant height, resource allocation, above ground biomass, flower production per plant, and fruit size, all of these intrinsic factors could have profound effect on the reproductive performance of this plant species. Therefore, further study including all the potential extrinsic and intrinsic factors would be required to generalize the current finding in a broader context.

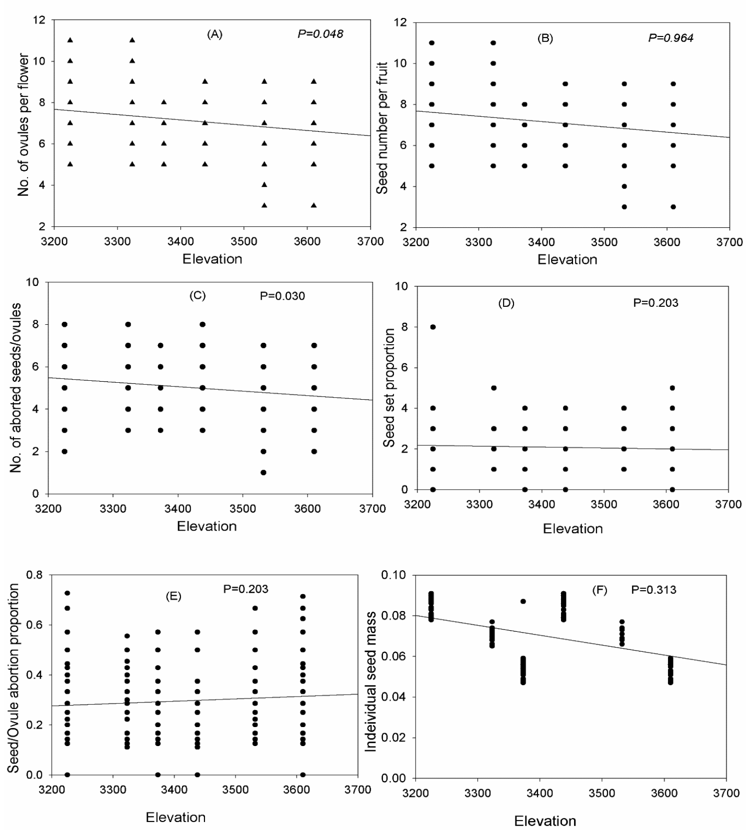

Figure 3: Generalized linear regression models showing the effect of elevation on various reproductive output parameters of P. *nepalensis*. A- Number of ovules per flower, B-seed number (production) per fruit C- Number of aborted ovules/ seeds per fruit, D- Seed set proportion, E- Ovule/ Seed abortion proportion, and F- Individual seed mass. Solid lines represent the regression function for each of the studied reproductive output parameters. P values indicate the level of significance.

Figure 4: Generalized linear regression model showing the effect of intrinsic factor (seed number per fruit) on the individual seed mass of P. *nepalensis*.

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