Variation in Adaptive Traits of an Endemic *Meconopsis napaulensis* DC. along an Elevation Gradient in Alpine Himalaya, Central Nepal

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
TUCH	Tribhuvan University Central Herbarium
KATH	National Herbarium and Plant Laboratories, Godavari
SPSS	Statistical Program for Social Science
GIS	Geographical Information System
TTC	Triphenyl Tetrazolium Chloride
PCA	Principle Component Analysis
LNP	Langtang National Park
GPS	Geographical Positioning System
DNPWC	Department of National Parks and Wildlife Conservation
BARDAN	Biodiversity Associates for Research, Development and Action, Nepal
DDC	District Development Committee
GoN	Government of Nepal
CDB	Central Development of Botany
m	Meter
cm	Centimeter
asl	Above Sea Level
^{0}C	Degree Celsius
mm	Millimeter
SE	Standard Error
SW	South West
TU	Tribhuvan University
e.g.	For example
LD	Lauribina Danda
LP	Lauribina Pass
GK	Gosainkunda

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ABSTRACT

Understanding the pattern of variation in adaptive traits of rare and endemic species along environmental gradient can suggest important implications for developing optimal strategies for species conservation and sustainable management.

In this study we aimed to study variation in life-history traits of an endemic species *M. napaulensis* DC. along an elevation gradient in Langtang National Park, Central Nepal covering three populations. Each population was investigated two times (2013 and 2014) covering different seasons. Population sampling was made to read habitat during peak growing period (during monsoon) except seed output, which was studied during late growing period (post monsoon). Entire area of each population was extensively surveyed to record all the individuals, including plants in flowering or fruiting and their detail vegetative characteristics and traits related to population fitness.

Analysis of the habitat features showed that *M. napaulensis* exhibited high habitat specificity. *M. napaulensis* was restricted to open and rocky habitats of high altitudes. *M. napaulensis* growing sites had low vegetation cover indicating decreased inter-specific competition. *M. napaulensis* showed high variation in traits due to altitudinal variation, climatic condition and disturbances. Specifically reproductive traits discriminated the populations. *M. napaulensis* was suffered from human disturbance mainly from livestock grazing, plant harvesting and garbage pollution as the study area is an important tourist destination and pilgrimage site. Conservation of rare and endemic species such as *M. napaulensis* requires strong provisions restricting human activities and minimizing the impact from harvesting and grazing; and implementing habitat restoration and population augmentation programs.

Key words: *Endemic, elevation gradient, population ecology, density, vegetative traits, reproductive traits.*

CHAPTER 1

INTRODUCTION

1.1. Background

Elevation gradient is correlated with several environmental factors. With the increasing elevation plant populations are subjected to gradually decreasing mean temperature, and shorter growing season (Landolt, 1967; Körner, 2003). Elevation gradient is therefore ideally suited for examining variations in species' traits, which strongly influence fitness (Minden, 2010), including growth and competition (Wright *et al.,* 2006). Plant adaptive traits are evolved in response to the changing environmental conditions, and therefore exhibit considerable variations along elevation gradients (Westoby and Wright, 2006), including gradients associated with disturbance (Daiz *et al*., 1999; Wana and Beierkuhnlein, 2009). When comparing plant populations growing on lower elevation areas with that of higher elevation, an obvious adaptation of high altitude plants to the adverse environment is found to be the reduced size (Jenny-Lips, 1948; Körner, 2003). Plant species growing along the elevation gradient show considerable variations in life history strategies, including structure of their populations and demography (Kim and Donohue, 2011). Studies pertaining to variation in adaptive traits along the elevation gradient may provide opportunities to examine performances of plant populations under environmental changes (Kim and Donohue, 2011).

High altitude areas, such as the high Himalaya, representing the uppermost ecosystem along the elevation gradient, are highly vulnerable to natural and human-induced drivers of change. In high altitude ecosystem, plant populations are subjected to increasing level of anthropogenic pressure, created by habitat destruction, deforestation, and overexploitation (Ghimire *et al.,* 2008; Sharma *et al*., 2009). Such areas are particularly vulnerable to natural variation in climate (Sano *et al.,* 2005; Cavaliere, 2009; Salick *et al.*, 2009). The Himalayan Mountains are vulnerable to extreme climate change in term of global warming in the last 150 years. Studies have revealed that the last 30 years has already affected the Himalaya as many of the mountain glaciers have thinned and retreated (Ives, 2006). In such scenario of environmental change, populations of rare endemic species are particularly vulnerable as their distribution is highly restricted (Korner, 2002; PAN, 2009; Sharma *et al*., 2009). Studies have shown that populations of rare endemic species exhibit reduced size, altered population structure, reduced fitness and ultimately faced greater extinction risk in response to altered environmental conditions at high altitudes (Brune and Kress, 2002; Lavergne, 2005; Colling and Matthies, 2006; Dar *et al.,* 2006). Reduced fitness in many rare and endemic species is associated with limited success in sexual reproduction, which can be attributed to reduced pollination, failure in the formation of viable seeds, increased herbivory and predation pressure (Morely, 1982; Menges *et al.,* 1986; Karran, 1987; Lavergne, 2005; Albert *et al.,* 2005).

Seed size is the prominent life history trait that affects seed dispersal, seedling establishment and survival (Leishman *et al.,* 2000). Under the increasingly adverse ecological conditions along altitudinal gradient, seedlings from heavy seeds might be more successful in establishment and survival (Leishman *et al*., 2000; Moles and Westoby, 2004). Studies have shown that alpine plant species exhibit reduction in seed size with increasing elevation. Low temperatures and short growing seasons at high elevations are the two most important factors affecting seed maturation and seed weight (Totland and Birks, 1996; Totland 1997a; Wagner and Reichegger, 1997; Baskin and Baskin 1998; Blionis and Vokou, 2005).

In alpine environments, plant demography is often characterized by low seedling recruitment and high mortality at early developmental stages compared with lower-elevation populations (Billings and Mooney, 1968). Demographic rates in plants are usually stage-dependent (Harper, 1977), the structure of a population may be indicative for its demographic future. In general, it has been widely regarded that population size is the most important factor influencing reproductive success, although the exact mechanisms remain often unclear. Moreover, lowered seed output is one of the major threats to plant life-history processes influencing the population age-stage structure directly, and may increase the probability of extinction of populations and species in the long-run (Lennartsson, 2002). Altitudinal differences in stage-specific mortality correspond with differences in life history, especially at the inter-specific level, such that long-lived, frequently iteroparous, perennials are dominant in alpine habitats (Billings and Mooney, 1968).

Long-term persistence of a species population depends on continuous regeneration (Thakuri, 2010). Regeneration of a species is greatly influenced by habitat conditions. However, natural regeneration of a species largely depends on production and germination of seeds and the establishment and survival of seedlings. Potentialities of a species' regeneration can be depicted through the analysis of population structure (i.e. the proportion of plant individuals classified into different stage/age classes) (Bharali *et al*., 2012). Study on the variation in population structure along elevation gradients would be helpful in understanding the influences of environmental factors on regeneration (Wang *et al.,* 2004).

The present study was conducted to assess the responses of *Meconopsis napaulensis* populations along the elevation gradient in north-central Nepal. *Meconopsis napaulensis* is an endemic species, distribution of which is restricted to the alpine areas of Langtang National Park, north-central Nepal. The conservation and management of rare and endemic species is a major challenge in the Himalaya. The genus *Meconopsis* as whole is one of the critically threatened taxa (Sulaiman and Babu, 1996). Among the species in *Meconopsis*, rare and endemic ones, such as *M. napaulensis*, are predicted to be more vulnerable. This study explores habitat properties, population structure and fitness-related traits of *M. napaulensis* along elevation gradient, which can provide a link between population responses to environmental change.

1.2 Objectives

The overall objective of this research is to investigate the variation in habitat characteristics, population structure, and fitness-related traits of *Meconopsis napaulensis* along elevation gradient in its native range in Langtang National Park, north-central Nepal.

Specific objective are:

a. To study habitat characteristics of *Meconopsis napaulensis*.

b. To study variation in fitness-related vegetative and reproductive traits of *Meconopsis napaulensis* along elevation gradient.
c. To study variation in population structure and density of *Meconopsis napaulensis* along the elevation gradient.

CHAPTER 2

MATERIALS AND METHOD

2.1 Study Species

2.1.1 Distribution

The genus *Meconopsis* Vig. (Family: Papaveraceae) consists of 55 species distributed in the Himalaya (Egan and Shrestha, 2011). In total, 22 species are reported from Nepal, out of which 11 are endemic to high altitude areas between 2400-4900 m (Grey-Wilson, 2006). Most of the endemic species of *Meconopsis* are restricted in Central Nepal; there are only three endemic species in Western Nepal (i.e., *M. chankheliensis*, *M. simikotensis* and *M. regia*) and one (*M. dhwojii*) in Eastern Nepal (Egan and Shrestha, 2011) (Appendix I). The endemic *M.* napaulensis is very local in distribution and is known only from Gosainkunda and Ganesh Himal area of Langtang National Park, and its adjoining region in Rasuwa district, central Nepal (Grey-Wilson, 2006). It is found in rocky, grassy slopes, open shrub berries, open rocky shrub, rocky grassland and stream- margins at 3200-4500 m asl (Egan and Shrestha, 2011).

2.1.2 Taxonomy

Meconopsis napaulensis DC., Prodr. **1**:121. (1824).
Type: Wallich, Nepal, 1821 (G-DC, Holotype; K; isotype).
Monocarpic herb with dauciform tap root. Stem ascending 30-160 cm in height and 0.8-2.0 cm in diameter. Indumentum barbellate-bristly. Leaves cauline and in basal rosette. Lamina pinnatisect towards the base and pinnatifid towards the leaf apex, with 6-9 sub

opposite pairs of segments. Inflorescence racemose or paniculate with 2 or 3-flowered lateral cymules, flowers solitary above, pedicels 3-14 cm. Petals 4, pale yellow, obovate, pappery, delicate and soft, anther yellow to orange-yellow, styles 9-14 mm, stigma yellow-green, ovary densely bristles, capitate with 6-lobed, capsule oval to narrow ellipsoidal. Seeds ellipsoid-oblong, smooth, rugose. Flowering in June–August; fruiting in August- September.

Meconopsis napaulensis in its natural habitat.

Meconopsis napaulensis in flowering

Meconopsis napaulensis in fruiting A bumble bee visiting the flower

Fig.1: Study species (*Meconopsis napaulensis)* in alpine rocky area of LNP.

2.1.3 Ecology

The members of the genus *Meconopsis* are poorly known with respect to their reproduction traits, fitness and population ecology. Sulaiman and Babu (1996) found that *Meconopsis* species are habitat specialist. In *Meconopsis* a large number of seeds are produced but most of them are exposed to high level of insects, fungal, and viral infections (Sulaiman and Babu, 1996). Germination and seedling recruitment have also been reported to be very low both in natural habitat and under laboratory conditions (Xie *et al.,* 2002). Several study conducted in different species of genus *Meconopsis* revealed existence of physiological dormancy hindering seed germination (Sulaiman 1993; Dar *et al.,* 2009). Some species of *Meconopsis* (i.e., *M.dhwojii, M.gracilipes, M. horridula* and *M.regia*) relatively required higher temperature for seed germination that resulted in a high germination percentage (Thompson, 1968). In *M. latifolia* embryo grows in autumn but physiological dormancy is not broken until seeds receive cold stratification during winter and seeds germinate only in spring (Dar *et al.,* 2009).

2.1.4 Uses

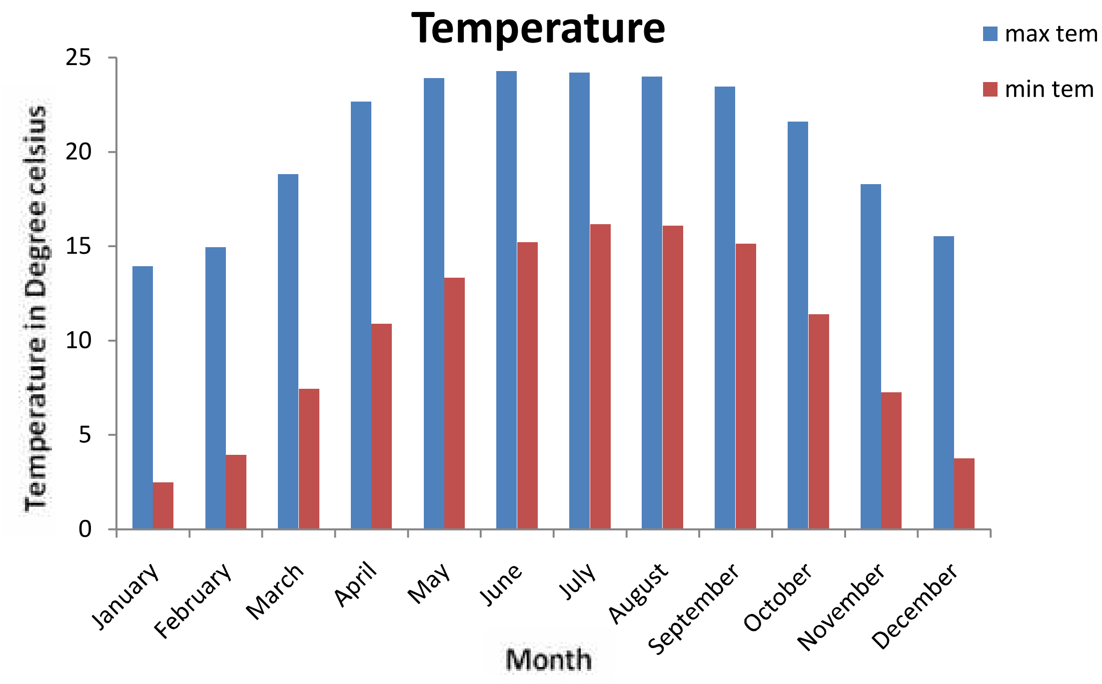
The members of genus *Meconopsis* are widely used as ornament and traditional medicine. Medicinally they are used for the treatment of inflammations, pain, hepatitis, fever, bile disorder, swelling of limbs and even for the broken bone to cancer (Xie *et al*., 2002; Ghimire *et al.,* 2008). In Gosainkunda region, root and leaf of *M. napaulensis* are used to treat digestive system disorder, chest pain, sore throat and headache (Poudeyal, 2010).

2.2 Study Area

The study was undertaken in upper Trishuli valley between Lauribina danda to Gosainkunda in North-central part of Langtang National Park (LNP). Langtang National Park (LNP) was established in 1976 to conserve the unique flora and fauna of the region. LNP covers an area 1,710 sq km. It is located between the latitude 28^{o}28’20”N and longitudes 85^{o} 15’86”E. Elevation in the park ranges from 792 to 7245 m. The area is rich in terms of flora and vegetation. Over 15 types of vegetation have been described belonging to tropical, subtropical, temperate, subalpine and alpine zones (Stainton 1972; Malla *et al*., 1976; Chaudhary, 1998). The focused study area covers alpine zone with elevation range of >4000-4500 m asl. *Meconopsis* species are the important component of vegetation on alpine zone in upper Trishuli valley.

2.2.1 Climate

The climate of the park is influenced by the south-west summer monsoon. Temperatures vary greatly due to the extreme differences in elevation in the entire area. Most of the precipitations occur from June to September. There was no meteorological station in the Gosainkunda area. Meteorological data of Department of Hydrology and Meteorology for the last 15 years (1995-2009) of nearest meteorology station (Dhunche, Rasuwa district; 1950 m asl), showed an average maximum temperature of 24.2^{0} C in June and average minimum temperature of 2.88^{0} C in January (Fig. 2). The average precipitation was 2053.53 mm. The study area falls on quite high altitude (>4000 m asl), therefore the precipitation is expected to depend on aspect, altitude and rain shadow effect. In high altitude region, the accumulation of snow occurs above 5500 m asl in summer and down to 4000 m asl in autumn, but in winter it starts accumulating from 2500 m asl.



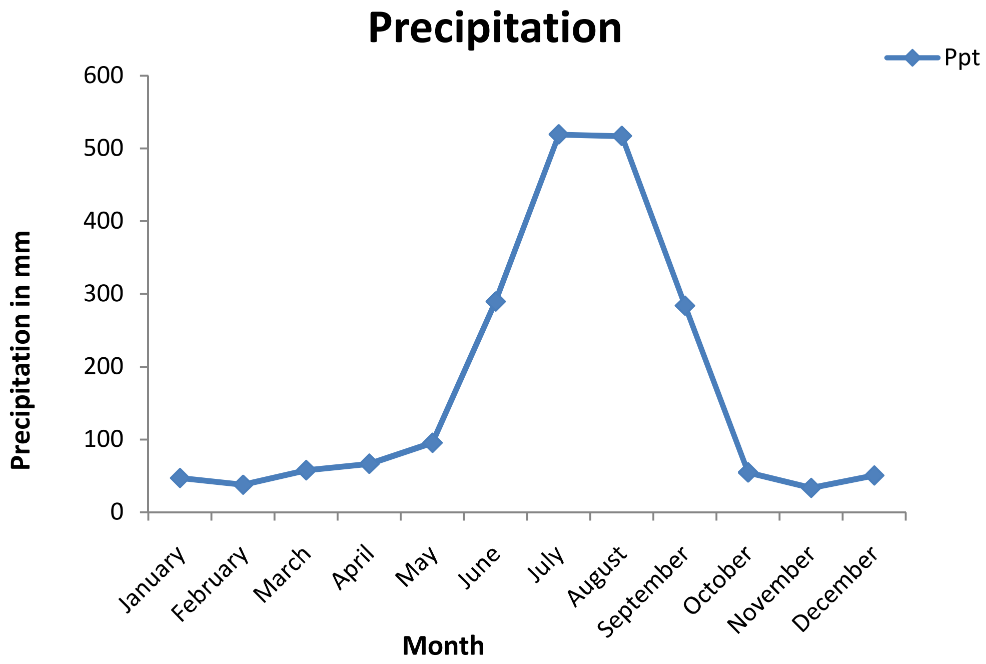


Fig 2: Fifteen years (1995-2009) average minimum and maximum temperature and average precipitation recorded at Dhunche, Rasuwa district (1950 m asl), Langtang National Park (*source:* Department of Hydrology and Meteorology, Government of Nepal, GoN, (2005).

2.2.2 Vegetation and flora

In Nepal, LNP is considered as one of the biodiversity hot-spot areas. The alpine meadows between 4000-5000 m asl consists of varied herbaceous and graminoid species including *Gentiana dipressa, Bistorta macrophylla, Pedicularis longiflora, Anemone demissa, Androsace tapete* and *Campanula* sp. LNP is particularly known for high diversity of rare endemic plants. A total of 47 species of narrow endemic taxa of flowering plants have been recorded within LNP and adjoining areas, out of which 18 species are endemic to this region (Ghimire *et al.,* 2008). The major endemic taxa confined to high-altitude region of Gosainkunda area include *Arenaria globiflora, Berberis mucriflora, Crotalaria kanaii, Delphinium himalayai, Heracleum lalli, Impatiens harae, Maharanga wallichaina, Meconopsis napaulensis, Pedicularis pseudoregeliana, Pleurospermum rotundatum, Primula aureate, Primula sharmae, Schulzia nepalensis and Thalictrum* (Malla *et al.*, 1976; Ghimire *et al.,* 2008). Despite the presence of unique flora and vegetation, LNP is experience rapid habitat loss due to high level of anthropogenic impacts. The most of the high altitude area in LNP is excessively used as seasonal pastoral land and for harvesting medicinal plants and other non-timber forest products (Chaudhary 1998; Myint *et al.*, 2000).

2.3. Selection of Study Populations and Sampling

2.3.1 Study populations

The study was completed in two field visits (first visit was made in 2013 and second visit in 2014). The distribution of *M. napaulensis* populations in the study area was recorded with the help of GPS device and consultation with local people (Fig. 3). Based on the information obtained from field survey, as well as from literature and herbarium study, the whole of the distribution range of *M. napaulensis* was divided into three distinct elevation levels each representing distinct population: (i) lower elevation (4117-4125 m) in Lauribina danda, (ii) mid elevation (4200-4255 m) in Lauribina pass, and (iii) higher elevation (4396-4417 m) in Gosainkunda.

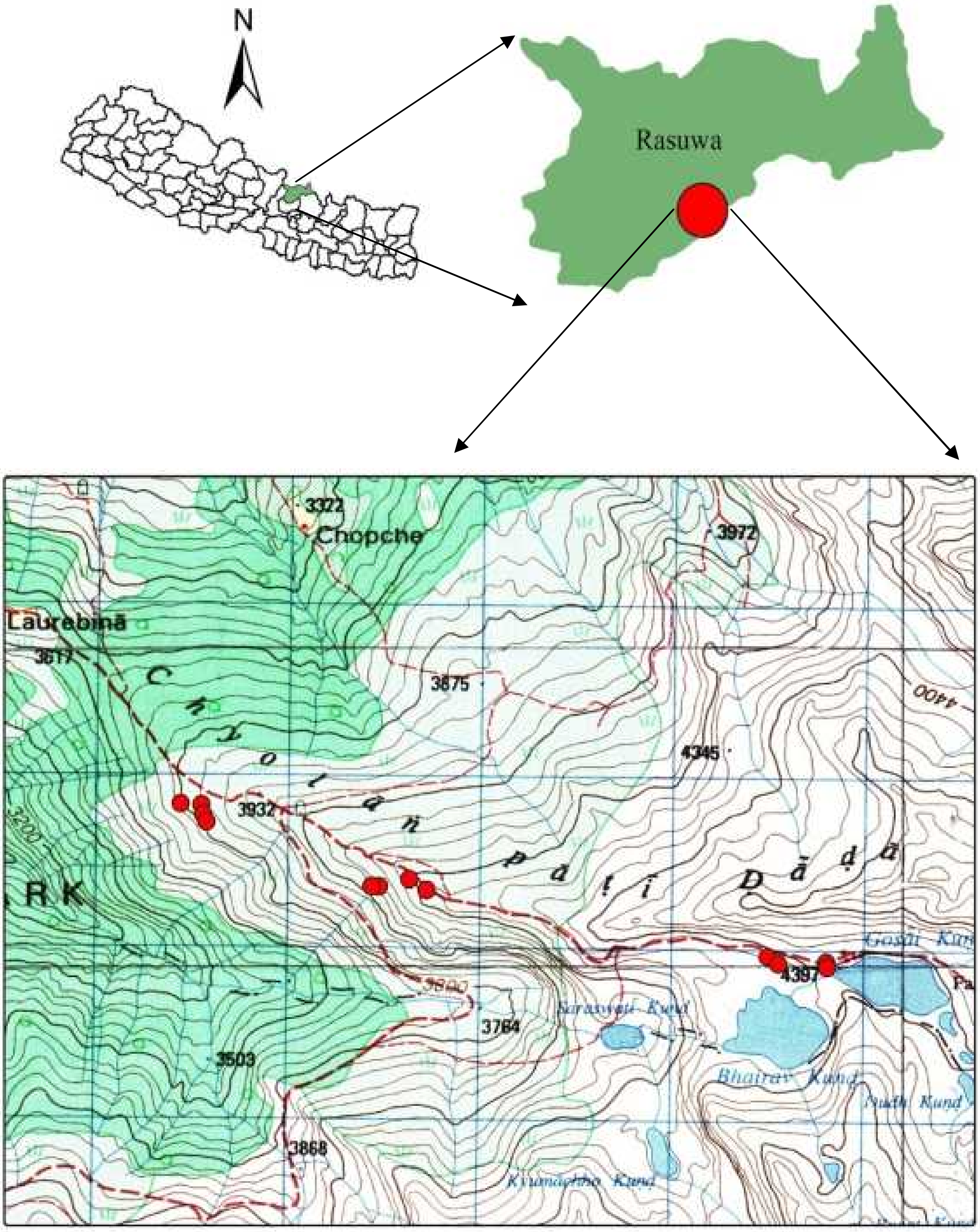


Fig 3: Map showing the location of study populations of *M. napaulensis*.

Table1: Physical parameters of sampling sites of *Meconopsis napaulensis*.

Elevation level (population)	**Elevation (m asl)**	**Aspect (^{0})**	**Habitat type**
Lower (Lauribina danda)	4117 - 4125	SW	*Rhododendron lepidotum* dominated open shrub land.
Mid (Lauribina pass)	4200 - 4255	SW	Open rocky shrub land dominated by *Berberis erythroclada* & *Potentilla coriandrifolia.*
Higher (Gosainkunda)	4396 - 4417	SW	Rocky grassland

2.3.2 Sampling strategy

In each elevation level, *M. napaulensis* was sampled in four plots of 10 × 10 m size. The plots were laid systematically in the area where *M. napaulensis* density was high. Each plot was divided into 4 subplots (5 × 5 m); and thus altogether 48 such subplots were sampled from all the three elevation levels. Most of the population parameters were collected during peak growing period in July (during monsoon) except seed output, which was studied during late growing period in September (post monsoon season).

2.4 Data Collection

2.4.1 Population censuses and change in density

In each subplot, presence/absence of plant species associated with *M. napaulensis* was recorded. In both the years (2013 and 2014), sampling consisted of recording all the individuals of *M. napaulensis*, classified into four different life cycle stages, on the basis of plant size, reproductive stage and the number and size (length) of the largest leaf; small rosette (leaf number 1-7; largest leaf length 1-6 cm), juvenile rosette (leaf number 3-28; largest leaf length >6-14 cm), large rosette (leaf number 4-45; largest leaf length >14 cm), and reproductive adult (with flowering/fruiting peduncle). Each of these individuals was tagged by aluminium tag in 2013 and was monitored for the change in population structure in 2014.

2.4.2 Habitat variations

Longitude, latitude and elevation were recorded for each sampling plot with the help of global positioning system device (GPS, eTrex Garmin). Elevation data was cross-checked with an altimeter. Slope and aspect were recorded by a clinometer-compass. Habitat parameters for each subplot further included soil pH, soil moisture, litter depth, vegetation composition, and ground surface cover by vegetation or physical components of the environment. Soil moisture and pH were recorded by using a gauge (soil pH and moisture tester; model DM 15) with a default scale of 1 to 8 for moisture and 1 to 7 for pH recording. Similarly, soil depth was measured by inserting an iron peg. In each subplot, pH, moisture and depth were measured diagonally at three different points.

Anthropogenic disturbance was recorded in each plot. The disturbance variables included harvesting, garbage pollution and grazing. As Gosainkunda is known for its pilgrimage, more pilgrims visit every year in August especially in 'Janaipurnima'. They pluck flowers for offering to God and also pluck roots, fruits and seeds for medicinal purposes (based on interview with local people) so there is high rate of harvesting. In addition, garbage pollution was recorded along the trekking route due to more flow of peoples for trekking which turned more use of things along the trekking route (based on interview with local people). Harvesting impact was recorded by direct observation of plant uprooting or by observing the scar left after plucking of flowers and/or fruits. Each type of disturbance was scored by categorical scale as no disturbance, moderate disturbance, high disturbance, very high disturbance.

2.4.3 Vegetative and reproductive attributes

All the individuals from each subplot were marked in 2013 by aluminium tag. Each individual was thoroughly inspected for recording a number of vegetative and reproductive traits having significant adaptive value to the plant. Parameters recorded from the individuals in rosette stage (non-reproductive) were total number of leaves, and length of largest leaf. Parameters recorded from the individuals in reproductive adult stage included plant height; and the number of buds, flowers and fruits (capsules).

The number of flowers and buds were counted in flowering period and number of capsule in fruiting period. During flowering period, 5 flowering individuals from each population were randomly selected and their flowers (n = 2) were collected for the study of pollen viability. Similarly, during fruiting period, 5 matured individuals were marked per population for capsule harvesting. From each such plant, at least five matured but unopened capsules were collected. Ten such capsules were randomly selected per population for the measurement of seed size (in terms of seed mass) and seed viability. Capsule size was measured with the help of Vernier calliper. Both length and diameter of capsule was measured. Diameter was recorded as the mean of upper, middle and lower portions of the capsule.

2.4.4 Pollen and seed viability

Glycero acetocermine (1:1) mixture was used for the treatment of pollen viability test assessed by Belling's Iron-Aceto-Carmine staining method (Singhal and Kumar, 2008). Pollen grains from each flower were treated with Glycero acetocermine (1:1) mixture and were studied under compound microscope. Well–filled pollen grains with stained nuclei were regarded as fertile/viable, while shriveled and unstained pollen were counted as sterile. Similarly, for the seed viability, Triphenyl Tetrazolium Chloride (TTC) test (Baskin and Baskin, 1998; Lin *et al*., 2001) was used. In this process, seeds of *M. napaulensis*, collected from different populations, were cut into two equal halves in such a way that each part got a portion of embryo. Then treated with 1% solution of TTC (Dar *et al*., 2009) and observed under stereo-microscope. Seed viability was revealed by pink TTC precipitation produced by dissected seeds. Embryos that turned pink were considered as viable and other as non viable.

2.5 Data Analysis

2.5.1 Habitat variation

Habitat characteristics of *M. napaulensis* were evaluated by non-parametric Kruskal-Wallis one-way analysis of variance (ANOVA) as the data was found not normal. Variables related to anthropogenic impact were combined by using Principle Component Analysis (PCA) to obtain overall measure of disturbance. In this process, impact of harvesting and garbage pollution was combined as an overall measure of human impact and was obtained explaining 63.49% variance and grazing as measure of livestock impact.

The number of associated species present per subplot were combined to calculate abundance in an ordinal scale from 0 to 4 where 0 (absent in all subplots) and 4 (present in all subplots). The abundance data of 71 associate species from all 48 subplots were used to calculate their frequency and dominance mean value in lower, middle and higher elevation sites.

2.5.2 Variation in population density and structure

Variations in population density and structure were studied at subplot level (5 x 5 m) Population structure indicated the proportions of individuals of different life cycle stage (small rosette, juvenile rosette, large rosette and adults). Variation in population density among elevation sites was compared using Kruskal-Wallis test.

2.5.3 Variation in vegetative and reproductive growth traits

Vegetative and reproductive traits were computed for each adult individual in the respective elevation site. The total data set comprised of 75 adult individuals. Sample size for estimating capsule production comprised of 69 individuals of which 25 each individual from lower and middle site and 19 individual from higher site, each individual seeds weight were recorded. Similarly, sample size for viability test for seed and pollen comprised of 30 individuals of which 10 each individual from lower elevation to higher elevation.

Kruskal-Wallis test and independent sample Wilcoxon test were used to assess the variation in vegetative and reproductive traits, including seed and pollen viability, among and between study sites. SPSS version 16.0 was used for all statistical analysis. Arc GIS 9.3 version was used for mapping.

CHAPTER 3

RESULTS

3.1 Habitat Characteristics of *Meconopsis napaulensis*

Meconopsis napaulensis was recorded from open rocky habitats in south-west facing slopes in Upper Trishuli Valley of Langtang with main vegetation type being dwarf *Rhododendron* and *Berberis* shrubland, subalpine to alpine meadows and rocky slopes. *M. napaulensis* occurred on gently south-west facing slopes towards higher elevations receiving high solar radiation. Altogether, 71 plant species (belonging to 47 genera and 33 families), associated with *M. napaulensis,* were identified in the study area (Appendix II). The dominant species associated with *M. napaulensis* in Lauribina danda (4117-4125 m asl) were *Potentilla coriandrifolia, Kobresia pygmaea, Kobresia nepalensis, Anaphalis nepalensis, Rhododendron lepidotum, Berberis erythroclada* (Appendix II a). Similarly, in Lauribina pass (4200-4255 m asl), the dominant species were *Rhododendron lepidotum, Potentilla coriandrifolia, Viola biflora, Saxifraga parnassifolia*., *Kobresia nepalensis* (Appendix II b) and those in Gosainkunda (4396-4417 m asl) were *Kobresia pygmaea, Bistorta vaccinnifolia, Taraxacum eriopodon, Aster himalaicus* and *Viola biflora* (Appendix II c). The most frequently found species in all the three sites were *Kobresia pygmaea, Kobresia nepalensis, Viola biflora* and *Anaphalis nepalensis.* Asteraceae was the dominant family in the study area comprising 11 species and 7 genera, followed by Rosaceae, Saxigragaceae, Polygonaceae, Crassulaceae, Rananculaceae, Cyperaceae and Lamiaceae (Appendix II).

The habitats of *M. napaulensis* differed significantly for 6 out of 15 variables studied (Table 2.). Coverage of herbs was greater at higher elevation site and that of shrubs was higher at lower elevation site (Table 2). The litter coverage was found significantly higher at lower elevation which showed that habitat at lower site was more fertile than upper site. Among the edaphic variables, value of soil depth was high at lower site. As the elevation increases, more rock and scree are found due to which the amount of soil and its depth reduced at high altitude.

Level of human disturbance was high in plots from higher elevation sites. In general, higher proportions of sub-plots in populations at Gosainkunda and Lauribina pass received higher levels of garbage pollution and high livestock grazing pressure (Fig 4).

Table: 2. Habitat characteristics of *Meconopsis napaulensis.* The values shown in the first four columns are mean ± SE.

Habitat Attributes	Lauribina danda (LD)	Lauribina pass (LP)	Gosainkunda (GK)	Overall	χ^2	p-value*
Elevation (m asl)	4121±1.03	4227.5±7.10	4406.5±2.71	4251.66±17.36	42.98	<0.001*
Litter cov%	1.16±0.09	0.97±0.06	0.75±0.12	0.96±0.06	10.98	0.004*
Solid rock cov%	36.69±2.64	30.94±3.64	41.81±5.67	36.48±2.45	3.04	ns
Scree cov%	0.00±0.00	0.84±0.33	0.50±0.17	0.45±0.14	11.76	0.003*
Lichen cov%	1.59±0.09	1.44±0.15	1.41±0.16	1.48±0.08	0.91	ns
Moss cov%	2.41±0.21	2.69±0.36	1.84±0.22	2.31±0.16	5.08	ns
Shrub cov%	14.75±1.48	16.00±2.76	1.19±0.72	10.65±1.43	28.22	<0.001*
Herb cov%	13.00 ± 1.45	20.57±2.36	23.13±2.98	18.89±1.47	8.62	0.013*
Grass cov%	29.25±1.61	25.87±2.65	28.88±4.39	28.00±1.77	1.67	ns
Tot vas cov%	57.00 ± 2.51	62.44±3.52	53.19±5.57	57.54±2.36	2.09	ns
No. of ass. species	33.25±1.39	33.50±1.07	31.25±2.01	32.67±0.88	0.32	ns
Soil pH	6.55±0.06	6.63±0.04	6.56±0.06	6.58±0.03	0.89	ns
Soil moist	6.18±0.21	5.84±0.38	6.34±0.15	6.12±0.15	1.90	ns
Soil depth	17.68±0.19	8.6±0.45	8.69±0.34	11.66±0.65	32.78	<0.001*
Litter depth(cm)	1.44±0.18	1.41±0.15	1.04±0.09	1.29±0.09	4.68	ns

*Significance test to detect differences among populations in the subset of environmental variables based on non parametric Kruskal –Wallis test (N=47; df=2); chi-square (¡ ^{2}) values and only significant p values are shown (ns= the difference is not significant at p=0.05 level).

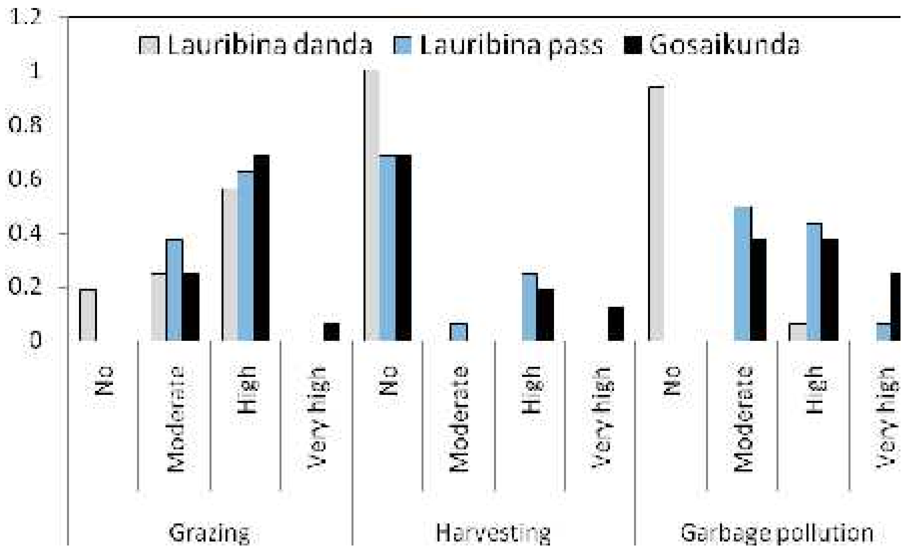


Fig 4: Level of disturbances in three study sites. Bar represents proportion of sub-plots receiving different levels of disturbance.

3.2 Population density

Density of small rosette (SR), juvenile rosette (JR), large rosette (LR) and adult of *M. napaulensis* in the entire study sites were found to be 0.072±0.016, 0.124±0.029, 0.194±0.027 and 0.080±0.018 (mean ± SE) individuals per 25 m^2. The overall density combining all four stage classes was 0.470±0.061 individuals per 25 m^2. The value of total plant density tended to be high at higher elevation site, but the result was statistically insignificant (Table 3).

Table 3: Density (number of individual per 5 x 5 m plot) of *M. napaulensis* in different stage classes recorded in three elevation sites in Upper Trishuli Valley. Data shown are mean ± SE.

	Lauribina danda (LD)	Lauribina pass (LP)	Gosainkunda (GK)	Overall	χ^2	p-value
Small rosette(SR)	0.07±0.03	0.08±0.03	0.07±0.02	0.07±0.02	0.371	0.831
Juvenile rosette(JR)	0.12±0.05	0.09±0.04	0.16±0.06	0.12±0.03	1.301	0.522
Large rosette(LR)	0.18±0.04	0.15±0.04	0.25±0.06	0.19±0.03	1.821	0.402
Reproductive(Rep)	0.03±0.01	0.13±0.04	0.07±0.03	0.08±0.02	8.479	0.014*
Total (Total)	0.41±0.10	0.46±0.10	0.55±0.12	0.47±0.06	0.632	0.729

¡ 2 and p-value based on non-parametric Kruskal-Wallis tests.

3.3 Population structure

The proportions of small rosette (SR), juvenile rosette (JR), large rosette (LR) and adult individual in the entire study sites were 0.175, 0.233, 0.438 and 0.154 respectively. In general, *M. napaulensis* exhibited almost similar population structure with high contribution of SR, JR and LR than adult (Fig. 5). Proportion of SR (mean ± SE= 0.175±0.040; χ^2=0.438, *P*=0.803) tended to decrease but adult proportion (0.154± 0.028), χ^2=12.977, *P*=0.002) significantly increased in populations from lower to higher elevation sites. Proportions of JR (0.233±0.036, χ^2=1.645, *P*=0.439) and LR (0.438±0.043, χ^2=2.000, *P*=0.368) remained almost identical in all the three sites.

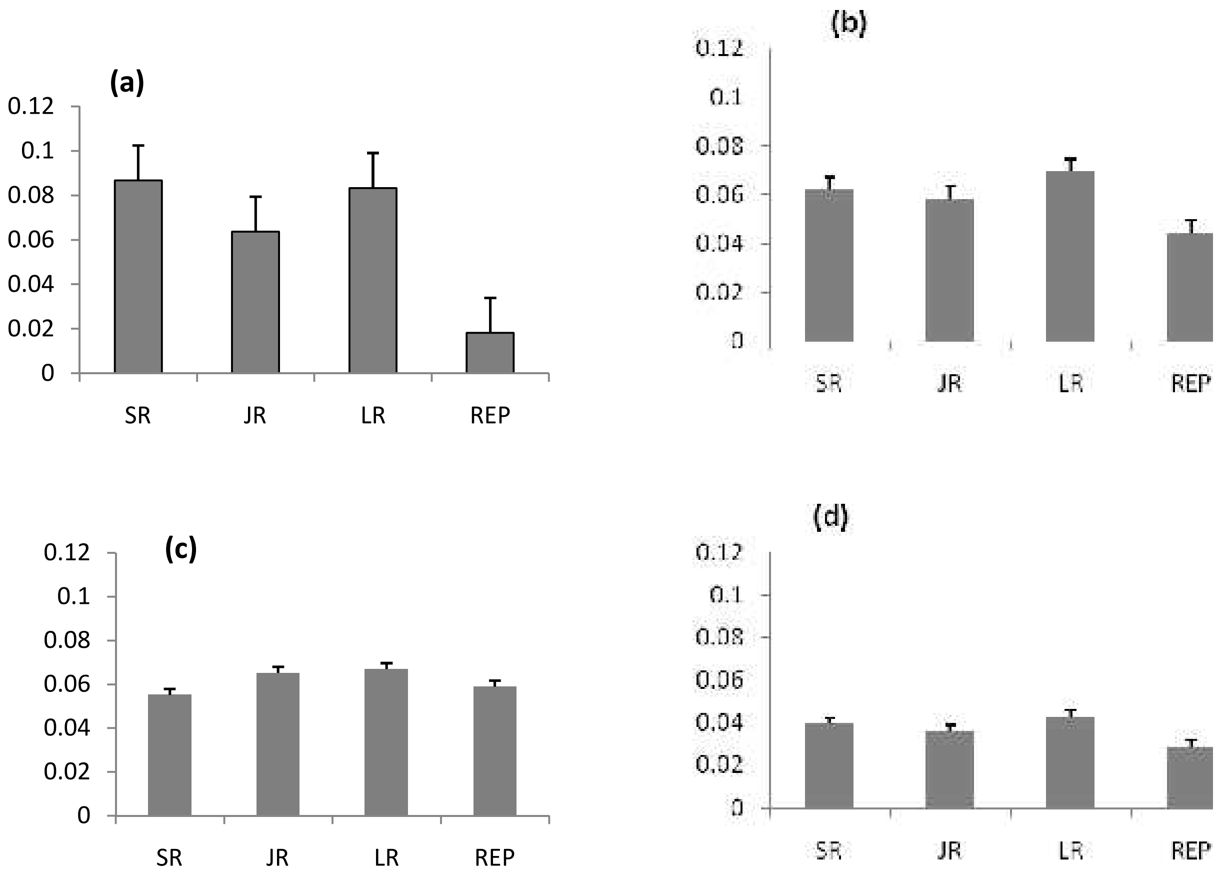


Fig 5: Population structure [proportions (mean ± SE) of small rosette, juvenile rosette, large rosette and adult] and overall pattern of *M. napaulensis* at three elevation sites in Upper Trishuli valley of Langtang. (a-c): (a)Lauribina-danda (4117-4125 m), (b)Lauribina pass (4200-4255 m), (c)Gosainkunda (4396-4417 m) and (d)Overall population structure.

3.4 Vegetative and reproductive traits

Among the vegetative and reproductive trait studied, 8 traits are statistically differed among 12 traits (Table 4). Also, among all the traits studied, number of bud per individual, number of capsule per individual and capsule length showed variation along the altitude. Secondly, number of flowers, plant height, seed viability, pollen viability and seed mass per capsule showed comparatively less variation along the altitude. The number of buds, flowers and capsules were reduced about 50% when the plants from Lauribina-danda(LD) and Gosainkunda(GK) sites were compared. But if the capsule mass without seed and number of seeds were compared between these two sites, these traits showed opposite trend. At least 50% increment was observed in number of seeds but found slight growth in capsule mass without seed. The traits studied between Lauribina danda and Lauribina pass did not show marked difference, except in case of plant height. Conclusively, the plant traits between Lauribina danda and Lauribina pass were markedly similar to each other but in contrary, plant traits from Lauribina danda and Gosainkunda showed marked difference between each other (Table 4).

Table 4: Variation in adaptive traits of *Meconopsis napaulensis* in three sites.

Variables	N	Mean ± SE at three sites			Overall Mean ± SE	Kruskal-Wallis test among sites*		Z-values indicating difference between[¥]		
		Lauribina danda (LD)	Lauribina pass (LP)	Gosainkunda (GK)		χ^2	p-value	LD &LP	LD & GK	LP & GK
Plant height (cm)	75	78.00±1.53	74.2±2.26	66.00±3.71	73.32±1.52	6.547	0.038*	-1.968*	-2.498*	-0.06
No. of bud per plant	189	17.2±1.8	13.4±1.83	8.05±1.01	13.30±1.05	15.166	0.001**	-1.716	-3.877***	-2.305*
No .of flower per plant	101	8.4±0.7	8.00±0.86	4.63±0.72	7.22±0.48	9.527	0.009**	-0.489	-2.993**	-2.394*
No. of capsule per plant	69	24.2±2.38	19.6±2.47	9.95±1.66	18.61±1.48	17.359	<0.001***	-1.716	-3.871***	-2.991***
Capsule length (cm)	69	3.91±0.12	3.8±0.00	3.00±0.00	3.62±0.06	36.337	<0.001***	-1.57	-4.732***	-6.557***
Capsule diameter (cm)	69	0.46±0.01	0.47±0.01	0.48±0.01	0.47±0.01	1.946	0.378	-0.679	-1.28	-0.948
Capsule mass in g (with seed)	69	0.16±0.01	0.15±0.01	0.14±0.02	0.15±0.01	3.59	0.166	-0.466	-1.885*	-1.339
Capsule mass in g (without seed)	69	0.08±0.01	0.09±0.01	0.1±0.01	0.09±0.01	2.176	0.337	-0.398	-1.328	-1.211
Seed mass/capsule in g	69	0.08±0.01	0.07±0.01	0.04±0.01	0.06±0.01	7.474	0.024	-0.68	-2.677**	-2.002*
No. of seed [§]	15	128.2±16.87	154.8±41.08	226±25.64	169.67±19.29	4.994	0.082*	000	-2.402*	-1.358
Seed viability (%)	30	4.1±0.35	3.5±0.34	2.6±0.22	3.4±0.21	8.734	0.013*	-1.258	-1.944*	-2.784**
Pollen viability (%)	30	7.6±0.49	7.3±0.54	5.9±0.28	6.93±0.28	6.475	0.039*	-0.501	-1.952*	-2.392*

[¥] Significance based on independent sample Wilcoxon test, rest of the values are based on Kruskal-Wallis test (non parametric of one way ANOVA).

[§] Number of seed based at constant weight of 0.01gm (number of seeds were counted present at constant weight of 0.01 gm).

Asterisk indicate that the medians for a particular parameter between sites are significantly different from one another at *****$p < 0.05$, ******$p<0.01$ and *******$p<0.001$.

CHAPTER 4

DISCUSSION

4.1 Habitat Characteristics

Plant population fitness is influenced by several ecological factors. Populations of *M. napaulensis* occupied alpine habitats (4117- 4417 m asl) experiencing strong ecological heterogeneity. In majority of cases, plant populations are grown in open rocky habitats in south-west facing slopes. The similarities in most of the edaphic characters (such as soil pH, moistures and litter depth) and substrate types (such as solid rock, non vascular plant cover, grass cover and total vascular plant cover) among sites revealed that *M. napaulensis* is habitat specific. Previous studies (Sulaiman and Babu, 1996; Lesica *et al.,* 2006 and Poudeyal, 2010) revealed that certain species of *Meconopsis* are restricted to extreme edaphic conditions (nutrient-poor, dry and open rocky substrates) where competition from dominant vegetation is reduced. Debussche and Thompson (2003) reported habitat specialization is the important relative traits for the rare endemic species. The habitat specific species like *M. napaulensis* should have strong correlation with edaphic endemism and other ecological specificities (Ghimire, 2005; Poudeyal and Ghimire, 2011).

Statistically insignificant difference in density value of individuals in different stage/state class (except reproductive size class) among three sites further supports that the population structure and stage class distribution of *Meconopsis napaulensis* largely depend upon the specific range of ecological amplitude. The regeneration capacity of rare endemic species generally depends upon the availability of suitable micro-habitat (Tilman *et al*., 1994). The patchy nature of *M. napaulensis* could also be linked to the limitation of suitable micro-habitat sites in surrounding areas. Thus, the distributions of such rare endemic plants are strongly influenced by availability of suitable micro-habitat (Tilman *et al.,* 1994).

Regarding the ground cover vegetation, shrubs coverage was found more at lower altitude site which create high competition for availability of soil nutrients and for sufficient amount of light (Sulaiman and Babu, 1996). It might be the reason of reduction of the density of reproductive individuals at lower altitude sites. The litter coverage was found significantly higher at lower elevation which showed that habitat at lower site was more fertile than upper site. In this study, the moss coverage was found to be high at lower elevation site. It retains high amount of moisture which enhances the vegetative growth of plants (Korner, 2003; Devkota, 2009). Thus, the higher level of vegetative growth of *M. napaulensis* was found to be associated to ecological integration including edaphic factors at lower altitude. Conversely, the seed production rate was negatively associated with surrounding vegetation. Although high altitude plant population tries to counteract allocating higher investment towards reproductive growth but due to harsh environmental conditions including poor nutrient substrates, the overall growth of the individual was substantially reduced in comparison to lower altitude plants.

4.1.1 Disturbances

Populations of *M. napaulensis* are also influenced by a various level of anthropogenic disturbance like in other parts of Himalaya (Ghimire *et al*., 2006 and 2008). The over-harvesting of medicinal plants, excessive grazing and trampling are the major anthropogenic factors which lead to the habitat fragmentation, destruction and ultimately cause local extinction. In case of *M. napaulensis* at Gosainkunda area, grazing seems to be driving factors for habitat fragmentation along with harvesting for medicinal and religious purpose. The availability of pastoral land towards high altitude provides the distribution of the grazing resources (Fox *et al*., 1996; Ghimire *et al*., 2006). Present findings also indicate that the anthropogenic effect was higher at Gosainkunda site in compare to other sites. As indicators to evaluate disturbances, higher levels of harvesting, grazing and garbage accumulation in Gosainkunda site proved the higher level of anthropogenic influence at high altitude population.

Further, most the populations of *M. napaulensis* are along the trekking (Lauribina and Gosainkunda) route and it is one of the important factors that plant population receive significant amount of anthropogenic disturbances. Similarly, the Gosainkunda Lake is one of religiously important lake among Hindus and Buddhists. Nearly 30,000 pilgrims visit per year in Gosainkunda Lake, during the festival 'Janaipurnima' and generate significant human disturbances (personal interview with locals). During the festival, people who visit there usually pluck the flowers and offer to the Gosainkunda deity. At the mean time collection of capsule for medicinal use was also prime factor for depletion of population. Previous study (Poudeyal, 2010) denoted that *M. napaulensis* roots, leaf and seeds are used to treat digestive system disorder, chest pain, sore throat and headache. Premature harvesting of capsules for medicinal purpose was prevalent in Gosainkunda and Lauribina. Thus, unsustainable harvesting of *M. napaulensis* could be one of the major challenges for the long-term population viability. Low to moderate level of grazing showed positive relationship with distribution and abundance but higher level of grazing showed negative relationship due to destruction and elimination of the species (Ghimire *et al*., 2006). The management plan for the pasture land (for grazing) and ecologically iconic species (for conservation) on the same area cannot go independently, so a system approach incorporating social management to accommodate the needs of different users at the landscape level is needed (Ghimire *et al*., 2004 and 2006).

4.2 Population structure, vegetative and reproductive traits

Based on the results, *M. napaulensis* showed identical densities in all stage classes, except reproductive class, among all the three sites. Reproductive (stage) density was significantly high at Lauribina pass. In comparison to other sites, Lauribina pass received lesser anthropogenic pressure because the site is located away from the human settlements. Previous study suggested that the extent of disturbance in alpine plant population is related to the distance from the settlements (Poudeyal and Ghimire 2011). Thus, Lauribina danda and Gosainkunda sites which are located nearer to the human settlements received higher amount of disturbances.

Meconopsis napaulensis showed variation in most of the vegetative and reproductive characters. The plant height is one of the discriminating characters among all the sites. Plants at lower altitude site (Lauribina danda) were taller in comparison to high altitude sites. Korner *et al.* (1983) and Brown *et al*. (2003) reported that reduction in plant height mostly related to corresponding decline in temperature and short growing period in alpine area. The poor availability of nutrients in the soil, thin soil profile and further higher level of anthropogenic influence also could be related to the stunted growth of plant in high altitude site (Gosainkunda). Similarly, the higher allocation on vegetative investment in lower altitude site supports the production of higher number of buds, flower and capsule. But in contrary to vegetative growth the number of seeds was significantly high at high altitude site. Xie *et al*. (2002) has reported that large number of seed production is the tendency of rare endemics. Similar is the case for *M. napaulensis* that produces innumerable number of tiny seeds. Plants at the high altitude site counteracted with that of lower altitude site by producing higher number of seeds and higher allocation towards reproduction in comparison to the vegetative growth. But seed viability tests suggest that high altitude site produced far less viable seeds. Thus the overall plant performance was found to be always higher in lower altitude populations.

M. napaulensis at high altitude produced light and ill developed seeds. Seed weight was significantly reduced at higher altitude if the plants from Gosainkunda (4417 m) and Lauribina danda (4117 m) were compared. The maturation and production of intact seeds in alpine plant is directly linked to the favorable environment (Totland, 1997b). Study revealed that alpine plants are more sensitive to temperature for seed feeling and maturation (Wagner and Reichegger, 1997). The frequently changing weather conditions, low temperature and short growing seasons hindered the production of healthy and heavier seeds at higher altitude (Pluess *et al*., 2005; Wagner and Reichegger, 1997; Totland, 1997b; Totland and Birks, 1996; Corbet, 1990; Galen, 1985). The production of intact seeds per plant was also related to the extent of disturbance. Escarre *et al*. (1999) reported that floral damage by herbivores limits the seed production. Similarly, suitable pollinator limitation, pollen limitation in fragmented habitat like *M. napaulensis* are also major challenges to developed well firmed seed in alpine habitat (Morely, 1982; Menges *et al*., 1986; Karran, 1987; Lavergne, 2005; Albert *et al*., 2005).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Based on above results following conclusions can be drawn:

- *Meconopsis napaulensis* prefer open rocky substrate, nutrient-poor, grassy slopes in south-west facing slopes. Habitat characters are directly linked to the vegetative and reproductive attributes. Lower altitudes provide fertile and nutrient-rich habitat for vegetative growth and reproductive outputs.

- Vegetative and reproductive traits of *Meconopsis napaulensis* showed variation along the elevation gradient. Lower altitude plants showed proliferation towards the vegetative outputs in comparison to higher altitude which could be linked to the favorable environment such as higher level of soil nutrients, moisture and higher temperature.

- In contrary to vegetative growth, high altitude plant tended to show higher number of seeds per capsule. Thus in this regard, a tradeoff among the plant traits was observed in which lower altitude plant invest higher allocation on vegetative growth and lesser investment on reproductive growth and development. Contrarily, high altitude plants showed higher investment on reproductive output in comparison to vegetative investment.

- Plant in four stage/size classes did not showed marked differences in density, but numerically the number of large rosette are slightly higher than that of all other stages. The population structure was identical in all sites studied. In regard to density of the plants, reproductive plant density was markedly differed among all the sites and stages. The reproductive density was far higher in Lauribina pass which could be linked to the safe habitat in comparison to the other two sites. The latter two sites were nearer to the human settlements and received higher level of disturbances.

5.2 RECOMMENDATION

Based on the results of present study, following recommendation have been made.

- The plant populations of rare endemic plants like *M. napaulensis* are very important and sensitive in terms of ecological and evolutionary points of view. But maintenance of their populations in natural habitat is challenging. Thus in this regard, development of conservation program in holistic approach is necessary. Thus Lauribina and Gosainkunda area should be given prime importance in term of conservation point of view.

- Awareness should be developed in local people about the importance of rare-endemic and ecologically susceptible species like *M. napaulensis*.

- The integration of local peoples' knowledge and scientific findings could be the best option for the sustainable management of plant population.

- Attention should be given for the proper mechanism to control garbage pollution. In this regard public awareness could be the best alternatives and also local people who are engaged in biodiversity conservation should be encouraged.

- For more detailed information like long-term viability of seed and pollen about rare endemic *M. napaulensis* extensive demographic (life stages and growth rate) study is recommended.

- Rotational grazing and harvesting as much as low amount in certain time interval can be other alternatives for conservation of valuable plant resources.

- Conservation by establishing *in situ* gene banks and habitat restoration for rare endemic species like *M. napaulenis* could be made possible.

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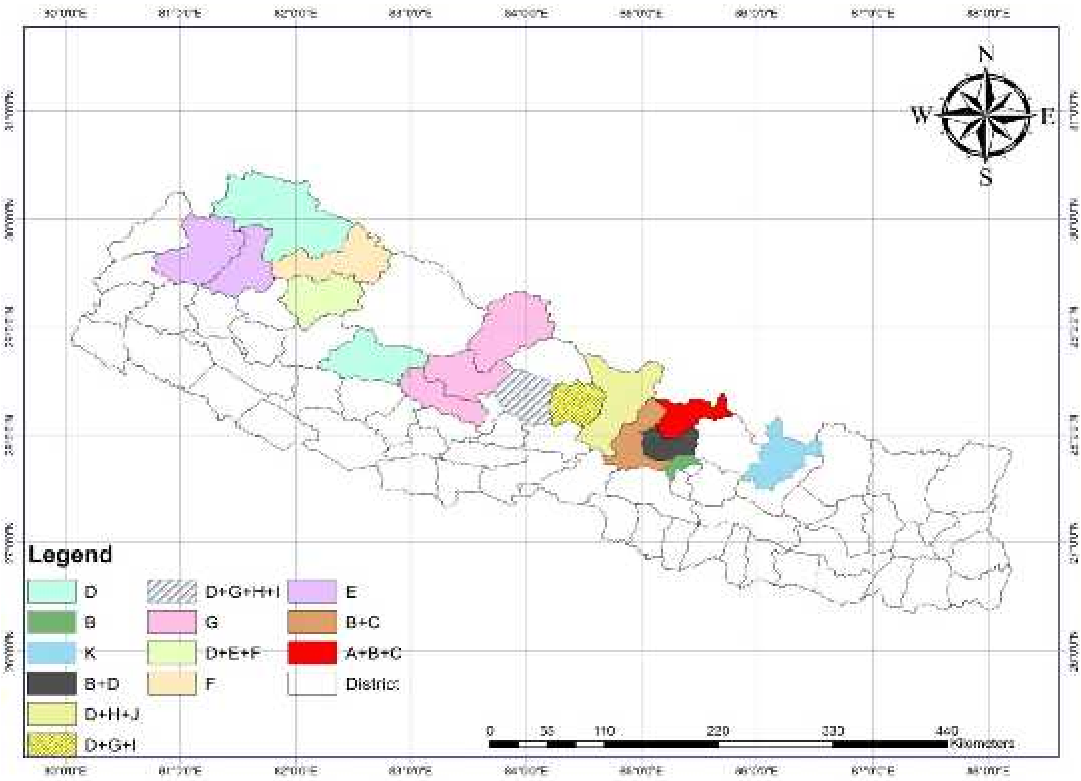
APPENDIX: 1

Distribution of endemic species of genus *Meconopsis* in Nepal.



Species are indicated by alphabet:

A= *M. napaulensis*

B= *M. ganeshensis*

C= *M. autumnalis*

D= *M. regia*

E= *M. simikotensis*

F= *M. chankheliensis*

G= *M. staintonii*

H= *M. gracilipe*

I= *M. taylorii*

J= *M. manasluensis*

K=*M. dhwojii*

APPENDIX: II

Appendix II a: Plant species associated with *M. napaulensis.*

Plant species associated with *M.napaulensis* their frequency and dominance mean value based on categorical (0-4) estimation in Lauribina-danda (LD).

S no.	Associate species	Family	Frequency	Dominance Mean
1	Aletris pauciflora (Klotzsch) Hand.-Mazz.	Liliaceae	62.5	2.5
2	Anaphalis contorta (D.Don) Hook.f.	Asteraceae	87.5	3.5
3	Anaphalis nepalensis Spreng.	Asteraceae	100	4.00
4	Anaphalis royleana DC.	Asteraceae	56.25	2.25
5	Anemone tetracepala Royle	Ranunculaceae	25.00	1.00
6	Aspidium sp.	Dryopteridaceae	31.25	1.25
7	Berberis erythroclada Ahrendt.	Berberidaceae	12.5	0.5
8	Bistorta macrophylla D.Don	Polygonaceae	18.75	0.75
9	Bistorta vaccinifolia Wallich ex Meissner	Polygonaceae	56.25	2.25
10	Carex sp.1.	Cyperaceae	62.5	2.25
11	Carex sp. 2.	Cyperaceae	68.75	2.75
12	Chesneya nubigena (D.Don) Ali	Fabaceae	37.5	1.5
13	Corydalis govaniana Wall.	Papaveraceae	56.25	2.25
14	Cyananthus himaliacus K.K. Shrestha	Campanulaceae	81.25	3.00
15	Cyananthus lobatus Wall ex Benth.	Campanulaceae	87.5	3.5
16	Elsholtzia eriostachya Benth.	Lamiaceae	56.25	2.25
17	Euphorbia stracheyi Boiss	Euphorbiaceae	62.5	2.5
18	Galium nepalense Ehrend. & schonb.	Rubiaceae	31.25	1.25
19	Gentiana ornata (D.Don) Griseb.	Gentianaceae	37.5	1.5
20	Geranium donianum Sweet.	Geraniaceae	62.5	2.5
21	Juniperus recurva Buch-Ham. ex D.Don	Cupressaceae	62.5	2.5
22	Kobresia nepalensis (Nees) Kuk.	Cyperaceae	100	4.00
23	Kobresia pygmaea C.B. Clarke	Cyperaceae	100	4.00
24	Koenigia delicatula (Meisn.) H.Hara	Polygonaceae	31.25	1.25
25	Leontopodium stracheyi (Hook.f.) C.B. Clarke	Asteraceae	68.75	2.75
26	Morina polyphylla Wall.ex DC.	Dipsacaceae	56.25	2.25
27	Nardostachys grandiflora DC.	Valerianaceae	87.5	3.5
28	Oxygraphis nepalensis Tamura	Ranunculaceae	68.75	2.5
29	Parnasia nubicola Wall.ex Royle.	Saxifragaceae	100	4.00
30	Pleurospermum benthamii (DC.) C.B. Clarke	Apiaceae	87.5	3.5
31	Poa bulbosa L.	Poaceae	93.75	3.5
32	Polygonatum hookeri Baker.	Liliaceae	31.25	1.25
33	Potentialla aristata Sajak.	Rosaceae	100	4.00
34	Potentilla coriandrifolia D.Don	Rosaceae	100	4.00
35	Potentilla fruticosa Lindle. ex Lehm.	Rosaceae	81.25	3.25
36	Potentilla microphylla D.Don	Rosaceae	93.75	3.75

S no.	Associate species	Family	Frequency	Dominance Mean
37	Potentilla pedicularis D.Don	Rosaceae	93.75	3.75
38	Primula primulina (Spreng.) H.Hara	Primulaceae	75.00	3.00
39	Primula rotundiflora Wall.	Primulaceae	31.25	1.25
40	Rhodiola cretinii (Raym.-Hamet) H. Ohba	Crassulaceae	25.00	1.00
41	Rhododendron lepidotum Wall. ex D.Don	Ericaceae	93.75	3.75
42	Saussurea nepalensis Spreng.	Asteraceae	6.25	0.25
43	Saxifraga caveana W.W.Sm.	Saxifragaceae	31.25	1.25
44	Saxifraga parnassifolia D.Don	Saxifragaceae	68.75	2.75
45	Saxifraga granulifera H.Sm.	Saxifragaceae	62.5	2.5
46	Taraxacum eriopodon DC.	Asteraceae	93.75	3.75
47	Thalictrum alpinum L.	Ranunculaceae	37.5	1.25
48	Valeriana hardwickii Wall.	Valerianaceae	31.25	1.25
49	Viola biflora L.	Violaceae	93.75	3.75

Appendix II b: Plant species associated with *M.napaulensis.*

Plant species associated with *M.napaulensis* their frequency and dominance mean value based on categorical (0-4) estimation in Lauribina pass (LP).

S no.	Associate species	Family	Frequency	Dominance Mean
1	Aconitum gammiei Stapf.	Ranunculaceae	6.25	0.25
2	Aletris pauciflora (Klotzsch) Hand.-Mazz.	Liliaceae	100	4.00
3	Anaphalis nepalensis Spreng.	Asteraceae	100	4.00
4	Anaphalis royleana DC.	Asteraceae	62.5	2.5
5	Aster himalaicus C.B.Clarke	Asteraceae	12.5	0.5
6	Bistorta macrophylla D.Don	Polygonaceae	100	4.00
7	Bistorta vaccinifolia Wallich ex Meissner	Polygonaceae	25.0	1.00
8	Carex sp.2	Cyperaceae	100	4.00
9	Chesneya nubigena (D.Don) Ali	Fabaceae	75.0	3.00
10	Cicerbita macrorhiza Royle	Asteraceae	6.25	0.25
11	Corydalis juncea Wall.	Papaveraceae	75.0	3.00
12	Cremanthodium nepalensis Kitam.	Asteraceae	12.5	0.5
13	Cyanantus lobatus Wall ex Benth.	Campanulaceae	100	4.00
14	Elsholtzia strobilifera Benth.	Lamiaceae	6.25	0.25
15	Elsholtzia fructicosa (D.Don) Rehder.	Lamiaceae	25	1.00
16	Epilobium palustre L.	Onagraceae	6.25	0.25
17	Euphorbia straychii Boiss	Euphorbiaceae	87.5	3.5
18	Fern 1	Pteridaceae	25.0	1.00
19	Fern 2	Pteridaceae	6.25	0.25
20	Gentiana ornata (D.Don) Griseb.	Gentianaceae	93.75	3.75
21	Geranium donianum Sweet.	Geraniaceae	87.5	3.5
22	Geranium nakaoanum H.Hara	Geraniaceae	75.0	3.00
23	Impatiens occultans Hook.f.	Balsaminaceae	6.25	0.25
24	Juncus thomsonii Buchenau.	Juncaceae	6.25	0.25
25	Kobresia nepalensis (Nees) Kuk.	Cyperaceae	100	4.00
26	Kobresia pygmaea C.B. Clarke	Cyperaceae	100	4.00
27	Koenigia delicatula (Meisn.) H.Hara	Polygonaceae	100	4.00
28	Leontopodium makianum Kitam.	Asteraceae	25.0	1.00
29	Nardostachys grandiflora DC.	Valerianaceae	75.0	3.00
30	Oxygraphis nepalensis Tamura	Ranunculaceae	100	4.00
31	Parnasia nubicola Wall. ex Royle	Saxifragaceae	100	4.00
32	Pleurosperm benthami (DC.) C.B.Clarke	Apiaceae	6.25	0.25
33	Poa bulbosa L.	Poaceae	100	4.00
34	Potentilla coriandrifolia D.Don	Rosaceae	100	4.00
35	Potentilla fruticosa Lindle. ex Lehm.	Rosaceae	56.25	2.00
36	Potentilla fulgens Wall.	Rosaceae	93.75	3.75
37	Potentilla microphylla D.Don	Rosaceae	75.0	3.00
38	Potentilla pedicularis D.Don	Rosaceae	43.75	1.75

S no.	Associate species	Family	Frequency	Dominance Mean
39	Primula denticulata Sm.	Primulaceae	6.25	0.25
40	Primula primulina (Spreng.) H.Hara	Primulaceae	75.0	3.00
41	Primula rotundiflora Wall.	Primulaceae	18.75	0.75
42	Rhodiola bupleuroides Wall. ex Hook.	Crassulaceae	25.0	1.00
43	Rhodiola crenulata (Hook.f. & Thomson) H. Ohba	Crassulaceae	25.0	1.00
44	Rhododendron setosum D.Don	Ericaceae	6.25	0.25
45	Rhododendron lepidotum Wall. ex D.Don	Ericaceae	100	4.00
46	Saussurea nepalensis Spreng.	Asteraceae	50.0	2.00
47	Saxifraga caveana W.W.Sm.	Saxifragaceae	31.25	0.75
48	Saxifraga parnassifolia D.Don	Saxifragaceae	12.5	0.5
49	Saxifraga granulifera H.Sm.	Saxifragaceae	100	4.00
50	Sedum gagei Raym-Hamet.	Crassulaceae	31.25	1.25
51	Taraxacum eriopodon DC.	Asteraceae	75.0	3.00
52	Viola biflora L.	Violaceae	100	4.00

Appendix II c: Plant species associated with *M. napaulensis.*

Plant species associated with *M.napaulensis* their frequency and dominance mean value based on categorical (0-4) estimation in Gosainkunda (GK).

S no.	Associate species	Family	Frequency	Dominance Mean
1	Anaphalis contorta (D.Don) Hook.f.	Asteraceae	75.0	2.75
2	Anaphalis nepalensis Spreng.	Asteraceae	68.75	2.75
3	Anaphalis royleana DC.	Asteraceae	37.5	1.5
4	Aster himalaicus C.B.Clarke.	Asteraceae	93.75	3.75
5	Bistorta macrophylla D.Don.	Polygonaceae	93.75	3.75
6	Bistorta vaccinifolia Wallich ex Meissner.	Polygonaceae	100	4.00
7	Bupleurum falcatum L.	Apiaceae	6.25	0.25
8	Carex sp.2.	Cyperaceae	75.0	3.00.
9	Chrysosplenium uniflorum Maxim.	Saxifragaceae	6.25	0.25
10	Cortia depressia (D.Don) C.Norman	Apiaceae	87.5	3.5
11	Corydalis juncea Wall.	Papaveraceae	75.0	3.00
12	Coryophylla sp.	Papaveraceae	6.25	0.25
13	Cremanthodium nepalensis Kitam.	Asteraceae	18.75	0.75
14	Cremanthodium vestitum	Asteraceae	25.00	1.00
15	Cyanantus lobatus Wall ex Benth.	Campanulaceae	43.75	1.75
16	Elsholtzia strobilifera Benth.	Lamiaceae	6.25	0.25
17	Elsholtzia fructicosa (D.Don) Rehder.	Lamiaceae	6.25	0.25
18	Epilobium pastustre L.	Onagraceae	25.00	1.00
19	Euphorbia stracheyi Boiss.	Euphorbiaceae	62.5	2.5
20	Fern	Pteridaceae	25.00	1.00
21	Fritillaria cirrhosa D.Don	Liliaceae	6.25	0.25
22	Gentiana ornata (D.Don) Griseb.	Gentianaceae	68.75	2.75
23	Geranium nakaonium H.Hara	Geraniaceae	81.25	3.25
24	Juncus thomsonii Buchenau.	Juncaceae	56.25	2.25
25	Kobresia nepalensis (Nees) Kuk.	Cyperaceae	93.75	3.75
26	Kobresia pygmaea C.B. Clarke	Cyperaceae	100	4.00
27	Koenigia delicatula (Meisn.) H.Hara	Polygonaceae	75.0	2.75
28	Leontopodium makianum Kitam.	Asteraceae	6.25	0.25
29	Leontopodium stracheyi (Hook.f.) C.B.Clarke	Asteraceae	6.25	0.25
30	Padicularis megalantha D.Don.	Scrophulariaceae	6.25	0.25
31	Pleurosperm benthami (DC.) C.B.Clarke	Apiaceae	18.75	0.75
32	Poa bulbosa L.	Poaceae	50.00	2.00
33	Polygonatum hookerii Baker	Polygonaceae	18.75	0.75
34	Potentialla forrestii W.W.Sm.	Rosaceae	37.5	1.5
35	Potentilla fruticosa Lindle. ex Lehm.	Rosaceae	6.25	0.25
36	Potentilla microphylla D.Don	Rosaceae	87.5	3.5
37	Potentilla pedicularis D.Don	Rosaceae	50.0	2.25

S no.	Associate species	Family	Frequency	Dominance Mean
38	Primula primulina (Spreng.) H.Hara	Primulaceae	43.75	1.75
39	Rhodiola crenulata (Hook.f.& Thomson) H.Ohba	Crassulaceae	18.75	0.75
40	Rhododendron setosum D.Don	Ericaceae	12.5	0.5
41	Rhododendron lepidotum Wall.ex D.Don	Ericaceae	6.25	0.25
42	Saussurea nepalensis Spreng.	Asteraceae	18.75	0.75
43	Saxifraga caveana W.W.Sm.	Asteraceae	18.75	0.75
44	Saxifraga parnassifolia D.Don	Saxifragaceae	68.75	2.75
45	Sedum gagei Raym.-Hamet	Crassulaceae	6.25	0.25
46	Silene gonosperma (Rupr.) Bocquet	Caryophyllaceae	18.75	0.75
47	Taraxacum eriopodon DC.	Asteraceae	93.75	3.75
48	Thalictrum alpinum L.	Ranunculaceae	6.25	0.25
49	Viola biflora L.	Violaceae	87.5	3.5

APPENDIX III

Appendix III: Families with number of genera and species recorded from the study area.

S.No.	Families	No. of Genera	No. of species
1	Asteraceae	7	11
2	Apiaceae	3	4
3	Balsaminaceae	1	1
4	Berberidaceae	1	1
5	Campanulaceae	1	2
6	Caprifoliaceae	1	1
7	Caryophyllaceae	1	1
8	Crassulaceae	2	4
9	Cupressaceae	1	1
10	Cyperaceae	2	3
11	Dazipsacaceae	1	1
12	Dryopteridaceae	1	1
13	Ericaceae	1	1
14	Euphorbiaceae	1	1
15	Fabaceae	1	1
16	Gentianaceae	1	1
17	Geraniaceae	1	1
18	Juncaceae	1	1
19	Lamiaceae	1	3
20	Liliaceae	1	1
21	Onagraceae	1	1
22	Papaveraceae	1	3
23	Poaceae	1	1
24	Polygonaceae	3	4
25	Primulaceae	1	2
26	Pteridaceae	1	1
27	Ranunculaceae	3	3
28	Rosaceae	1	7
29	Rubiaceae	1	1
30	Scrophulariaceae	1	1
31	Saxifragaceae	2	5
32	Valerianaceae	1	1
33	Violaceae	1	1
Total	33 families	47 genera	71 species

APPENDIX: V

Table: Variation in adaptive traits of *Meconopsis napaulensis* in three sites.

Variables	N	mean ± SE at three sites: Lauribina danda (LD)	Lauribina pass (LP)	Gosainkunda (GK)	Overall mean ± SE	Kruskal-Wallis test among site*: χ^2	p-value	Z and P-values indicating difference between[¥]: LD and LP: Z	p	LD and GK: Z	p	LP and Gk: Z	p
Plant ht (cm)	75	78.00±1.53	74.2±2.26	66.00±3.71	73.32±1.52	6.547	0.038*	-1.968	0.049*	-2.498	0.012*	-0.06	0.953
Number of bud/ plant	180	17.2±1.8	13.4±1.83	8.05±1.01	13.30±1.05	15.166	0.001**	-1.716	0.086	-3.877	0.00***	-2.305	0.021*
No. of flower/pant	101	8.4±0.7	8.00±0.86	4.63±0.72	7.22±0.48	9.527	0.009**	-0.489	0.625	-2.993	0.003**	-2.394	0.017*
No. of capsule/plant	69	24.2±2.38	19.6±2.47	9.95±1.66	18.61±1.48	17.359	<0.001*	-1.716	0.086	-3.871	0.00***	-2.991	0.003*
Cap length(cm)	69	3.91±0.12	3.8±0.00	3.00±0.00	3.62±0.06	36.337	<0.001*	-1.57	0.116	-4.732	0.00***	-6.557	0.00***
Cap dia(cm)	69	0.46±0.01	0.47±0.01	0.48±0.01	0.47±0.01	1.946	0.378	-0.679	0.497	-1.28	0.201	-0.948	0.343
Cap weight with seed(g)	69	0.16±0.01	0.15±0.01	0.14±0.02	0.15±0.01	3.59	0.166	-0.466	0.641	-1.885	0.059*	-1.339	0.181
Cap wt without sd(g)	69	0.08±0.01	0.09±0.01	0.1±0.01	0.09±0.01	2.176	0.337	-0.398	0.691	-1.328	0.184	-1.211	0.226
Seed mass per capsule(g)	69	0.08±0.01	0.07±0.01	0.04±0.01	0.06±0.01	7.474	0.024*	-0.68	0.497	-2.677	0.007**	-2.002	0.045*
No of seed[§]	15	128.2±16.87	155.8±41.08	226±25.64	169.67±19.29	4.994	0.082	0.00	1.00	-2.402	0.016*	-1.358	0.175
Seed viability	30	4.1±0.35	3.5±0.34	2.6±0.22	3.4±0.21	8.734	0.013*	-1.258	0.209	-1.944	0.052*	-2.784	0.005**
Pollen viability	30	7.6±0.49	7.3±0.54	5.9±0.28	6.93±0.28	6.475	0.039*	-0.501	0.616	-1.952	0.051*	-2.392	0.017*

[¥]Significance based on independent sample Wilcoxon test, rest of the values are based on Kruskal-Wallis test (non parametric of one way ANOVA).

[§] Number of seed based at constant weight of 0.01gm (number of seeds were counted present at constant weight of 0.01 gm).

Asterisk indicate that the medians for a particular parameter between sites are significantly different from one another at *$p < 0.05$, **$p<0.01$ and ***$p<0.001$.

Photo plate 1

Seeds of *M. napaulensis*

Seed viability test of *M. napaulensis* in lab

Viable seed of *M.napaulensis*

Seed germination

Photo plate 2

Sheep on the way to study site

Habitat of *M. napaulensis*

Way to study site

Study site at Gosainkunda