Growth Strategy and Population Structure in a Threatened Medicinal Herb (*Neopicrorhiza scrophulariiflora*) in Alpine Himalaya

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Submitted By

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Recommendation

This is to certify that the dissertation work entitled "**Growth Strategy and Population Structure in a Threatened Herb (*Neopicrorhiza scrophulariiflora*) in Alpine Himalaya**" submitted by **Mr. Balak Devkota** has been carried out under my supervision. The entire work is based on the results of his research work and has not been submitted for any other degree. I recommend this dissertation work to be accepted for the partial fulfillment of Masters of Science in Botany (Plant Systematics and Phytogeography).

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Approval Letter

The dissertation work submitted by **Mr. Balak Devkota** entitled "**Growth Strategy and Population Structure in a Threatened Herb (*Neopicrorhiza scrophulariiflora*) in Alpine Himalaya**" has been accepted as a partial fulfillment of the requirements for Masters of Science in Botany (Plant Systematics and Phytogeography).

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Contents

Acronyms and Abbreviations

ANOVA	Analysis of Variance
asl	Above Sea Level
CAMP	Conservation Assessment and Management Plan
CCF	Conservation Community Forest
CCFUG	Conservation Community Forest User Group
CDA	Canonical Discriminant Analysis
CF	Community Forest
CITES	Convention on International Trade in Endangered Species and Wild Flora and Fauna
DDC	District Development Committee
DoA	Department of Agriculture
DPR	Department of Plant Resources
GDP	Gross Domestic Product
GN	Government of Nepal
GPS	Geographical Positioning System
IUCN	International Union for Nature Conservation
KCA	Kangchenjunga Conservation Area
KCAP	Kangchenjunga Conservation Area Project
MAP	Medicinal and Aromatic Plant
MoAC	Ministry of Agriculture and Cooperatives
MP	Medicinal Plant
NARC	National Agricultural Research Council
NTFP	Non-Timber Forest Product
PCA	Principle Component Analysis
PRA	Participatory Rapid Appraisal
RRI	Relative Radiation Index
SD	Standard Deviation
SE	Standard Error
SPSS	Statistical Program for Social Science
US	United States
VDC	Village Development Committee
WWF	World Wide Fund for Nature
WWF-NP	World Wide Fund for Nature Nepal

ABSTRACT

Variation in growth strategy, plant performance and population structure was studied in a threatened long-lived clonal medicinal herb [(*Neopicrorhiza scrophulariiflora* (Pennell) D.Y. Hong)] in relation to altitude and other environmental variables. Seven populations were selected from lower alpine and upper alpine habitats in Walangchung Gola and Ghunsa sectors within Kangchenjunga Conservation Area, East Nepal. The two sectors differ in terms of degree of protection at landscape level (Ghunsa sector being more protected than Walangchung Gola sector) and level of precipitation (higher precipitation in Ghunsa sector). The habitat of *N. scrophulariiflora* ranged from shrubland to open rocky/scree or grassy slopes on increasing altitude. There were strong geographical (sectorial) and altitudinal differences in the relative contribution of sexual and vegetative growth strategies in population persistence. Altitude was also related to the inter-population variation in other plant performance traits as well as in population size and structure. Sexual allocation and sexual reproductive effort were high in lower alpine populations. As compared to Ghunsa sector (moist sites); populations from Walangchung Gola sector (drier sites) produced large-sized genets (except in highly disturbed site). Ghunsa populations on the other hand showed significantly large number of flowering ramets per genet than did by Walangchung Gola populations. This shows that at high altitudes and at drier sites, plants tend to invest less in sexual fecundity and more in traits ensuring persistence of vegetative offshoots. Populations from Walangchung Gola sector showed higher proportions of juvenile and vegetative adult ramets, with poor representation of very young and reproductive adult ramets. In highly disturbed populations, proportion of reproductive adult was even greatly reduced. On the other hand, populations from Ghunsa sector showed almost equal proportions of young, juvenile and vegetative adult ramets. Proportion of adult reproductive ramets was also significantly high in Ghunsa populations than in Walangchung Gola populations. This showed that Ghunsa populations were stable in density and self-replacing. The strong relationship between environmental conditions and plant growth strategy, adult performance and population structure of *N. scrophulariiflora* has important implications for its *in situ* management. The lower performance of plants (in terms of growth-related traits), and low sexual reproductive efforts in higher alpine sites (especially Walangchung Gola sector) indicate that these populations need immediate action to protect them from illegal and premature harvesting. In such populations management should focus on increasing seedling recruitment and reducing damage to the reproductive adults. Thus long-term plan with a strong measure for sustainable utilization system is needed to manage the target species populations simultaneously respecting traditional access rights of the local users. Extremely low seedling recruitment makes this species highly vulnerable if harvesting is applied at fairly higher level, because in such condition population genetic diversity can be expected to be very low. Thus applying low level of harvesting with fairly long rotation is a good strategy for population persistence. Level of harvesting should be even low in harsh higher alpine habitats of Walangchung Gola sector.

Key words: altitude, disturbance, medicinal plant, plant performance, soil variable, sustainable management.

Chapter 1

INTRODUCTION

1.1 Background

Many plants are used by man for relieving and curing diseases since ancient times. As in many developing countries, medicinal plants (MPs) in Nepal have still played an important role in local health care. In addition, MP trade is one of the major income generating activities in high mountains of Nepal, where agricultural land is limited with scarce livelihood opportunities. In Nepal, over 161 species of useful plants, including MPs, have been estimated to be harvested (as non-timber forest products or NTFPs) for commercial purpose, with a total export value of over US$ 16 million (Subedi 2006). Among the traded high-altitude species, *Neopicrorhiza scrophulariiflora* together with *Nardostachys grandiflora* stand highest in terms of value and volume in trade (Olsen 2005; Ghimire 2008).

Many of the Himalayan MPs are widely considered to be threatened due to commercial harvesting pressure (Shrestha and Joshi 1996; Tandon *et al*. 2001; Olsen 2005). In addition to this, habitat destruction, livestock grazing, forest fires etc. are other important factors responsible for the depletion of many valuable species. Unmanaged harvesting may alter ecological processes at individual, population, species, ecosystem and landscape levels (Hall and Bawa 1993; Cunningham 2001; Tiktin 2004; Ghimire 2008). Conservation Assessment and Management Plan (CAMP) workshops held in different parts of the Himalaya have prioritized a number of high-altitude species (including *Neopicrorhiza scrophulariiflora*) for immediate conservation and management through detail study of their population biology (Ved and Tandon 1998; Tandon *et al*. 2001). In the Himalaya, population level assessments are available for only limited species and locations (Kala 2000; Pandit and Babu 1998, 2003; Ghimire *et al*. 2005, 2008; Shrestha and Jha 2009).

Plant populations growing on different habitats may have different responses to ecological heterogeneity and human management (Cunningham 2001; Ghimire *et al*. 2005). Anthropogenic factors, like grazing, trampling and plant part harvesting affect the size, structure and reproductive strategy of plant populations. Plant populations also exhibit patterns of variability in morphology, reproductive strategy and persistence in relation to ecological conditions. The diversity of growth patterns and life forms of MPs, which may vary among varying habitat conditions, may have diverse responses to human management (Ticktin 2004; Ghimire *et al*. 2005, 2008). Therefore, understanding of population biology of MPs in varying environmental conditions, including human management, is crucial in formulating management strategies of such plant species.

Neopicrorhiza scrophulariiflora (family: Scrophulariaceae) is a high valued MP, distributed in lower alpine to upper alpine regions (at an altitudinal range of 3500 to 4800 masl) in the Himalaya (Press *et al*. 2000). It is found in diverse habitat types, such as forests, shrublands, meadows, rocky slopes and screes (Gahire 2003; Ghimire *et al*. 2005; Shrestha and Jha 2009). It is a long-lived perennial herb exhibiting 'guerrilla strategy' (*sensu* Lovett Doust 1981) of clonal growth, where the vegetative multiplication and growth of ramets occur more rapidly and successfully (Ghimire *et al*. 2005). Clonal growth strategy has been considered to be an advantage for rapid growth of *N. scrophulariiflora* in shrubland habitat in north-west Nepal (Ghimire *et al*. 2005; Ghimire and Aumeeruddy-Thomas 2005). In spite of its rapid vegetative growth and high habitat amplitude, *N. scrophulariiflora* has become a highly threatened species due to habitat encroachment and over harvesting. Premature harvesting of this species at the peak growing period (June-August) is the major issue for the long-term survival of its populations. In addition, a number of natural factors are also responsible for the decline of *N. scrophulariiflora* populations. In the high Himalaya, populations of *N. scrophulariiflora* are highly fragmented and its population sizes are reported to be declining even in the area where there is no commercial collection pressure at present (Shrestha and Jha 2009).

Because of its long creeping rhizomes, rate of ramet recruitment in *N. scrophulariiflora* through vegetative means is high, but seedling bear extremely low recruitment rate (Ghimire *et al*. 2005; Ghimire and Aumeeruddy-Thomas 2005). In clonal plants, sexual reproduction is generally poor and the plant invests more on vegetative growth (MacArthur and Wilson 1967; Cook 1979; Eriksson 1989). Sexual reproduction in clonal plants is favored by favorable site conditions (Loehle 1987). However, the relative contribution of sexual and clonal recruitment may vary widely among populations in relation to habitat conditions (e.g., light, nutrients, etc) (Bierzychudek 1982; Lovett-Doust and Cavers 1982; Eckert 2002; Kanno and Seiwa 2004). In environments with limited resources vegetative propagation is thought to be more advantageous than sexual reproduction for maintenance of populations (Kanno and Seiwa 2004). Reduced sexual reproduction may be particularly common at the geographical margins of species' ranges (Eckert 2002).

In the case of *N. scrophulariiflora*, study on the variation in vegetative and sexual reproductive modes, in relation to habitat conditions, and their relative contribution on population persistence is greatly lacking. In this species, only few studies have been carried out at the population-level (Ghimire *et al*. 2005; Shrestha and Jha 2009), and these studies are further limited by the small geographical/altitudinal range of the species covered. As distribution of *N. scrophulariiflora* cover wide altitudinal range (3500 to 4800 masl), study on the effect of environmental factors on persistence of its populations covering this range will have important implications in formulating management recommendations. This study, therefore, aims at assessing growth pattern, plant performance (vegetative and reproductive response), and population size (density) and structure of *N. scrophulariiflora* in relation to habitat heterogeneity and human management, covering populations from upper alpine (>4500 masl) and lower alpine (<4500 masl) levels in north-east Nepal. The main research questions are: (i) Do the population size (density), population structure, and adult plant performance vary among populations from upper alpine (>4500 masl) and lower alpine (<4500 masl) habitats experiencing different human management regimes? (ii) Which environmental factors are more associated with the variation in population size and structure and plant performance in *N. scrophulariiflora*?

1.2 Objectives

The overall aim of the research is to assess growth strategy, plant performance, and population size and structure of *N. scrophulariiflora* in relation to habitat heterogeneity and human management in different altitudes. The specific objectives are to:

1. Study variation in growth strategies and plant performance (plant size, reproductive output and sexual and vegetative allocation) in relation to altitude, disturbance and other environmental factors
2. Study variation in population size (density) and structure (proportions of different life stages) in relation to altitude, disturbance and other environmental factors
3. Assess and discuss the patterns of utilization and conservation status of *N. scrophulariiflora* and suggest strategy for its long-term management.

Chapter 2

LITERATURE REVIEW

2.1 Medicinal Plants in the Nepal Himalaya: Status and Conservation

Nepal has been regarded as one of the important reservoir for the supply of MPs in South Asia (Malla *et al.* 1995). However, the exact figure of diversity of MPs is not known. Previously, Malla and Shakya (1999) compiled a list of 630 species of MPs from Nepal. Baral and Kurmi (2006) later compiled 1792 species of MPs, including lichens and fungi. Recent works have estimated over 1900 species of MPs in Nepal (Ghimire 2008) which is almost 27.10% of total flora[1] of the country. The diversity of MPs is high in subtropical (1000-2000 m) region and highest proportion of MP species has been described from Central Nepal (Malla and Shakya 1999; Ghimire 2008). About 28.5% of the naturally growing MP species from the Nepal Himalaya are endemic to the Himalayan region (Ghimire 2008). About 120 species of MPs are cultivated in Nepal (Malla and Shakya 1999) and over 150 species are commercially traded (Edwards 1996; Bhattarai 1999; Subedi 2006 cited in Ghimire 2008).

Conservation Assessment Management Plan Workshop (CAMP) held in Nepal identified 51 species of threatened MPs (Tandon *et al.* 2001); later Government of Nepal listed 60 species of threatened MPs. A total of 30 species of NTFPs, including MPs, have been prioritized by the Government of Nepal for research and economic development (DPR 2006)[2]. Under the Forest Act of 1993, Government of Nepal imposed restriction for the collection of 17 species of plants including NTFPs[3] and still 13 species are included in CITES Appendices (Appendix II and III).

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[1] Press *et al* (2000) enumerated 5345 species of flowering plants from Nepal belonging to 1534 genera and 216 families.

[2] Government of Nepal has enlisted 701 medicinal plants (DPR 2007), out of which 30 species were prioritized for research and development and 12 species for agro-technology development (including *Neopicrorhiza scrophulariiflora*) (DPR 2006).

[3] Using the authority provided by the Forest Act (1993), Government of Nepal has imposed following restrictions on the NTFP species: (A) Ban for collection, transportation and trade of ‘panch aunle’ (*Dactylorhiza hatagirea*) and ‘okhar ko bokra’ (bark of *Juglans regia*). Initially, ‘kutki’ (*Neopicrorhiza scrophulariiflora*) was also banned for collection, but its ban has been lifted for products legally harvested from sustainably managed forests. (B) Ban for export outside the country without processing: (i) talishpatra (*Abies spectabilis*), (ii) ‘sugandha kokila’ (*Cinnamomum glaucescens*), (iii)

Trade of MPs and other NTFPs has contributed significantly to the rural and national economy. In certain rural areas of Nepal, this trade provides up to 50% of the family income (Edwards 1993). It has been reported that the revenue from the medicinal herbs in Nepal is over 10% of the total revenue generated from the forest-based products (reviewed by Ghimire 1999). The export of MPs and other NTFPs from Nepal to the neighboring countries is very old practice. Historically, MPs of Nepal have been traded to India and China. Since 1960s, the trade of MPs has intensified and extended to overseas countries as well, though the bulk of the trade (>90%) is still with India (Malla *et al.* 1995; Edwards 1996; Olsen and Larsen 2003; Ghimire 2008). Subedi (2006) estimated a total of 161 plant-based NTFP species involved in trade from Nepal, later Bhattarai and Ghimire (2006) listed a total of 143 species as commercial MPs. The global trade value of NTFPs was over of US $ 11 billion in 1995, and about 60% of the plant material was imported by USA, Japan and European community (Kunwar 2006). The global herbal market trade is increasing day by day with growth rate of 7% per annum (Kunwar 2006). Based on this growth rate the global herbal market is estimated to reach US$ 5 trillion by 2050 and the estimated amount for Nepal is US$ 10-18 million as revenue from the wild collection of MPs. In Nepal, the annual growth rate of NTFP trade is about 20% (Kunwar 2006).

Among the traded species of MPs, some high altitude species like *Neopicrorhiza scrophulariiflora* and *Nardostachys grandiflora* contribute significantly to the value and volume of international trade. Olsen (2005) stated that these two high altitude species along with other three MP species, such as *Sapindus mukorosi*, *Swertia chirayita*, and *Zanthoxylum armatum* have top five market and trade value in international market. They together make up more than 52% of the total value. In fiscal year 2003/04, the government of Nepal collected royalty of 5200 kg rhizome of *N. scrophulariiflora* (DoF 2004; Shrestha and Jha 2009). Annual trade levels from Nepal are estimated at 100-500 tones of air-dried rhizomes of *N. grandiflora* and 175-770 tones of air-dried rhizomes of *N. scrophulariiflora* (Olsen 2005). Rhizomes of *Picrorhiza kurrooa* are mixed with *N. scrophulariiflora* and traded under the same trade name as 'kutki'. Nepal is the main supplier of *N. grandiflora* (82 ± 5%) and *N. scrophulariiflora* (66 ± 12%) in the international market, followed by

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jhyau (lichens), (iv) 'jatamansi' (*Nardostachys grandiflora*), (v) 'sarpagandha' (*Rauwolfia serpentina*), (vi) 'lauth salla' (*Taxus wallichiana*), (vii)'sugandhawal' (*Valeriana jatamansii*). (C) Ban for felling, transportation and export: (i) 'khayar' (*Acacia catechu*), (ii) 'simal' (*Bombax ceiba*), (iii) 'satisal' (*Dalbergia latifolia*), (iv) 'okhar' (*Juglans regia*), (v) 'chaamp' (*Michelia champaca*), (vi) 'bijaya sal' (*Pterocarpus marsupium*), and (vii) 'sal' (*Shorea robusta*).

India (13 ± 5% and 19 ± 12%) and Bhutan (5 ± 4% and 14 ± 8%) respectively (Olsen 2005).

The best means of conservation of MPs are to ensure that the population of species continue to grow and evolved in the wild in their natural habitat. In Nepal, *in-situ* conservation of MPs has been maintained mainly within the protected area systems. But, in practice, protection of wild populations and cultivation for commercial purpose are the two prioritized methods for the prevention of species from being extinct or decline (Shrestha and Jha 2009). According to Ghimire (2008) "*in situ* conservation and maintaining resources for future generations can be achieved through understanding of the biological/ecological, economic, socio-cultural and political aspects of resource base; understanding of the complex interactions between many of these factors; and with careful planning and management grounded in ecological principles". The ecological principle of resource conservation and sustainable management includes four fundamental tasks (reviewed by Ghimire 2008): (i) the species or resources to be exploited are first selected; (ii) baseline data about the current resource base, their abundance (density and productivity) and distribution, local use and management practices are then collected though inventory, (iii) demographic studies are conducted under different management regimes to assess the population growth rates, the effect of management and environmental factors on life history parameters, and estimate the optimal rate of use for the long-term persistence of plant populations, (iv) finally, the impact of use is carefully monitored and use levels are adjusted as necessary to minimize biological over-utilization. In Nepal, with the initiatives of government and non-government organizations, some species of MPs and other NTFPs have been brought under *in-situ* management by developing species-specific action plan in different community forests and protected areas (Lawrence 2006; Ghimire and Nepal 2007).

Besides *in-situ* conservation, *ex-situ* conservation measures, like extension of botanic gardens or herbal farms, and commercial cultivation of MPs have been adopted to some extent to reduce pressure on wild resource base. Domestication and cultivation are widely accepted means for the decrease of harvesting pressure of wild population of MPs (Chhetri 2005; Rawat *et al.* 1992). But cultivation practices can creates some problems. The quality and medicinal value of plant materials may decrease and there may be logistic problems on cultivation of MPs in high Himalayas (Schippmann *et al.* 2002; Hamilton 2004). Only 20% of native Himalayan MPs are under cultivation. This proves that there is severe lack of research for the domestication of Himalayan MPs (Dhar *et al.* 2000; Shrestha and Jha 2009).

For the successful cultivation of MPs without decaying any quality in medicinal properties, wild habitat condition must be replicated in farm land. This practice may be less practicable but can be maximized by acquiring detail information about habitat and distribution of target species (Dhar *et al*. 2000). In Nepal, research on domestication and cultivation of MPs was initiated by the government since 1960s' through the establishment of herbal farms in different ecological regions, but this practice could not be replicated in large extent (Bista 1976; Ghimire 1999). But cultivation practices could be raised as per the need of market demand due to the lack of institutional support for the production, fluctuating export market and low price paid by traders, lack of highly technical man power, lack of research for the cultivation techniques and knowledge of species biology etc. are the reasons for paying less effort for cultivation (Ghimire 1999).

2.2 Population Ecology and Management of MP Resources *in situ*

2.2.1 Disturbances and ecology of MPs

MPs are affected by diverse factors of both anthropogenic and natural origin. Over harvesting, livestock grazing and fire are some of the important anthropogenic factors affecting population ecology of a number of MP species. Harvesting of MPs may alter ecological processes at individual, population, species, ecosystem and landscape levels (Hall and Bawa 1993; Tiktin 2004; Ghimire *et al.* 2005). So for the estimation of sustainability of MP harvesting, the study of population dynamics is important. Effects of harvesting on population dynamics depend on the plant part harvested (Cunningham 2001; Tiktin 2004). Ghimire *et al.* (2005) stated that harvesting period, amounts harvested and methods applied are the important factors that differentially affect the population ecology of MP species. In contrast to the harvesting for health care, commercial harvesting leads to the premature harvesting due to high demand in local as well as regional markets. So it is essential to establish more sustainable approaches based on sound knowledge of the biology and ecology of the target species as well as focusing on socioeconomic relationship between collectors and traders (Ghimire *et al.* 2004; Subedi 2006).

Forest fire influences not only the diversity and abundance of plant species but also their distribution pattern. In Manang valley, north-western region of Nepal, Shrestha and Jha (2009) observed that in the post fire site, the individuals of *N. scrophulariiflora* mostly formed compact mat on the exposed soil surface, and other species were poorly represented within the mat. But in other types of habitats, like scree and debris deposited sites the patches were comparatively loose. In these habitats other species like *Rhododendron anthopogon, Bistorta macrophylla*, *Primula* spp., *Aster* spp., *Geranium* spp., *Salix calyculata*, *Bistorta affinis* and *Potentilla fruticosa* etc. are well represented (Shrestha and Jha 2009).

Grazing of domestic animals influences the diversity and abundance of plant species (Austrheim and Eriksson 2001; Oostermeijer *et al.* 2003; Ghimire *et al.* 2006). Heavy grazing leads the simplification of ecosystem structure, life forms and species diversity (Austrheim and Eriksson 2001; Ghimire *et al.* 2006). The abundance of plant species is also influenced by trampling of livestock and wild animals. Trampling and repeated grazing causes damage to the plant reducing seed production. Lama *et al*. (2001) reported a number of MPs that are not eaten by livestock, for example *Aconitum spicatum, Geranium pratense, Rumex nepalensis, Artemisia* spp., *Elsholtzia* spp etc., are grazing resistant species in high Himalayas. Ghimire *et al.* (2004, 2005) also reported that the two commercially threatened MP species recorded in Dolpo region (*Nardostachys grandiflora* and *Neopicrorhiza scrophulariiflora*) are unpalatable and grazing tolerant. But both species shows decreasing abundance with increasing proximity to a summer house, indicating higher sensitivity to harvesting pressure (Ghimire *et al.* 2004). The grazing resistant plant species were most abundant in heavily grazed sites, which lead to the conclusion that species may be positively influenced to a certain extent by grazing. However the combined effect of grazing and high level of harvesting has imposed negative impact on the diversity and abundance of rare and economically exploited MP species (Ghimire *et al.* 2004). Managing grazing and resource harvesting at ecologically and economically sustainable level is of great challenge for the maintenance of plant diversity and abundance in high Himalaya.

2.2.2 Population size and structure in relation to habitat heterogeneity and human impact

There is strong relationship between environmental conditions, and population size and structure of plants and this relationship has been reported to have important implications for conservation (Colling *et al.* 2002). In fragmented landscape, such as high Himalaya, plant populations become both smaller and more isolated from each other. In Manang district, for example, populations of *N. scrophulariiflora* are fragmented and its population sizes appear to be declining though there is no commercial collection at present (Shrestha and Jha 2009). Smaller populations are more prone to demographic, environmental and genetic stochasticity as well as allee and edge effects (Lande 1988, 1998). Compared to demographic stochasticity, populations need to be larger to be buffered against environmental stochasticity (Menges 1991; Menges 1992; Lande 1993, 1998). In particular, catastrophes, environmental stochasticity with high impact and low frequency, may easily cause local extinction (Lande 1993). Habitat fragmentation is the main cause for the rarity of species and small population generally has less variation than large population. Genetic variation is significantly correlated with population size, pollination system (Oostermeijer *et al*. 2000, 2003) among others. Very small populations, with fewer than 50 flowering plants, suffer from the allee-effect and larger populations, however, are also pollen-limited due to the effect of lesser present of pollinator (Oostermeijer *et al*. 2000, 2003).

Local populations of short-lived species are even more sensitive to the deteriorating environmental conditions than the long-lived species in their response to habitat change. Thus local populations of short-lived species will decline and become extinct quickly because of the effect of any demographic change on population size (Schemske *et al*. 1994; Fischer and Matthies 1998a,b). Although long life spans in long-lived species allow populations to withstand long periods of unfavorable environmental conditions, they are strongly affected by human use as recovery in such species can be slow due to recruitment limitations (Schemske *et al*. 1994; Eriksson 1996; Ghimire *et al*. 2008). In long-lived species, the size and number of populations may thus not be good indicators of the status of a species. However, Oostermeijer *et al*. (1994, 2001) suggested that the analysis of population structure in perennial plant may give valuable insights into population processes and may help to assess the status of population.

2.3 Morphology and Ecology of Clonal Plants

2.3.1 Clonal plant architecture and growth strategies

Plant architecture refers to vegetative characteristics, such as size or growth form, patterns of seasonal development, or the persistence and variety of above ground parts (Lawton 1983). The concept of plant architecture suggests that each plant species has its own growth form (Bell 1984). Plants are modular in structure, and so clonal plants add modules at its base and extend horizontally. Clonal growth may be defined as the horizontal extension of a plant by the addition of vegetative offshoots which develop their own roots. These vegetative offshoots are initially physically attached to the parent and each capable of independent existence if they are detached. These morphological units with the potential for an independent existence are called ramets. The plant originated from seed and which divide into ramets is called genet as all parts share exactly the same gene. The behavior of genet which is physiologically integrated with each other to some degree is likely to be different from the behavior of unconnected genet. Populations of ramets have birth, death and reproductive rates just as genets do.

Perennial plants are typical example, with a genetic individual consisting of numerous modules or ramets arising separately from a basal crown or from rhizomes or stolons. So that growth of genet will be dependent on the number of ramet produced. Many plant species, like *Cynodon dactylon*, *Festuca rubra, Helianthus divaricatus, Nardostachys grandiflora, Neopicrorhiza scrophulariiflora, Potamogeton perfoliatus, Rhus aromaticus*, *Trifolium repens,* show clonal growth (Silvertown 1987; Nantel and Gagnon 1999; Wolfer and Straile 2004; Ghimire *et al.* 2005). In many species, like *Viola blanda, Aralia nudicaulis* ramets form clumps, though excavations have revealed that these are frequently formed by the convergence of rhizomes or stolen from different genet.

Compared to non-clonal plants, clonal plant architecture comprises additional growth rules such as number of ramets, branching patterns (frequency and angle) of rhizome, and the rhizome spacer length in between consecutive shoots (Callaghan *et al.* 1990; Huber *et al.* 1999). These rules are plastic to a certain degree allowing for adaptive responses to a spatially heterogeneous environment (Marbà and Durte 1998). The knowledge of plant architecture is essential for understanding the different rates and patterns at which these plants occupies space. Information on clonal plant growth rules is also important for the understanding and management of clonal species. Plant species also differ in the capacity of their roots to respond to unpredictable soil nutrient enrichment. Different edaphic, environmental, topographical, harvesting patterns etc are the important factor.

Lovett Doust (1981) used the term ‘guerrilla’ and ‘phalanx’ to describe the growth strategies of clonal plants. The clonal plant species with widely spaced ramets that rapidly spread into new areas are called ‘guerrilla species’ and clonal plants with closely spaced ramets resulting in an unbroken advancing front and slow spread into new areas are called ‘phalanx’. The biomass of clonal plants that bear guerrilla strategy increased more rapidly when the space is available and also guerrilla type genotypes also decline more rapidly under severe competition than phalanx-like genotypes (David Humphrey and Pyke 1998).

Guerrilla species are better adapted to compete effectively with other species and phalanx species are better able to withstand intraspecific competition (Schmid and Harper 1985; David Humphrey and Pyke 1998). Guerrilla species should have an advantage over phalanx species in exploiting open space (Lovett Doust 1981; de Kroon and Schieving 1990; Bazzaz 1991; de Kroon and Hutchings 1995).

2.3.2 Clonality, reproductive success and population persistence

Sexual reproduction is important to allow organisms to produce genetically diverse offspring with higher potential to adapt to new or changing environments (Silvertown 1987), and is less susceptible than the genetically uniform progeny of clonal growth because resistance to disease often has heritable component (Silvertown 1987). The clonal plant species are generally expected to invest relatively more in vegetative growth (MacArthur and Wilson 1967). Sexual reproduction in clonal plants is favored by the low density or favorable site conditions (Loehle 1987). The probability of flowering and seed production declines on increasing the density and competition in clonal plants (Newell and Tramer 1978; Eriksson 1989).

The challenging question regarding clonality is whether reliance on vegetative propagation with its inherent reduction in genetic diversity allows the flexibility for a species to adapt to a changing environment and avoid extinction. The establishment of clonal plants from seed is initially very much important because it lays foundation for a whole dynasty of vegetative propagating genet. Natural selection acts heavily at the establishment phase of clonal population and that prolonged competition between different clones over several years allows only those best adapted to local condition to survive (Silvertown 1987). So that the genetic differences should be found between clones occupying different micro-environments within a habitat. For example, *Erythronium propullans* (Minnesota trout lily) reproduces by means of a dropper (propagation bulb), which allows the species to colonize in relatively large areas; this may prevent its extinction. In this species diversity increases with geographic distance as the number of dominant clones also increases (Ellstrand and Roose 1987). Clonal reproduction becomes the dominant reproduction unit when clonal progeny (ramet) is very adaptive to particular environment.

In clonal plants, established genet survival is the most important factor for the determination of population growth (Eriksson 1992). The mortality rate of clonal plants is relatively low; clonal growth can enhance the genet survival by sharing the risk of death between ramets (Cook 1979; Harnett and Bazzaz 1985; Callaghan *et al.* 1990; de Kroon *et al.* 1992; Eriksson 1993). The tendency towards a reduction in the number of genet in a population may be accompanied by an increase in number of ramet per genet. Clonal plants have also developed the ability to maintain genet survival in the face of competition (Cook 1979; de Kroon *et al.* 1992). However, clonal growth can lead to large dense stands of a single genotype dominating a population. Both high density and genetic uniformity appear to increase the susceptibility of plant population to epidemic disease and to insect attack (Silvertown 1987).

2.3.3 Clonal behavior on contrasting environmental gradients

The interaction of clonal plants with other plants and their abiotic environment is influenced by ramet numbers and spatial spread of ramets by means of rhizomes and stolons. Interconnected ramets are less affected by intraspecific competition at high density than are individual shoots grown from seeds (Harnett and Bazzaz 1985). This may have been because the connected shoots possessed a rhizome which contained stored assimilate unavailable to single shoots. Clonality could be an effective means of placing ramets into unoccupied resource patches (David Humphrey and Pyke 1998). The ability to translocate assimilates from ramet to ramet allows clone to even out spatial heterogeneity (Silvertown 1987). The clonal plants which bear guerrilla strategy are more effective at exploiting resource heterogeneity. They have greater competitive ability and ability to exploit more quickly larger patches of resources due to rapid rhizomatous growth, but are ineffective at smaller patch sizes (Schmid and Harper 1985; Thompson 1993; Cheplick 1997).

Increased productivity had contrasting effects at the genet and ramet (rosette) levels. For example, at nutrient enriched sites, fertilization is favored in species such as *Scorzonera humilis* (Asteraceae), a European endangered clonal plant, because of the higher ramet density. Site productivity and soil moisture were found to be the main variables that influenced the proportion of small genets, such as in *Scorzonera humilis* (Colling *et al.* 2002). On increasing the productivity and decreasing the soil moisture, the proportion of small genet decreases and that of large genet increases. On decreasing the genet density, ramet density also decreases indicating that if a site is fertilized, recruitment and survival of genet is reduced but growth of surviving genet is increased (Colling *et al.* 2002). Experiments have shown that increased productivity can strongly reduce or even completely prevent seedling establishment of such clonal species because of increased competition and litter accumulation (Tilman 1993; Foster and Gross 1998). It has been shown for several other species of meadows or grassland habitats that competition by the established vegetation is an important factor affecting seedling establishment (Krenova and Leps 1996; Spackova *et al*. 1998; Kotorova and Leps 1999). There has been positive effect of reducing competition by the established vegetation on seedling survival of clonal plants in the grasslands (Oostermeijer *et al*. 1994; Krenova and Leps 1996; Morgan 1997; Fischer and Matthies 1998a, b).

Chapter 3

MATERIALS AND METHODS

3.1 Study Species

Neopicrorhiza scrophulariiflora (Pennell.) Hong. [synonym: *Picrorhiza scrophulariiflora* Pennell; vernacular names: Gentian, Hellebore (English); Gorki, Gurki (Gurung); Katuko, Kutki (Nepali); Katukaa, Aristha, Katavi, Matsyapitta, Tikta, Vamaghni (Sanskrit); Hunglen (Sherpa); Kuraki (Tamang); Honglen, Honglen naak-po (Tibetan); Kuru, Kutki (Hindi)] is a commercially threatened medicinal plant species, distributed in sub-alpine to alpine region of the Himalaya and China from Garhwal Himalaya, westernmost Nepal to Bhutan, SE Tibet and SW China from 3500 to 4800 masl (Press *et al*. 2000). The habitat varies from high altitude rocky shrubland (mainly dominated by *Potentilla fruticosa*, *Rhododendron anthopogon* and *Rhododendron lepidotum*), forests, meadows, gravel areas of moraine to rocky slopes (Fig. 3.1). It prefers moist north-facing slopes with richer and partial shady soil (Shrestha and Jha 2009). Partial shades of small shrubs especially those of *Rhododendron anthopogon, Potentilla fructicosa* have been considered to be better for its growth and productivity (Ghimire *et al*. 2005).

It is a long-lived, rhizome bearing perennial herb and exhibits 'guerrilla strategy' of clonal growth. The morphology, growth and regeneration of this species have been described by earlier workers (Ghimire *et al*. 2005; Ghimire and Nepal 2007; Shrestha and Jha 2009). It reproduces both by sexual and vegetative means (clonally through the production of vegetative off shoots, i.e. ramets). It has highly bitter creeping rhizomes. It develops long rhizomes from the bases of old rosettes, with new rosettes being established at some distance from the mother plant (Ghimire *et al*. 2005). The growing season is short, starting in May and ending by early October, flowering occurs in June-July and fruiting from August on wards. Flowering period of *N. scrophulariiflora* varies depending upon altitude and climatic factors; generally flowering takes place from June to August and fruiting from July to September. The flowers are bisexual in *N. scrophulariiflora* and pollination is entomophilous specially takes place by *Bombus* sp. (bumble bees). The dehiscence mechanism is septicidal (Ghimire *et al*. 2005; Ghimire and Nepal 2007). Seeds are minute and winged so disperse by wind, anthropogenic factors and snow. Seeds germinate by May to June of the following year. The seed germination is phanerocotylar (Ghimire and Nepal 2007). Seed viability is very short. Seedling recruitment is very low in natural conditions but in controlled environment (laboratory conditions), germination of seeds range from 75-100% in 3-4 weeks (Ghimire *et al*. 2005).

Rhizome is traded highly for medicinal propose. In regional market, the rhizomes of *N. scrophulariiflora*, together with those of *Picrorhiza kurrooa* Royle (which is listed in CITES Appendix II) have been traded under a common trade name kutki (Mulliken 2000). Nepal is the main supplier of *N. scrophulariiflora* to international market. Government of Nepal has listed *N. scrophulariiflora* as a protected MP and has prioritized it for research and agro-technology development (DPR 2006).

Kutkin (a glucosidal principle), kurrin (a non-bitter product), kutkiol (an alcohol) and kutkisterol are the important biochemical constituents of *N. scrophulariiflora* rhizome and rhizomes and roots contain picroside I, picroside II, kutkoside, minecoside, phenolglycoside (picein and androsin) and 4-hydroxy-3-methoxyacetophenon (reviewed in Ghimire *et al* 2005; DPR 2007). Its rhizome is used to control fever and gastralgia, root powder as laxative, also prescribed in liver complaints, anemia and jaundice. In Nepal, the creeping rhizomes of *N. scrophulariiflora* have been used for treating cold, fever, headache, anemia, high blood pressure, sore throat, gastritis, intestinal pains and conjunctivitis in traditional medicine practice by amchies and healers (Pohle 1990; Lama *et al*. 2001; Manandhar 2002; Bhattarai *et al*. 2006; Shrestha and Jha 2009). So due to its high medicinal value, it is on over exploitation in different areas of Nepal. A thorough study of the biology of such plant species is needed for conservation and suggesting sustainable harvesting regime.

Fig 3.1. Photographs showing habitat of *N. scrophulariiflora.*

3.2 Study Area: Kangchenjunga Conservation Area

3.2.1 Location and physiography

The study area consists of the Walangchung Gola and Ghunsa villages and their surrounding river valleys in Kangchenjunga Conservation Area (KCA), in Taplejung district in the north-east corner of Nepal (Fig. 3.2-3.4). KCA (latitude: $27^0$30' to $28^0$00'N and longitude: $87^0$45'E to $88^0$15'E) represents unique floral and faunal diversity and is rich in cultural heritage, encompassing a total area of 2,035 sq. km within the altitudinal range 1200 to above 8500 masl. KCA shares an international boarder with Sikkim of India in the east and the Tibet Autonomous Region of China in the north. It covers several majestic peaks including Mt. Kangchenjunga (8586 m), the third-highest peak of the world. In recognition of its rich natural and cultural resources, the Kangchenjunga Conservation Area was declared as "A Gift to the Earth" on April 1997 by Government of Nepal in support of WWF's Living Planet Campaign. The area is characterized by great variation in elevation, landscape, climate, habitat, vegetation and ethno cultural diversity. About 65% of the area of KCA is represented by rocks and ice-covered mountains, rivers and glaciers, 14% by forests, 10% by shrubs, 9% by meadows or pastures and only 1.6% by agricultural land (Amatya *et al.* 1995; KCA-MC 2005).

KCA covers four Village Development Committees (VDCs), namely Tapethok, Lelep, Yamphudin and Walangchung Gola. The focus of the present study was alpine pastures and surrounding habitats in Walangchung Gola (Walangchung Gola VDC) and Ghunsa (Lelep VDC) (hereinafter referred to as Walangchung Gola sector and Ghunsa sector respectively). Two U-shaped river valleys, Singjema and Syamdo were surveyed in Walangchung Gola sector and one river valley, Yamatari was surveyed in Ghunsa sector (see Fig. 3.2, 3.3 and 3.4; Table 3.3). Singjema khola and Syamdo khola are the major tributaries of Tamur River and Yamatari khola is the tributary of Ghunsa River. The study sites in Walangchung Gola fall within the Tiptala Bhanjyang Conservation Community Forest. Similarly, the study sites in Ghunsa fall within the Kumbhakarna Conservation Community Forest.

3.2.2 Climate

Because of an extreme altitudinal gradient of over 7000 m within a short distance of <100 km, the climate of KCA ranges from subtropical to alpine. The higher altitude (above 2500 m) areas such as Ghunsa and Walangchung Gola go through a mild summer and cold winter with snow and frosts. Annual precipitation recorded in the nearest meteorological station (Taplejung, altitude: 1840 m) range 1500-2500 mm with an average of 1965 mm (between 1990 and 2000) (Ghimire and Nepal 2007). About 75%-80% of the rainfall in KCA occurs during monsoon (mainly June-September) (Shrestha and Ghimire 1996; KCA-MC, 2005). Among the two sectors studied, Ghunsa sector lies towards the far eastern corner of Taplejung district which can be assumed to receive generally higher precipitation. Walangchung Gola sector receives relatively lower precipitation as most of its areas, particularly lying at high altitude, are sheltered from monsoon by high mountain ranges towards the east. However, in Ghunsa as well, the inner Khambachen valley is very much dry. Only sporadic data on precipitation of Ghunsa sector is available. Regmi (2003) recorded precipitation for monsoon period at three different altitudes in Ghunsa sector: 3407 masl (in Ghunsa village area), 4648 masl (Kangchenjunga Glacier) and 5235 masl (mid-slope). The total amount of rainfall from May to September was 603.4, 239.2, and 308.0 mm at Ghunsa, Kanchenjunga Glacier, and the mid-slope, respectively (Regmi 2003).

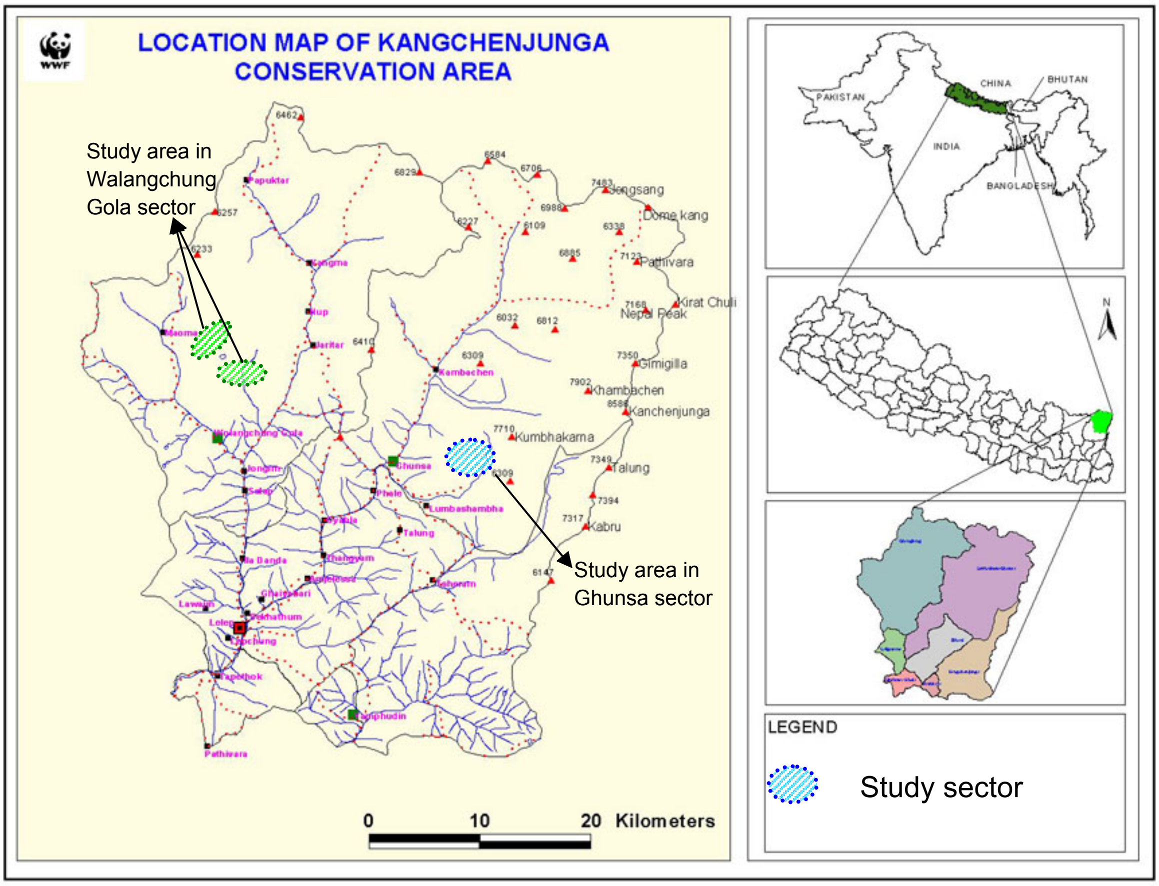

Fig 3.2. Map of Kangchenjunga Conservation Area, Taplejung, East Nepal (study sites in Walangchung Gola and Ghunsa sectors are shown in the figure). (Source: WWF Nepal)

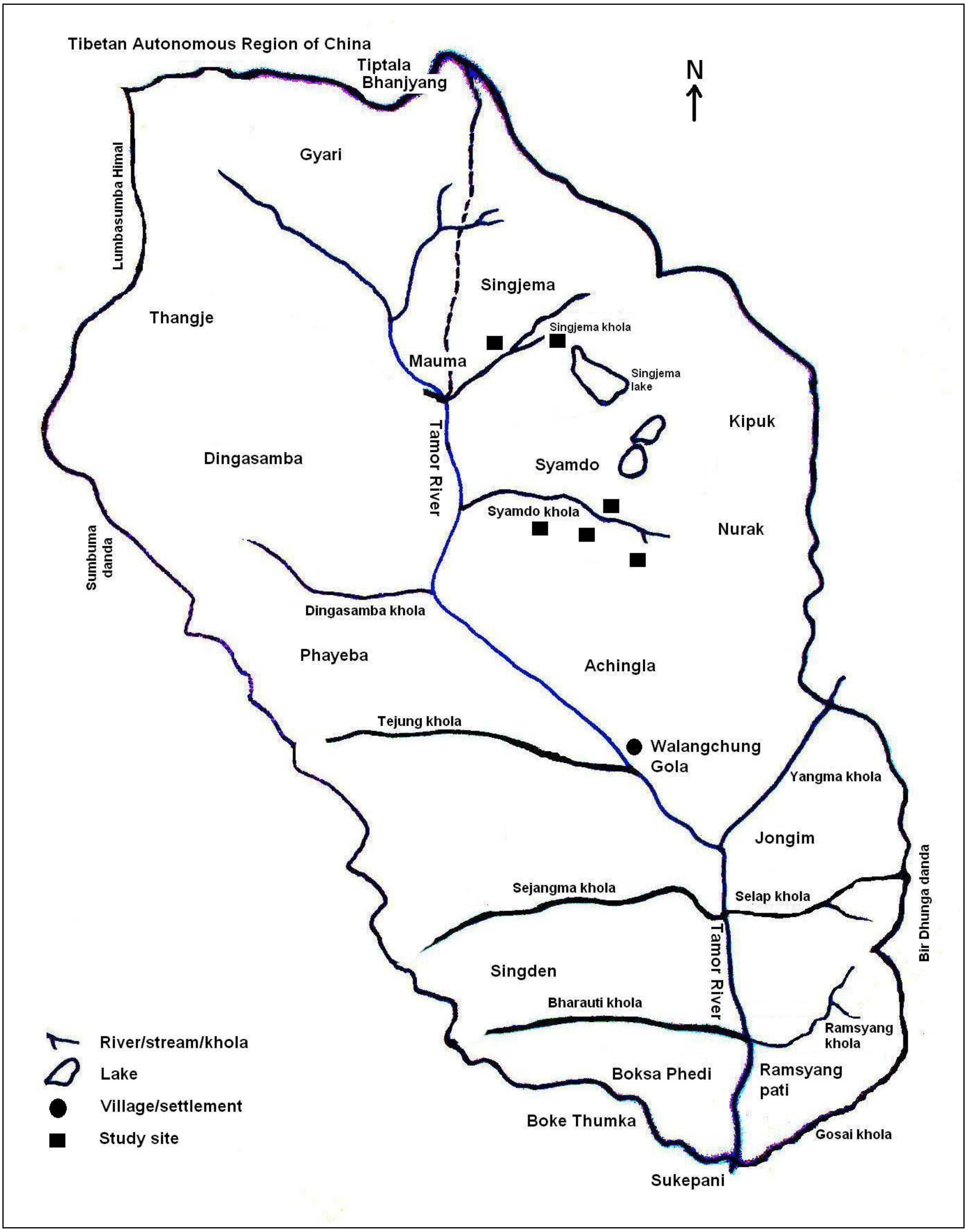

Fig 3.3. Map of Tiptala Bhanjyang Conservation Community Forest, Walangchung Gola.

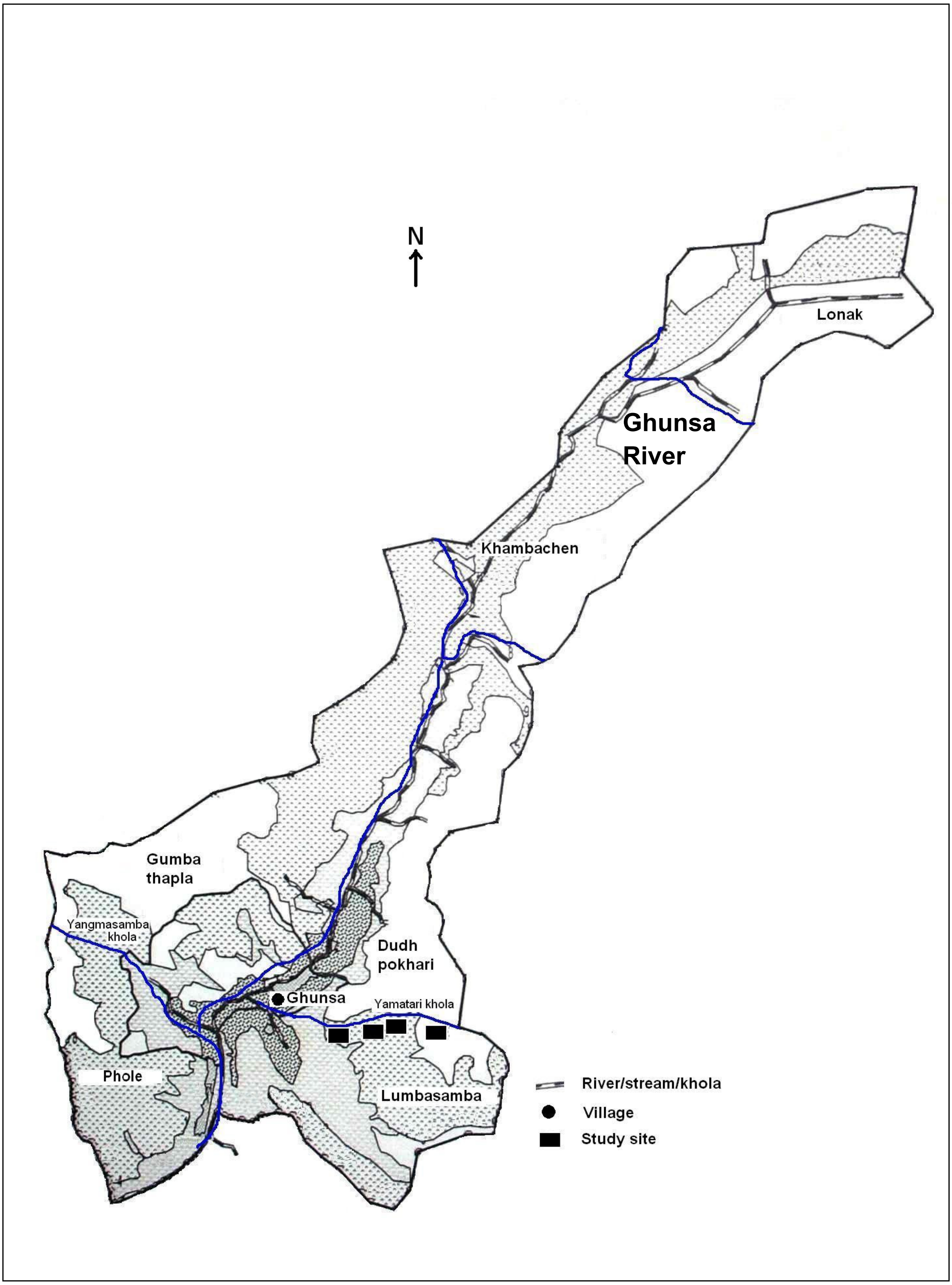

Fig 3.4. Map of Kumbhakarna Conservation Community Forest, Ghunsa, Lelep.

3.2.3 Vegetation and plant biodiversity

KCA is regarded as one of the important 'biodiversity hotspot' sites of eastern Himalaya. Phytogeographically, the area forms the meeting ground of the Indo-Malasian and Sino-Japanese (E. Asian) elements with typical east Himalayan types of vegetation. Several scientists visited KCA, after the first botanical exploration made by J.D. Hooker in 1952 (Hooker 1854; 1883-1897). Some of the important botanical expeditions were those of H. Hara (University of Tokyo), H. Kanai (University of Tokyo), S. Kurosawa (University of Tokyo), G. Murata (Kyoto University), M. Togassi (Tokeda Pharmaceutical Laboratory, Osaka), T. Tuyama (Ochanomizu University, Tokyo) (Hara 1966). Aspects of the flora and vegetation of the region is treated by Banerji (1955), Stainton (1972), Carpenter *et al.* (1994 a, b), Shrestha and Ghimire (1996).

Table 3.1. Biodiversity of KCA compared to national and global biodiversity.

Species	KCA	Nepal	World
Plants	844	5884	2200559
Birds	253	861	9040
Mammals	22	181	4000

Source: Shrestha and Ghimire (1996); KCA-MC (2005).

KCA harbors diverse vegetation types [16 vegetation communities have been described so far; Shrestha and Ghimire (1996)], and over 844 species of flora and over 275 species of fauna (Table 3.1). KCA consists of two WWF's Global Eco-regions, i.e. Eastern Himalayan Alpine Meadows and the Eastern Himalayan Broadleaf and Conifer forests (WWF-NP 2001). There are 31 species of endemic flowering plants in KCA and its surrounding areas (Shrestha and Ghimire 1996). The area is also rich in diversity of rhododendrons and orchids. Out of 31 *Rhododendron* species recorded so far from Nepal, 24 species occur in KCA; similarly, 69 out of 250 orchids found in Nepal occur in KCA (Amatya *et al.* 1995; KCA-MC, 2005). The area is particularly rich in Himalayan Larch (*Larix griffithiana*); and many other endangered (e.g., *Michelia kisopa*) and vulnerable (e.g., *Neopicrorhiza scrophulariiflora*) plant species of Nepal.

Distribution of forest types show distinct pattern along altitudinal and other micro-environmental gradients. Shrestha and Ghimire (1996) identified 16 vegetation communities in four bioclimatic zones. At subtropical to lower temperate elevations (1000-

2500 m), the forest consists of evergreen broad-leaved forest made up of *Schima-Castanopsis-Quercus* which is generally replaced by conifer forest (generally *Abies spectabilis, Tsuga dumosa, Juniperus indica and Larix griffithiana* at higher altitudes). Coniferous forests extend as high as altitudinal timber line (about 4000 m). Above this line in the upper sub alpine to alpine zones, vegetation consists of the species of *Rhododendron* (e.g., *R. anthopogon, R. lepidotum, and R. setosum*), *Potentilla* (e.g., *P. fructicosa, P. microphylla*), *Bistorta*, *Geranium*, *Saxifraga*, etc. The south facing slopes are dominated by *Juniperus indica* and north facing slopes are dominated by *Rhododendron* spp.

3.2.4 Biodiversity conservation

KCA is one of the most important protected areas of Nepal managed by local communities. The main objective of KCA is to safeguard biological and cultural treasurers ensuring the traditional rights over sustainable resource utilization and enhancing livelihoods of mountain people. However, the biodiversity of KCA is under great threat from unsustainable harvesting and illegal trade of natural resources (such as NTFP), overgrazing, forest fire, etc. Similarly, other issues and challenges related to biodiversity conservation and sustainable livelihoods are inadequate research and documentation of biodiversity (particularly the status of endangered and endemic species, traditional knowledge and skills on biodiversity use), lack of awareness on sustainable natural resources management, lack/inadequacy of transportation and communication infrastructure, absence of advocacy/advertising/poor entrepreneurship/marketing skills of local communities, etc.

Recently, with the initiation of Department of National Park and Wildlife Conservation (DNPWC) and Kangchenjunga Conservation Area Project (KCAP), 16 conservation community forests (CCFs) have been delineated and successfully handed over to the respective communities ensuring better management of natural resources. In both Walangchung Gola and Ghunsa sectors of KCA, the land tenure regime of *N. scrophulariiflora* growing sites has been handed over to the communities as Tiptala-Bhanjyang and Kumbhakarna CCFs, respectively. In both CCFs, forest operation plan has set operational rules and conditions how the forest will be managed. In addition, NTFP Action Plans have also been prepared for the management of specific NTFPs in both the CCFs. Illegal collection activities have been well regulated in Kumbhakarna CCF, while in Tiptala-Bhanjyang CCF still the enforcement of forest management rules and regulations is weak. This is the area where illegal harvesting of resources being the main issue especially from the people coming from Tibet as this area is linked to Tibet via Tiptala pass. As revealed by previous studies, the outsiders (cross border communities) do collect 3-4 times more than that the local collectors do (Ghimire and Nepal 2007).

3.2.5 Socio-economy and culture

In KCA, about 4941 people belonging to 975 households (with average family size of 5.1 per household) live in different settlements within four VDCs (Ghimire and Nepal 2008). Bhotia (Sherpa) from Tibetan origin at higher altitudes, and Limbu, Rai, Gurung and Tamang of Tibeto-Burman origin at lower altitudes are the major communities of KCA each with their own language, culture and religion. Bhotia people of Walangchung Gola are popularly known as Walung and they speak their own Walung dialect slightly different from other Sherpa dialects. Agriculture, animal husbandry, seasonal trade and tourism are the major economic activities of the people. Over 50% of the households earn less than NRs. 15000 (or US$ 200) per year which indicates the poverty level and is the average gross domestic product (GDP) per capita in Nepal (WWF-NP 2002). Animal husbandry and trade of medicinal plant is the major economic activities in high altitude areas of KCA.

3.2.6 Medicinal plants in KCA: diversity, trade and conservation status

Diversity of MPs

A total of 253 species of NTFPs have been documented from KCA, including some high value species, such as *Dactylorhiza hatagirea*, *Daphne* spp., *Edgeworthia gardneri, Hippophae tibetana*, *Nardostachys grandiflora, Neopicrorhiza scrophulariiflora, Saussurea* sp., *Swertia chirayita* etc. (Ghimire and Nepal 2007, 2008). Highest number of species has been cited for medicine (50% of total species) and food (42%) and about 35% of total species have multiple uses (Ghimire and Nepal 2007; Devkota B. unpublished data). Alpine vegetation, most threatened by environmental and land use changes, is rich in biodiversity and harbor most useful plant species. Among most potential NTFPs, *Daphne* spp., *Edgeworthia gardneri* and *Swertia chirayita* from the lower altitude and *Neopicrorhiza scrophulariiflora, Saussurea* sp. and *Hippophae tibetana* from higher altitude have been identified by WWF as potentially important for research, monitoring and management in community forests in KCA (Ghimire and Nepal 2007).

Medicinal plant trade and collection pattern

Medicinal plants and other NTFPs provide valuable income to the rural people. Shrestha and Ghimire (1996) described that Taplejung district is one of the most important areas of Eastern Nepal from where large amounts of MPs have been exported to India since long. Due to the lack of arable land and other opportunities of employment and poverty, MP trade is the primary sources of income (Sherpa 2001). Walangchung Gola is one of the important trading routes in north-eastern parts of Nepal. The trade of MPs via Walangchung Gola to Tibet is decades long and is still in existence. For centuries, trans-Himalayan trade through Koshi hills was centered on the village of Walangchung Gola (Edwards 1993). A huge amount of MPs like *Neopicrorhiza scrophulariiflora*, *Saussurea* spp., *Swertia chirayita* etc. and animal products like butter, dried cheese (churpi, a hard sweet made from yak milk), etc. are used to be exported to Tibet. Because of high demand, the market of certain MPs has expanded in large quantities every year (Sherpa 2001). *Neopicrorhiza scrophulariiflora* (Kutki) and *Saussurea gossipiphora* (Yazembawa) are the two species which have highest demand in Tibet. Sometimes Tibetan traders come themselves to Walangchung Gola to buy MPs and sometimes barted with food materials and other household consumer goods. Almost trade of MPs is performed in fresh condition.

Collection of high-altitude MPs is generally carried out in groups during November (after monsoon) immediately after seed reaches maturity (Sherpa 2001). Collection at this time permits seed dispersal and trade portion may be larger and may also yield more active ingredients per unit weight (Edwards 1995). However many local harvesters of KCA are forced to collect early because they have to compete for the scarce resource not only themselves but also with the people from Tibet.

To import food and other accessories from Tibet before the snow fall in high Himalayan pass, people harvest MPs prematurely though the quality is poor. This practice is believed to be serious as the species are declining from the area once they flourished and people have found it more difficult to collect the products. Thus the unsystematic, unscientific collection and over exploitation are the major threats to the continued existence of medicinal and aromatic plants in high altitude areas of KCA (Sherpa 2001).

Table 3.2. Highly prioritized species of MPs found in Walangchung Gola with ranking scores assigned by local people, availability, threat, collection period and uses.

Botanical name	Local Name*	Average ranking score for different uses			Total score		Period of collection	Parts use	Local avail-ability	Threat	Main local uses
		Medicinal	Socio-cultural	Trade	Sum (in 30)	%					
Aconitum bisma	Bikhma (N), Pungnak (W)	6.75	1.00	1.58	9.33	31.11	Aug-Nov	Root	rare	Over harvesting for local use	Anti poison, common cold
Aconitum spicatum	Bikh (N), Chenduk (W)	0.00	0.00	0.00	0.00	0.00	Aug-Nov	Root	rare	Naturally rare	Not used locally
Dactylorhiza hatagirea	Panchawale (N), Angulakpa (W)	4.58	0.00	0.33	4.92	16.39	Sept-Nov	Root	common	Over harvesting for local use & trade	Wound healing
Hippophae tibetana	Bhuichuk Tara	0.00	0.00	0.00	0.00	0.00	April-May	Root	rare	Collection for local use and trade	Not found in W. Gola but is available in Ghunsa valley used to make fruit juice
Nardostachys grandiflora	Jatamansii (N), Pangboe (W)	0.83	3.83	1.75	6.42	21.39	Aug-Nov	Root	rare	Over harvesting for local use & trade	Incense
Neopicrorhiza scrohulariiflora	Kutki (N), Hungle (W)	4.83	0.00	6.08	10.92	36.39	Aug-Nov	Root	common	Over harvesting for local use & trade	Fever, cold, cough, trade
Rhododendron anthopogon	Sunpati (N), Phalu (W)	0.00	6.42	0.92	7.33	24.44	All year	Leaves	common	Not threatened	Incense
Saussurea gossipiphora	Maikopila (N), Yazembawa (W)	1.00	1.17	1.83	4.00	13.33	Sept-Nov	Whole plant	common	Over harvesting for local use & trade	Trade, wound healing, ritual
Swertia chirayita	Chirayito (N), Tikta (W)	0.83	0.00	3.17	4.00	13.33	July-Aug	Whole plant	common	Over harvesting for local use & trade	Brewing local alcohol (rum), fever, trade

*N = Nepali name; W = Walung name.

Conservation status

A total of 38 species of MPs found in KCA fall under threat category assigned under different designations (Appendix 1). Similarly, 21 species fall under protection or prioritization categories of Government of Nepal. Out of these species, 6 species are prioritized for agro-technology development, 2 species including *Neopicrorhiza scrophulariiflora* are banned for collection and trade, 2 species are banned for export outside the country without processing, 14 species including *N. scrophulariiflora* are prioritized for research and development and 2 species are included as threatened by trade. A total of 33 species are locally threatened, out of which 11 are threatened due to natural factors (i.e. they are naturally rare) and 22 are threatened due to anthropogenic activities, mainly harvesting for trade (Appendix 1). The local status of different plant species was based on field observation and data from secondary sources.

3.3 Methods

3.3.1 Prioritization of most important MP species in KCA

Field studies were conducted during the peak growing season (June/July) in 2006 and 2007. Prior to primary data collection, inception workshops and community discussions were held in Walangchung Gola (Tamur valley) in order to identify the most potential species of MPs in terms of local use and conservation value. In this process, a total of nine highly prioritized species of MPs were selected to assess their relative importance in Walangchung Gola (Table 3.2). These species were ranked (with a ranking score from 1-10) by local people in terms of their medicinal, other socio-cultural and trade values. Among these species, *N. scrophulariiflora* received the highest total score (10.92 out of total score of 30, i.e. 36.39%) with highest ranking score for trade. *Aconitum bisma* was second highest in terms of total value with highest score for medicinal use. *N. scrophulariiflora* received second highest score for medicinal use. Ranking exercise was not conducted in upper Ghunsa valley. However, based on the interaction with local people and from previous studies it was revealed that *N. scrophulariiflora*, together with some other species of MPs, is also highly valued in upper Ghunsa valley in terms of medicine and trade. Although the local people did not mention other socio-cultural uses of *N. scrophulariiflora* except medicinal use, this species is highly threatened due to over harvesting for medicine and trade in both areas. This is the reason why *N. scrophulariiflora* was selected in this study.

3.3.2 Utilization and management practices of *N. scrophulariiflora*

Local people, traders, herders, and local healers were interviewed in Walangchung Gola (upper Tamur valley) and Ghunsa village (upper Ghunsa valley) to assess the patterns of utilization and management of *N. scrophulariiflora*. In this process a total of 15 households were interviewed *in situ* as well as during transect walk.

3.3.3 Identification of study populations and their geographical range

A total of seven sites (each representing distinct population of *N. scrophulariiflora*) were selected from Walangchung Gola and Ghunsa sectors of KCA covering different altitude levels and human disturbance regimes (mainly in terms of harvesting pressure) (Appendix 2). Study populations were selected from Walangchung Gola and Ghunsa sectors because these two sectors differ in terms of degree of protection at the landscape level (Ghunsa sector is more protected than Walangchung Gola sector, see section 3.2.4) and level of precipitation (see section 3.2.2) thus exhibiting situations of two different microclimates which may have differential effect on the population biology of the target species.

On the basis of altitude, the studied populations were grouped as upper alpine (mean altitude >4500 masl; total 3 populations) and lower alpine (mean altitude <4500 masl; total 4 populations) populations. In each altitude level and in each sector, at least one population was selected from relatively undisturbed site. In Walangchung Gola sector, three populations were selected from upper alpine (mean altitude >4500 masl) and two populations from lower alpine (mean altitude <4500 masl) levels, whereas in Ghunsa sector both of the studied populations were selected from lower alpine level. Sampling of upper alpine populations was not possible in Ghunsa sector due to time constraints. Populations of *N. scrophulariiflora* were selected with the help of local people by participatory resource mapping activities and informal interviews. Study populations were differentiated from each other by geographical barriers made by river and mountain peak.

3.3.4 Variation in growth strategy and plant performance

Variation in growth strategy and plant performance was studied in five populations of *N. scrophulariiflora*: Semjoyunjo (mean altitude 4718 masl), Chhuwapangsong (mean altitude 4570 masl), Chherpari (mean altitude 4390 masl), Chherchen (mean altitude 4368 masl; all from Walangchung Gola sector), and Phukpadep (mean altitude 4316 masl; from Ghunsa sector). In each population, 15 matured clumps or putative genets of *N. scrophulariiflora* were identified and excavated at widely spaced intervals along transect, so as to minimize the chances of sampling clones (except in Semjoyunjo, where 9 clumps were studied). Attempts were made to select clumps having at least one flowering ramet. For each genet a variety of plant performance traits [size; pattern of ramification; number of vegetative and reproductive ramets; length, circumference (girth) and branching pattern of rhizomes; length of peduncle, length of inflorescence and seed output) were measured. A descriptive approach was used for the study of architectural pattern (mostly below ground) and its influence on performance.

Biomass allocation pattern was assessed in three populations (Semjoyunjo, Chherpari, and Phukpadep) to know (although indirectly) the relative contribution of vegetative and sexual reproduction in population persistence (Levine and Feller 2004). In each population, 6-15 matured clumps (genets) of *N. scrophulariiflora* were randomly selected for harvesting different plant parts (rhizome, root, above ground shoot with leaves, and inflorescence). The plant parts were air dried and brought to the laboratory and dried in an oven at 80^0C for 48 hours until constant weight. After 48 hours, oven dry weight of each plant part was measured and following parameters were estimated: sexual allocation (biomass of inflorescence), sexual reproductive effort (biomass of inflorescence/total biomass), allocation to vegetative (clonal) growth (biomass of rhizome branch or vegetative shoot) and vegetative reproductive effort (biomass of rhizome branch or vegetative shoot/total biomass).

To know the pollination mechanism, 20 flowering individuals from two populations (Chherpari and Semjoyunjo) were randomly selected and marked at the bud stage in June 2006. Among these, flower heads (at bud stage) of 10 individuals were bagged and rests of the 10 individuals were left open to assess autogamy and open pollination, respectively. After one year (July 2007), the bagged and open-pollinated flower heads were inspected for seed set. Unfortunately, all the individuals with pollination bag were found dead and were lying on the ground. Some of the individuals were even disturbed by trampling and grazing. Particularly, the experimental site in Semjoyunjo population was totally disturbed because of plant collection and trampling by livestock. In the individuals left for open pollination in Chherpari population, 2-5 capsules were recorded in each inflorescence. The capsules were already dehisced so that only a total of 4-11 seeds were recorded per inflorescence. Therefore, the results of bagging experiment are not further elaborated in the result section.

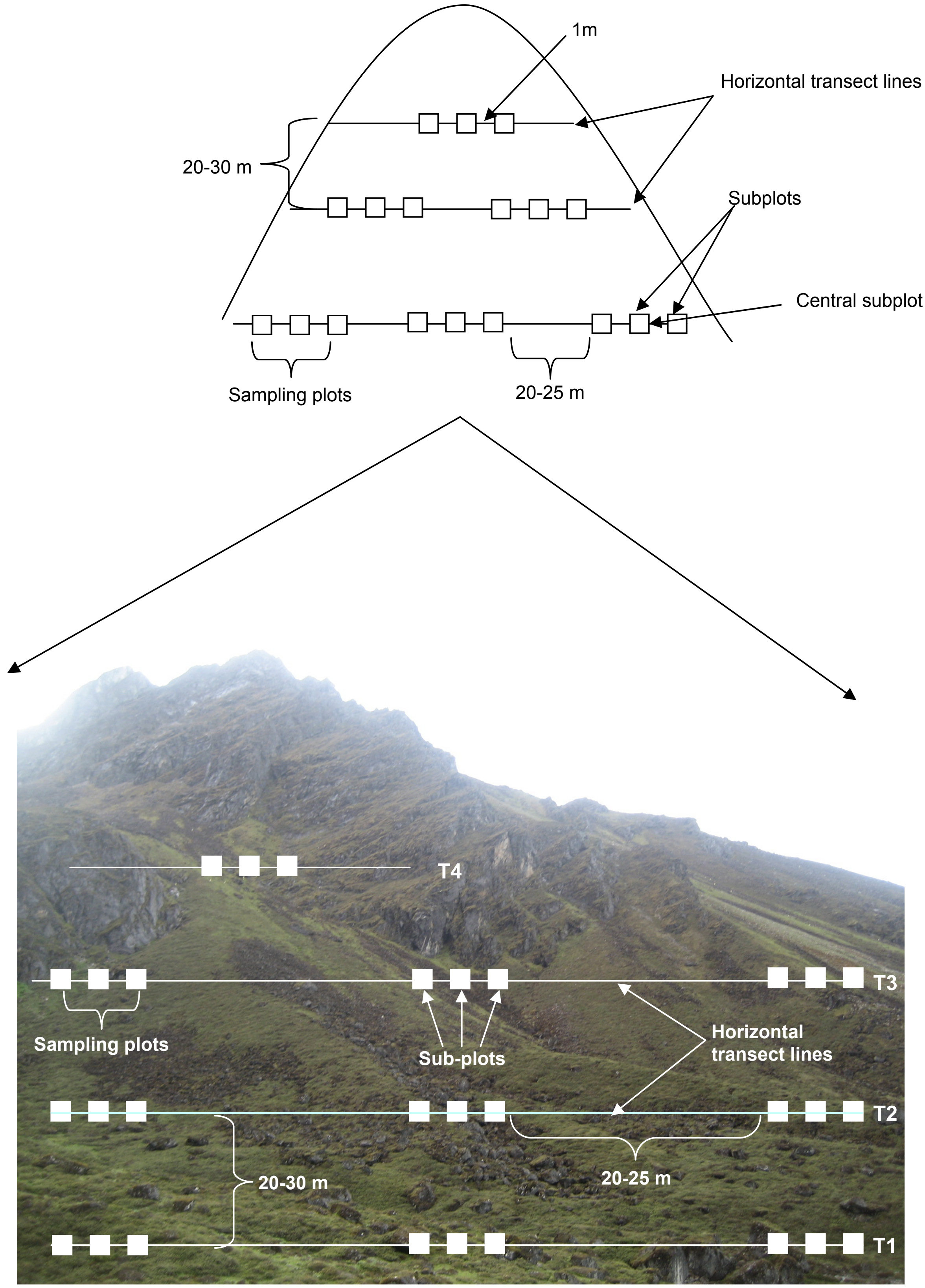

Fig 3.5. Plot layout for sampling *Neopicrorhiza scrophulariiflora* populations (T1-T4 are transects).

3.3.5 Variations in population density and structure

Population density and structure of *N. scrophulariiflora* were studied in each of the identified populations (total seven populations). Population density and structure was studied both at the ramet and genet level. Stratified sampling method was applied covering the area occupied by the target species. In each population, sampling plots were established along horizontal transect lines (see Fig. 3.5). The distance between two successive transects was 20 to 30 m depending upon the field condition. In each transect, 2-4 sampling plots were studied depending upon the field conditions and availability of the target species. Each sampling plot represented 3 consecutive sub-plots of 1 m × 1 m size separated from each other by 1 m. The distance between successive sampling plots was 20 to 25 m, depending upon the field conditions. In total, 136 plots (consisting of 408 sub-plots) were studied in 39 transects in 7 populations. In each sub-plot, number of ramets, at different growth stages (Table 3.3; Fig. 4.2), was counted separately for each genet. Data was recorded carefully to avoid the chances of recording ramets from another genet (clone). At the putative genet level, an individual of *N. scrophulariiflora* was defined as all ramets connected together into a clump. Size of a genet was thus determined based on the number of interconnected ramet.

Table 3.3. Ramet stage class categories of *N. scrophulariiflora*.

Stage classes	Characters
Very young small-sized ramet	Immature, bearing 1-3 smaller-sized leaves
Juvenile/medium-sized ramjet	Immature, bearing >3 smaller-sized leaves
Adult vegetative ramet	Mature but not in flowering stage, bearing large number of leaves
Adult reproductive ramet	Mature flowering or fruiting, bearing large number of leaves

3.3.6 Habitat conditions

Physical, topographical and human management variables were recorded as far as possible for each sampling plot. Impacts of harvesting and other management regimes were assessed by interviewing local people, herders and collectors. Impacts of harvesting, grazing and fire were also studied by direct field observation. Among the other variables cover abundance of all other associated species were recorded in each plot. Similarly, percentage cover of rocks and mosses was also recorded. Soil moisture and pH values were noted in the field, at least in two end sub-plots of each plot, with the help of portable pH-moisture meter. Similarly, soil depth was recorded with the help of iron peg and scale.

However, study of soil parameters was limited to the populations of Walangchung Gola sector. Latitude, longitude and altitude were taken at the two ends of each horizontal transect with the help of GPS and Altimeter. Similarly, slope and aspect were recorded for each plot. Slope was recorded with the help of clinometer. The values of slope, aspect and latitude were combined to estimate relative radiation index (RRI) (which gives the relative value of how much solar radiation a particular spot receives at noon at equinox; the RRI value ranges from +1 to -1) by using the following formula (Ôke 1987):

$RRI = \cos(180^{\circ} - \Omega).\sin\beta.\sin\theta + \cos\beta.\cos\theta$ (where, Ω is aspect, β is the slope, and θ is the latitude of each plot).

Detail soil chemical analysis was done for four representative populations of *N. scrophulariiflora* from Walangchung Gola sector. For this, soil samples at the depth of 10 cm were collected randomly from two locations of each horizontal transects. Soil samples from each transect were mixed and packed in a clean ziplock polythene bag. The samples were immediately brought to the laboratory for further analysis. Different soil parameters [nitrogen (N), phosphorous (P), potassium (K), organic matter (OM), and pH] were analyzed in the regional Soil Testing Laboratory, Department of Agriculture (DoA), Ministry of Agriculture and Cooperatives (MoAC), Government of Nepal, Pokhara, Nepal. Soil organic matter was analyzed by Walkey-Black Method, nitrogen by Kjeldahl method, phosphorous by modified Trug's Method (Ayres–Hagihara) and potassium by flame photometric method (PCARRD 1980).

3.3.7 Data analysis

Non-parametric Kruskall-Wallis ANOVAs and associated pair wise comparisons (Mann-Witney U tests) were executed to detect differences among populations and between altitude levels in the subset of environmental variables. Detrended Correspondence Analysis (DCA) was performed on the species abundance (percent cover) data to assess gradients in the associated species composition, and relationships between species and environmental variables among the *Neopicrorhiza scrophulariiflora* growing sites from upper alpine and lower alpine levels. All the disturbance variables were combined to an overall measure of disturbance (Dis-PCA); similarly all the soil variables were combined to an overall measure of soil variable (Soil-PCA) using principal component analysis (PCA).

Both of these combined variables were then used as predictor variables in multivariate and regression analyses to reduce the effect of multicoliniarity among the individual variables.

One way analysis of variance (ANOVA) was used to assess the differences in plant performance traits, population density and structure among populations. The data were first checked for normality and when distinct skewness exists, the data were normalized by transformation as described by Fowler *et al*. (2001). In addition, nested analysis of variance (ANOVA) model was used to detect differences in plant performance traits, density of ramets and genets across altitude levels with site (population) nested in altitude level (lower alpine vs. upper alpine). Variation in sexual and vegetative allocations among populations was analyzed with Kruskal Wallis one-way ANOVA.

The data on plant performance traits were subjected to Canonical Discriminant Analysis (CDA) to compare groups of individuals from various populations. CDA tests for a significant difference between samples with multivariate data assigned to groups *a priori*. This procedure generates a set of discriminant functions based on linear combinations of the predictor variables that provide the best discrimination between the groups. CDA was used to determine if the populations of *N. scrophulariiflora* were different from one another in terms of plant performance. This technique also was useful for identifying which trait(s) could be useful for distinguishing the populations or taxa.

General ideas about the relationships between environmental variables and population parameters were first developed by using non-parametric correlation analysis. In this regard, Spearman rank correlation coefficients among major environmental variables and between environmental and population variables were analyzed. The significant environmental variables, based on Spearman rank correlation analyses, were then entered into linear or quadratic regression models to isolate the variables that best explains plant density, genet composition, and population structure.

DCA was executed in PC-ORD 4.25 (McCune and Mefford 1999). All other statistical analyses were executed in Statistical Program for Social Science 11.5 (SPSS, 2002).

Chapter 4

RESULTS

4.1 Differences in Habitat Characteristics

The major environmental (physical and disturbance) variables recorded in the study sites, and the relationships between variables in the entire data set are presented in Appendix 2a and Appendix 3 respectively. The altitudinal range of studied populations of *N. scrophulariiflora* was 3993-4734 masl. The slope was found to range between 27.5^0 and 47.0^0. A total of 33 species of angiosperms were recorded as associated species in the *N. scrophulariiflora* growing sites. *Rhododendron anthopogon, Bistorta emodi, Salix calyculata* and *Salix lindleana* were the frequent species found in most of the study sites (Appendix 4); among these, *Rhododendron anthopogon* was most abundant.

Strong habitat differentiation between upper alpine and lower alpine sites could be detected in terms of stand structure and vegetation composition (Fig. 4.1). The habitat of *N. scrophulariiflora* ranged from shrubland to open rocky/scree or grassy slopes on increasing altitude. At the lower alpine level, the habitat was found to be tall shrubland mostly on moist north or north-west facing slopes, dominated by thick canopy of *Rhododendron anthopogon* and *Rhododendron lepidotum.* Upper alpine sites, on the other hand, were mainly represented by scattered thickets of *Rhododendron anthopogon* in association with other close mat-forming dwarf shrubs or shrublets (mainly *Salix lindleana, Cassiope fastigiata* and *Gaultheria trichophyla*) on steep or fairly steep slopes (for cover value, see Appendix 4).

In general, lower alpine (<4500 masl) and upper alpine (>4500 masl) sites did not vary much in terms of total woody species (tall shrub and dwarf shrub/shrublets) cover (Mann-Whitney U = 1774.5, p = 0.350), but dwarf shrubs or shrublets contributed much in the community composition at the upper alpine sites, whereas tall shrubs had high contribution at lower alpine sites (Table 4.1). The upper alpine sites also showed high cover values for dicotyledonous herb, grass and rock (Table 4.1). Cover of non-vascular plants (mostly moss and lichen), on the other hand, tended to be high in lower alpine sites. Mean richness of associated species (mean number of species per plot) was almost the same in the sites from both altitude levels (Table 4.1).

Table 4.1. Mean richness of associated species, mean cover (%) of different vegetation layer and mean cover (%) of rock in sampling plots in seven populations of *N. scrophulariiflora* from upper alpine and lower alpine levels. Values shown are mean ± SE.

Vegetation Variables	**Upper alpine populations (>4500 masl)**				**Lower alpine populations (<4500 masl)**					**Overall Mean**	**Population difference[†]**	**Altitudinal difference[‡]**
	Walangchung Gola sector			**Mean**	**Walangchung Gola sector**		**Ghunsa sector**		**Mean**			
	Semjoyunjo	**Chhuwapangsong**	**Chherchung**		**Chherpari**	**Chherchen**	**Lumbasamba**	**Phukpadep**			χ^2	***'p'** based on Mann-Whitney U*
mean altitude (masl) →	4718	4570	4554		4390	4368	4373	4316				
Associated species richness (only vascular)	2.42 ± 0.19	4.62 ± 0.59	3.78 ± 0.29	3.67 ± 0.24	2.55 ± 0.21	5.44 ± 0.73	3.63 ± 0.23	3.37 ± 0.2	3.59 ± 0.16	3.62 ± 0.14	31.23**	ns
Tall shrub cover (%)	30.31 ± 5.99	29.03 ± 4.2	36.75 ± 5.26	33.05 ± 3.13	52.24 ± 5.51	51.67 ± 4.66	16.97 ± 2.58	40.11 ± 4.11	33.98 ± 2.55	33.63 ± 1.97	37.21**	ns
Dwarf shrub/shrublet cover (%)	9.58 ± 2.29	16.01 ± 4.12	11.77 ± 2.8	12.37 ± 1.83	0.30 ± 0.30	3.85 ± 1.92	4.89 ± 0.7	15.28 ± 2.18	7.96 ± 1.07	9.59 ± 0.97	39.10**	0.028
Total woody plant cover (%)	39.89 ± 5.97	45.04 ± 6.22	48.52 ± 5.07	45.42 ± 3.28	52.55 ± 5.53	55.52 ± 4.49	21.85 ± 2.61	55.39 ± 4.19	41.93 ± 2.69	43.22 ± 2.08	40.50**	ns
Herb cover (%)	1.58 ± 0.97	10.37 ± 2.11	14.47 ± 3.24	10.14 ± 1.81	1.61 ± 0.82	7.69 ± 6.28	6.71 ± 1.14	3.95 ± 1.06	5.12 ± 0.91	6.97 ± 0.9	35.19**	0.032
Grass cover (%)	34.78 ± 7.3	19.95 ± 6.12	9.26 ± 1.38	18.54 ± 2.9	6.74 ± 3.34	14.52 ± 2.11	0.24 ± 0.09	0.45 ± 0.13	2.75 ± 0.71	8.58 ± 1.34	89.31**	<0.001
Total vascular Plant cover (%)	41.47 ± 5.57	55.40 ± 5.65	63.00 ± 4.4	55.56 ± 3.16	54.15 ± 5.46	63.21 ± 8.41	28.56 ± 2.53	59.34 ± 4.3	47.06 ± 2.72	50.2 ± 2.1	44.26**	0.039
Non-vascular plant cover (%)	27.86 ± 3.73	28.94 ± 6.71	38.36 ± 3.48	33.19 ± 2.68	54.12 ± 6.89	63.97 ± 4.79	25.49 ± 1.85	41.77 ± 3.21	39.51 ± 2.24	37.17 ± 1.74	41.04**	ns
Rock cover (%)	7.78 ± 2.7	16.23 ± 1.82	31.3 ± 5.07	21.34 ± 2.93	7.85 ± 3.95	15.69 ± 4.58	5.46 ± 0.88	17.87 ± 3.53	11.44 ± 1.62	15.1 ± 1.54	38.26**	<0.001

[†] Significance test to detect differences among populations in the subset of environmental variables based on non-parametric Kruskal-Wallis one-way ANOVA (df = 6); chi-square values (**$p<0.001$) are shown.

[‡] Significance test to detect differences between altitude levels in the subset of environmental variables based on Mann-Whitney U tests; only significant p values are shown (ns = the difference is not significant at $p = 0.05$ level).

Soil chemical analysis of four representative sites of *N. scrophulariiflora* showed acidic nature of the soil (Appendix 2b). Soil pH ranged from 4.20-4.50 (overall mean 4.36 ± 0.11). Soil nutrient content was found to be higher as per the standard of Nepal Agricultural Research Council (NARC 2002). Soil nitrogen content varied from 0.47% (in Chherchung) to 0.87% (in Chherchen) (overall mean = 0.73 ± 0.11%). Phosphorus content of soil varied from 7.0 kg/ha (in Chhuwapangsong) to 86.5 kg/ha (in Chherpari) (overall mean = 58.09 ± 17.40 kg/ha). Potassium content varied from 574.33 kg/ha (in Chherchung) to 774.00 kg/ha (in Chherchen) (overall mean = 669.91 ±57.90 kg/ha). Soil organic matter content varied from 9.33% (in Chherchung) to 17.37% (in Chherchen) (overall mean = 14.62 ± 2.25%).

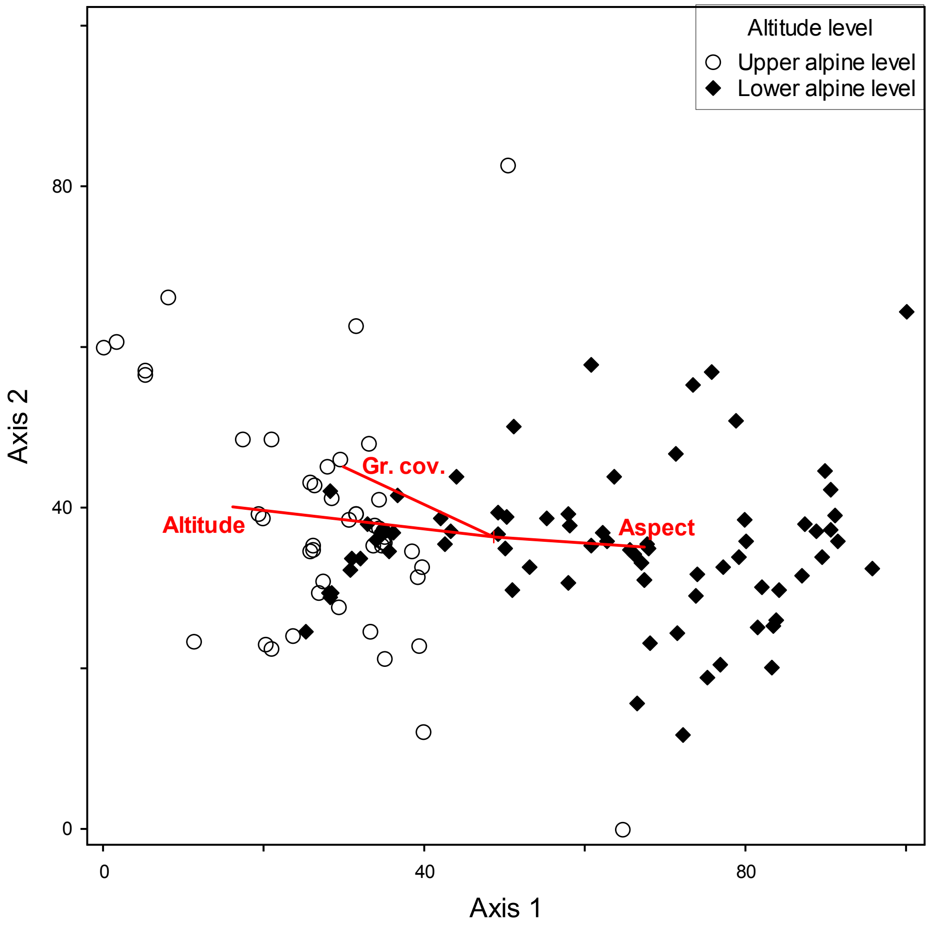

Fig. 4.1. DCA ordination of sample plots (length of gradient = 3.761; Eigen value = 0.671 for axis 1, and 0.262 for axis 2; the first two DCA axes cumulatively explained 70.1% of variance in species data). Symbols refer to sample plots from upper alpine and lower alpine sites. Environmental variables significantly correlated with the ordination axes are shown as biplot vectors [and they are: altitude (Person correlations (*r*) = – 0.608 with the first axis), grass cover (*r* = – 0.463 with the first axis), and aspect (*r* = 0.463 with the first axis)].

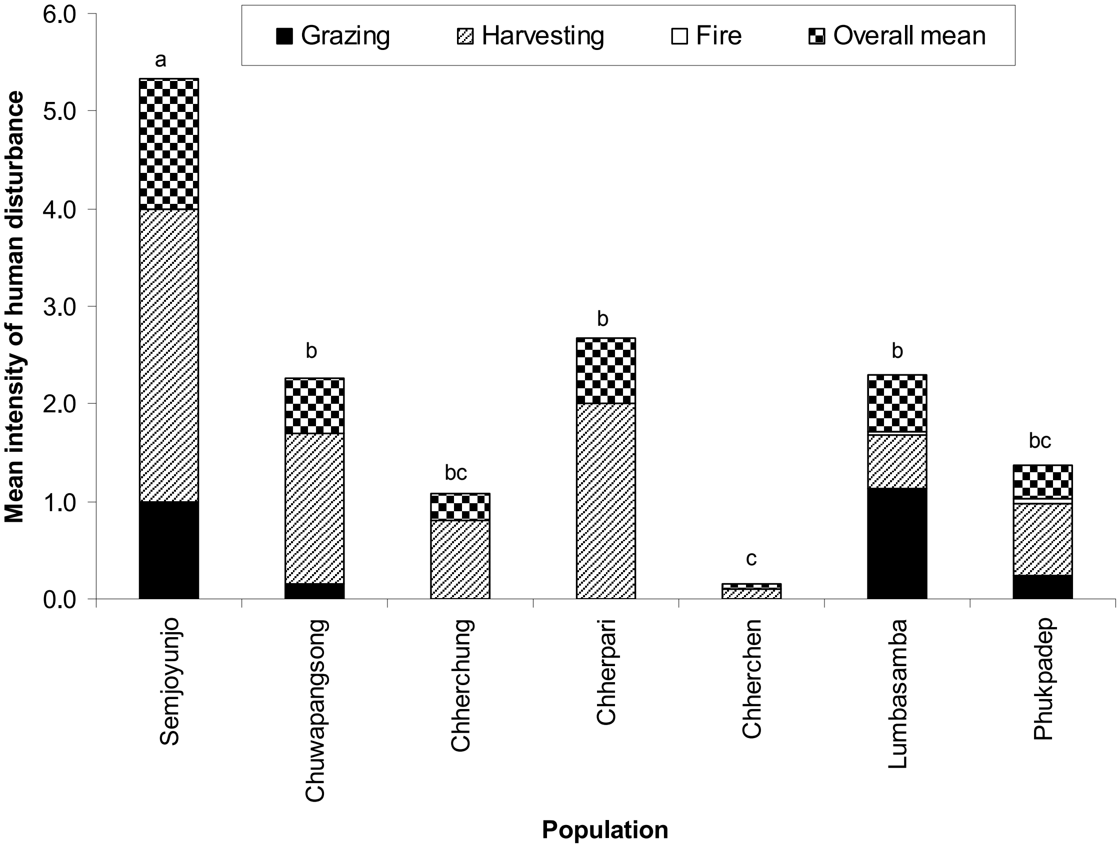

Fig. 4.2. Levels of human impact (grazing, harvesting and fire) in different sites/populations (for each type of disturbance, categorical intensity values were assigned for each plot as 0 – low, <1 – medium, >1-2 high, and >2 – very high). Bars with different letters at the top indicate significant difference in overall mean of grazing, harvesting and fire intensities at $p = 0.05$ level.

Majority of the populations received moderate intensity of human disturbance (Fig. 4.2; in most populations overall mean intensity of human disturbance per plot ranged >0.1-<1.0). Forest fire (although in very low extent) was observed in some populations of Ghunsa sector but no fire disturbances were observed in Walangchung Gola sector. Grazing was mainly observed in Lumbasamba population of Ghunsa sector and Semjoyunjo population of Walangchung Gola sector. Grazing was moderate in Phukpadep and Chhuwapangsong, high in Lumbasamba and Semjoyunjo, but in other three populations (Chherpari, Chherchung and Chherchen) grazing was totally absent.

Intensity of human disturbance showed positive relationship with altitude, indicating higher human pressure at higher altitude and lower pressure at lower altitudes (Appendix 3a). As compared to Ghunsa sector, populations from Walangchung Gola sector received higher harvesting pressure (except Chherchen). Among the populations from Walangchung

Gola sector, very negligible disturbance (overall mean = 0.037) was observed in Chherchen population (growing at lower altitude) and comparatively very high disturbance (overall mean = 1.33) was recorded in Semjoyunjo population (growing at higher altitude); and rest of the three populations from Walangchung Gola sector received moderate pressure. Among the populations from Ghunsa sector, Lumbasamba population received higher grazing pressure than did by Phukpadep population; but harvesting pressure was slightly higher in Phukpadep.

4.2 Growth Strategy and Plant Performance

4.2.1 Plant architecture and growth pattern

In *N. scrophulariiflora*, a ramet consists of above-ground (orthotropic) shoot bearing rosette of leaves which is connected to long creeping below-ground plagiotropic stem or rhizome (Fig. 4.3, 4.4 and 4.5). Rhizomes are long-lived and grow horizontally just (few centimeters) below the ground with regular production of nodes and giving buds apically and laterally. Branching is usually sympodial. Branching occurs at the tip and formation of a new ramet occurs at the end of the branch. Most of the apical buds are capable of producing new branch of below-ground stem (plagiotropic rhizome) which by growing horizontally after certain distance from initial point of origin becomes orthotropic and eventually grows forming above-ground shoots bearing rosette of leaves. The plagiotropic part of the stem bears scales, remnants of old leaf bases, and a few roots at nodes. Since the growing season in the alpine region is very short, some of the buds, however, remain dormant and does not grow in the same growing season; these are sprout out only in the next season. Sometimes, lateral branching also occur forming lateral rhizomes. These rhizomes are probably formed when density of the neighbors is low and resource is sufficient.

The rhizome produces new branch at an average angle of 33.76^0 ± 11.21 (mean ± SD). At the point of branching, the rhizome first produces a long internodes, and thereafter nodes and very short internodes at regular intervals. Towards the proximal end, internodes become indistinct and the rhizome appears to have crowns of old leaf bases without distinction between nodes and internodes. Some mortality (decaying) occurs in November when snow started to cover the area. Decaying started at the distal older part of the rhizome and proceeded towards the proximal portion. Based on rhizome morphology, its pattern of growth and decay, life-span of the rhizome has been expected to be very long (>10 years).

Fig. 4.3. Different growth stages of ramet in *N. scrophulariiflora*.

Fig. 4.4. Branching pattern of *N. scrophulariiflora*.

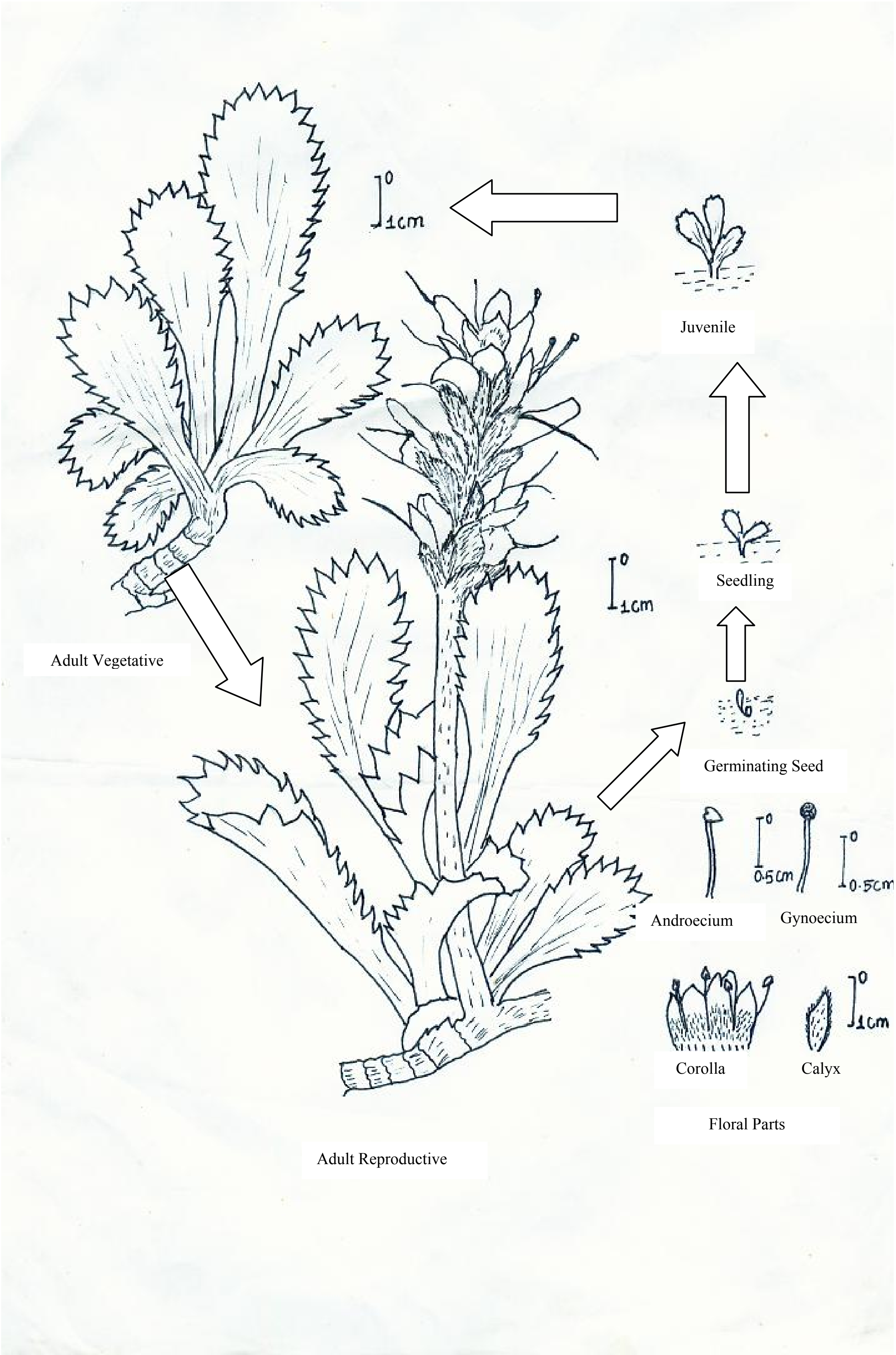

Fig. 4.5. Life cycle of *N. scrophulariiflora*.

Table 4.2. Variation in plant performance traits (vegetative and sexual growth parameters) among different populations of *N. scrophulariiflora* from upper alpine and lower alpine levels (values are mean ± SE).

SN	Morphological parameters	Upper alpine populations (>4500 masl)			Lower alpine populations (<4500 masl)				Overall mean	Nested ANOVA Pop(alt. level)
		Semjoyunjo	Chhuwapang song	Mean	Chherpari	Chherchen	Phukpadep	Mean		
	Mean altitude (masl) ⟶	4718	4570		4390	4368	4316			***F***
1.	No of rhizome branch per genet[a]	4.22 ± 0.81	7.87 ± 0.83	6.50 ± 0.70	6.40 ± 0.77	9.60 ± 1.19	7.00 ± 0.71	7.67 ± 0.56	7.26 ± 0.44	5.41** (1,64)
2.	Total length of all rhizome branches (cm)[b]	47.11 ± 7.10	85.57 ± 12.39	71.15 ± 8.95	90.18 ± 14.04	108.57 ± 16.78	124.83±28.40	107.86 ± 11.87	95.09 ± 8.56	3.73** (1,64)
3.	Average length of rhizome branch (cm)	14.37 ± 2.60	10.95 ± 1.01	12.23 ± 1.18	14.53 ± 1.61	11.85 ± 1.18	16.84 ± 2.18	14.40 ± 1.01	13.65 ± 0.78	1.70 (1,64)
4.	Length of longest rhizome branch (cm)	30.22 ± 4.69	32.97 ± 4.24	31.94 ± 3.13	47.47 ± 5.51	52.70 ± 7.58	44.80 ± 6.67	48.32 ± 3.78	42.62 ± 2.84	2.90* (1,64)
5.	Average girth of rhizome (mm)	4.92 ± 0.31	4.93 ± 0.25	4.92 ± 0.19	5.70 ± 0.32	6.33 ± 0.22	5.93 ± 0.27	5.99 ± 0.16	5.62 ± 0.14	4.96**(1,64)
6.	No. of ramets per rhizome branch	1.07 ± 0.06	1.10 ± 0.16	1.09 ± 0.10	1.02 ± 0.07	1.07 ± 0.06	1.41 ± 0.14	1.17 ± 0.06	1.14 ± 0.05	2.06 (1,64)
7.	Proportion of flowering ramet per genet	0.37 ± 0.11	0.36 ± 0.05	0.36 ± 0.05	0.41 ± 0.06	0.34 ± 0.05	0.40 ± 0.04	0.38 ± 0.03	0.38 ± 0.03	0.30 (1,64)
8.	Average no. of flower per inflorescence	7.28 ± 0.59	9.64 ± 0.44	8.97 ± 0.42	8.10 ± 0.43	9.38 ± 0.39	11.50 ± 0.29	9.05 ± 0.32	9.02 ± 0.25	6.31***(1,49)
9.	Average length of peduncle (cm)	2.63 ± 0.43	4.43 ± 0.5	3.8 ± 0.4	5.83 ± 0.78	5.55 ± 0.37	6.26 ± 0.57	5.85 ± 0.33	5.09 ± 0.28	7.91***(1,57)
10.	Total length of inflorescence (cm)	3.63 ± 0.43	5.83 ± 0.55	5.07 ± 0.44	7.51 ± 0.72	6.99 ± 0.43	11.45 ± 1.1	8.40 ± 0.51	7.18 ± 0.41	15.14***(1,58)
11.	Length of fertile part of inflorescence (cm)	0.9 ± 0.12	1.39 ± 0.30	1.21 ± 0.20	1.54 ± 0.28	1.44 ± 0.10	5.19 ± 0.69	2.51 ± 0.34	2.02 ± 0.24	11.35***(1,58)
12.	Number of seed per fruit	10.78 ± 1.05	-	10.78 ± 1.05	11.05 ± 1.06	-	16.87 ± 1.72	14.90 ± 1.43	13.67 ± 1.1	6.00**(1,27)

[a] A rhizome branch (or spacer) refers to each rhizome segment starting from one branching node to another.

[b] Total length of all rhizome branches: calculated as $\Sigma(b_1+b_2+ \ldots b_n)$, where b_1 = length of first rhizome branch, b_2 = length of second rhizome branch, …. b_n= length of n^{th} rhizome branch.

[‡] The means are significantly different from one another at $*p < 0.05$, $**p<0.01$ and $***p<0.001$ levels based on nested ANOVA (for detail see text), degree of freedom is given in the parenthesis.

4.2.2 Variation in plant performance traits

Variation in plant performance traits (sexual and vegetative growth-related parameters) was studied among five populations of *N. scrophulariiflora* growing at two different altitude levels (Table 4.2). Out of a total of 12 traits compared, *N. scrophulariiflora* showed significant inter-population variation in 9 growth-related traits (one-way ANOVA: $p \leq 0.05$; ANOVA results not shown). Similar variation was observed between populations from upper alpine and lower alpine levels (Table 4.2). The populations growing at lower alpine level had significantly longer and thicker rhizomes and produced significantly larger inflorescences than did the populations growing at upper alpine level (Table 4.2). Total length of inflorescence and that of rhizome branch were highest in Phukpadep population (<4500 masl) and lowest in Semjoyunjo population (>4500 masl). But the highest value of rhizome girth was recorded in Chherchen population (<4500 masl). Mean number of flowers per inflorescence and number of seeds per fruit also showed significant altitudinal trend, with highest value of both of these traits in Phukpadep population (<4500 masl) and lowest in Semjoyunjo population (>4500 m). Thus, higher the altitude shorter would be the inflorescence with concomitant decrease in number of flowers and number of seed set. It is to be noted that the Semjoyunjo population had very few flowering individuals, as in sampling plot no flowering individuals were recorded (see section 4.4).

Canonical Discriminant Analysis revealed that *N. scrophulariiflora* populations were distinct from one another in several discriminant functions (Table 4.3; Fig. 4.6). The first discriminant function, shown on the horizontal axis of Fig. 4.6, placed Phukpadep population (of lower alpine level <4500 m) from Ghunsa sector at the positive end of the performance traits' variation and all the four populations from Walangchung Gola sector (Chherchen, Chherpari, Chhuwapangsong and Semjoyunjo) were placed at the negative end. The second function, on the other hand, separated the four populations of Walangchung Gola sector. The second function distinguished Chherchen and Chherpari (of lower alpine level <4500 m) at the positive end and other two populations (of upper alpine level >4500 m) at the negative end (Fig. 4.6). However, the closeness of the group centroids in Fig. 4.6 suggests that the separation between all four populations from Walangchung Gola sector is not very strong indicating some close affinity.

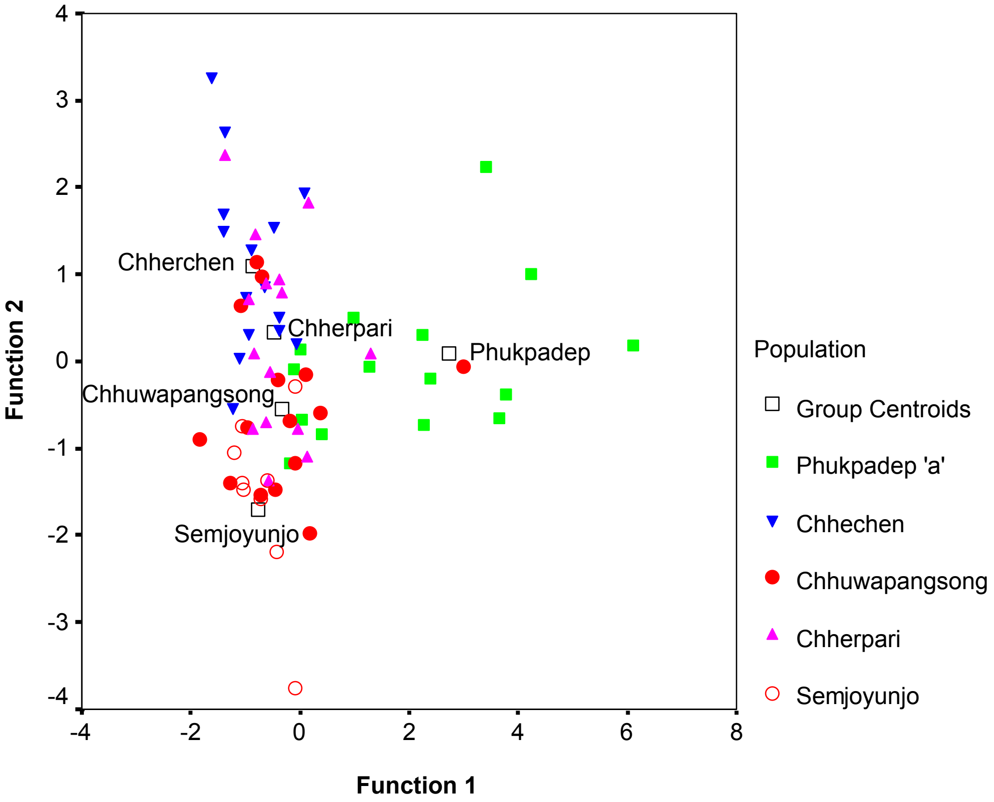

Fig. 4.6. Canonical discriminant function analysis of plant performance traits of *N. scrophulariiflora*. Only the first two discriminant functions were plotted, the third and fourth functions were found to be rather insignificant. The first and second functions cumulatively explained 89.5 % of variance.

Table 4.3. Structure matrix showing the correlation of each predictor variable (plant performance trait) with the discriminant function based on canonical discriminant analysis.

PPT variables	**Function**			
	1	**2**	**3**	**4**
Length of fertile part of inflorescence (cm)	0.910(*)	0.193	-0.195	0.131
Total length of inflorescence (cm)	0.627(*)	0.539	-0.121	-0.454
Average girth of rhizome (mm)	0.074	0.528(*)	-0.469	0.444
Length of longest rhizome branch (cm)	0.014	0.381(*)	-0.209	0.371
No of rhizome branch per genet	-0.022	0.453	0.604(*)	0.499
Average length of rhizome branch (cm)	0.197	-0.117	-0.536(*)	0.135
Average length of peduncle (cm)	0.213	0.547	-0.032	-0.643(*)
Eigen value	1.806	0.825	0.231	0.078
% of Variance	61.40	28.00	7.90	2.60
Cumulative %	61.40	89.50	97.40	100.00
Canonical correlation	0.802	0.672	0.433	0.268

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions. Variables ordered by absolute size of correlation within function.

(*) Largest absolute correlation between each variable and any discriminant function.

Inflorescence length (length of fertile part and total length) was most strongly correlated with the first function (Table 4.3). Average girth of rhizome and length of longest rhizome branch were most strongly correlated with the second function. Some of these traits appear to vary substantially among populations (Table 4.2). Number of rhizome branch per genet and average length of peduncle were most strongly correlated with the third and fourth discriminant functions, respectively; but these functions were found to be rather insignificant (low Eigenvalue and % of variance explained), so these variables were nearly useless predictor.

4.3 Allocation Pattern

In *N. scrophulariiflora*, resource (biomass) was highly centralized in underground parts (mainly rhizome; about 64% of the biomass was centered in rhizome). In all the three populations studied, below ground vegetative (asexual) allocation (mean ratio 0.76) was much higher than above ground vegetative allocation (mean ratio 0.22); and allocation to the sexual parts was very low (mean ratio 0.02). The three populations differ significantly in allocation pattern (Table 4.4). The total mean allocation and allocation for different components (sexual, asexual above ground and below ground) per genet were highest for Phukpadep population (of Ghunsa sector, growing at lower altitude), followed by Chherpari (of Walangchung Gola sector, growing also at lower altitude), and Semjoyunjo (of Walangchung Gola sector, but growing at higher altitude) (Table 4.4). Similarly, the sexual reproductive effort (in terms of inflorescence biomass/total biomass) was also highest for Phukpadep population (Fig. 4.7). But the three populations did not differ much in terms of vegetative reproductive effort (aboveground or belowground biomass/total biomass), although below ground vegetative effort tended to be higher in the populations from Walangchung Gola sector (Fig. 4.7).

Table 4.4. Variation in biomass allocation: mean plant dry weight (g) across 3 populations of sexual allocation (flower + flower stalk), asexual above ground allocation (sum of vegetative ramet mass), below ground allocation (sum of rhizome and root mass) and total allocation.

Biomass components	**Populations**			**Overall Mean**
	Phukpadep	**Chherpari**	**Semjoyunjo**	
Sexual allocation	0.46 ± 0.14^{a}	0.06 ± 0.03^{b}	0.04 ± 0.02^{b}	0.25 ± 0.07
Asexual above-ground allocation	6.48 ± 2.70^{a}	1.41 ± 0.24^{ab}	0.75 ± 0.17^{b}	3.66 ± 1.33
Below-ground allocation	9.87 ± 2.86^{a}	6.44 ± 0.94^{ab}	3.47 ± 0.86^{b}	7.49 ± 1.43
Total allocation	16.80 ± 5.57^{a}	7.92 ± 1.11^{a}	4.27 ± 1.02^{b}	11.40 ± 2.76

The different superscript letters in a row represent that the medians are significantly different from one another at $p = 0.05$ based on Mann-Whitney U Test.

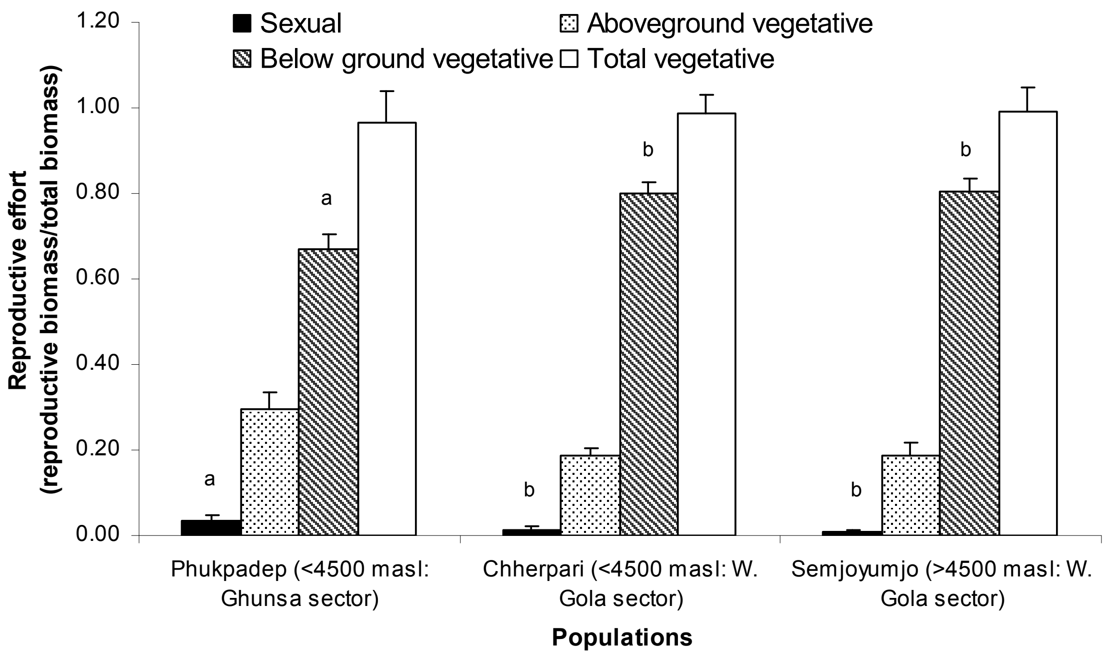

Fig. 4.7. Variation in reproductive effort among different populations of *N. scrophulariiflora*. Bars with different letters represent significant difference in reproductive effort among populations based on Mann-Whitney U Test.

4.4 Population Density and Structure

4.4.1 Variation in population density

Population density was estimated both in terms of ramet and putative genet densities, the values of which significantly varied among the studied populations and between two altitude levels (Table 4.5). Ramet density varied from 11.25 to 46.78 ramet per m^2 (overall mean 25.01 ± 1.85) and density of putative genet varied from 1.88 to 4.67 genet per m^2 (overall mean 3.15 ± 0.19). The number of ramet per genet (overall mean 8.17 ± 0.58) also varied significantly among the studied populations.

In general, densities of both ramets and genets were significantly high in populations from lower alpine level (mean ramet density: 29.98 ± 2.24 per m^2; genet density: 3.64 ± 0.20 per m^2) than in populations from upper alpine level (mean ramet density: 16.72 ± 2.90 per m^2; genet density: 2.33 ± 0.33 per m^2) (Table 4.5). At the same altitude level (when comparison was made among populations growing <4500 masl), populations from Walangchung Gola and Ghunsa sectors did not differ significantly in terms of ramet (one-way ANOVA $F_{1,\ 84}$ = 0.392, p = 0.533) and genet (one-way ANOVA $F_{1,\ 84}$ = 1.92, p = 0.169) densities, although these values tended to be high in populations from Ghunsa sector.

Table 4.5. Comparison of ramet and genet densities and genet composition (in terms of total number of ramets and number of flowering ramets per genet) of *N. scrophulariiflora* among different populations growing at two different altitudinal levels in Walangchung Gola (W. Gola) and Ghunsa sectors.

Altitude level	**Sector**	**Population**	**Ramet density (ramet m^{-2})**	**Genet density (genet m^{-2})**	**No of ramet per genet**		**No of flowering ramets per genet**
					Mean	**Range**	
Upper alpine (>4500 masl)	W. Gola	Semjoyunjo	11.25 ± 4.20	1.92 ± 0.57	5.19 ± 0.89	1-54	0.00 ± 0.00
		Chhuwapangsong	28.79 ± 8.09	2.64 ± 0.58	12.53 ± 3.60	1-78	1.46 ± 0.69
		Chherchung	13.21 ± 3.11	2.36 ± 0.52	6.37 ± 0.93	1-44	0.42 ± 0.16
	Mean for upper alpine level		*16.72 ± 2.90*	*2.33 ± 0.33*	*7.80 ± 1.17*		*0.59 ± 0.20*
Lower alpine (<4500 masl)	W. Gola	Chherpari	11.64 ± 2.59	1.88 ± 0.25	6.05 ± 0.88	1-44	0.27 ± 0.19
		Chherchen	46.78 ± 11.49	4.67 ± 0.82	13.50 ± 4.19	1-217	2.00 ± 0.33
	Ghunsa	Lumbasamba'	39.00 ± 2.02	3.99 ± 0.13	9.89 ± 0.53	1-41	9.22 ± 0.34
		Phukpadep	22.78 ± 3.21	3.62 ± 0.41	5.88 ± 0.46	1-19	4.64 ± 0.76
	Mean for lower alpine level		*29.98 ± 2.24*	*3.64 ± 0.20*	*8.40 ± 0.61*		*5.52 ± 0.48*
	Overall mean		**25.01 ± 1.85**	**3.15 ± 0.19**	**8.17 ± 0.58**		**3.67 ± 0.37**
nested ANOVA Pop(alt. level)		$F_{1,133}$	13.07	12.68	2.90		106.02
		P	<0.001	<0.001	0.059		<0.001

Table 4.6. Density of *N. scrophulariiflora* ramet in different stage classes in different populations.

Altitude level	**Sector**	**Population**	**Mean density in different stage classes***			
			Very young	**Juvenile**	**Adult vegetative**	**Adult reproductive**
Upper alpine (>4500 masl)	W. Gola	Semjoyunjo	1.472 ± 0.653	4.583 ± 1.776	5.194 ± 2.194	0.00 ± 0.00
		Chhuwapangsong	1.026 ± 0.400	8.051 ± 2.003	18.615 ± 5.688	1.103 ± 0.624
		Chherchung'	1.526 ± 0.494	3.679 ± 1.053	7.436 ± 1.905	0.564 ± 0.281
	Mean for upper alpine level		*1.386 ± 0.308*	*5.007 ± 0.87*	*9.758 ± 1.925*	*0.569 ± 0.217*
Lower alpine (<4500 masl)	W. Gola	Chherpari	0.939 ± 0.269	3.939 ± 0.984	6.454 ± 1.837	0.303 ± 0.145
		Chherchen	4.000 ± 1.137	8.814 ± 2.296	30.925 ± 8.422	3.037 ± 1.512
	Ghunsa	Phukpadep	4.727 ± 0.784	8.111 ± 1.146	7.566 ± 1.024	2.374 ± 0.445
		Lumbasamba	10.771 ± 0.623	11.979 ± 0.586	11.094 ± 0.609	5.156 ± 0.476
	Mean for lower alpine level		*6.435 ± 0.557*	*9.102 ± 0.627*	*11.224 ± 1.249*	*3.224 ± 0.343*
	Overall mean		**4.542 ± 0.422**	**7.566 ± 0.535**	**10.674 ± 1.060**	**2.228 ± 0.254**
nested ANOVA Pop(alt. level)		$F_{1,133}$	48.97	16.95	5.70	61.27
		P	< 0.001	< 0.001	0.004	< 0.001

*For definition of stage classes see Table 3.3.

Number of flowering ramets per genet was significantly high in lower alpine populations (mostly in the population from Ghunsa sector) (Table 4.5). On the other hand, the populations from two different altitude levels did not differ much in terms of total number of ramets per genet (i.e. genet size), although the value tended to be high in populations from lower alpine sites (Table 4.5). Variation in number of flowering ramets per genet and genet size (in terms of total number of ramets per genet) was strongly related with harvesting pressure than barely explained by altitude levels. Populations receiving high harvesting pressure (e.g., Semjoyunjo) showed small-sized genet and produced small number of flowering ramets than the populations receiving low harvesting pressure.

Similarly, there was strong sectorial difference in genet size and number of flowering ramets per genet. As compared to Ghunsa sector, populations from Walangchung Gola sector had large-sized genets (Table 4.5). Among the studied populations, genet size was largest in Chherchen population (mean 13.50 ± 4.19; range 1-217 ramets per genet) of Walangchung Gola sector; whereas smallest genets were recorded in Phukpadep population (5.88 ± 0.46; range 1-19 ramets per genet) of Ghunsa sector (Table 4.5). On the contrary, at the same altitude level (<4500 masl), Ghunsa populations showed significantly large number of flowering ramets per genet (mean 6.89 ± 0.51) than did by Walangchung Gola populations (mean 1.05 ± 0.27) (one-way ANOVA $F_{1,84} = 39.707$, $p < 0.001$).

4.4.2 Variation in population structure

The density and proportion of *N. scrophulariiflora* ramets at different stage classes significantly varied among populations, between altitude levels and between two sectors (Table 4.6). Populations from lower alpine areas showed significantly high densities of very young, juvenile, adult vegetative and adult reproductive ramets than did by populations from upper alpine areas (Table 4.6). However, at the same altitude level, ramet population structure differed between Walangchung Gola and Ghunsa sectors. All the populations from Walangchung Gola sector showed higher proportions of juvenile and vegetative adult ramets, but very young and reproductive adult (flowering) ramets were poorly represented (Fig. 4.8). On the other hand, the populations from Ghunsa sector showed almost equal proportions of young, juvenile and vegetative adult ramets. Proportion of adult reproductive ramets was also significantly high in the populations from Ghunsa sector than in the populations from Walangchung Gola sector.

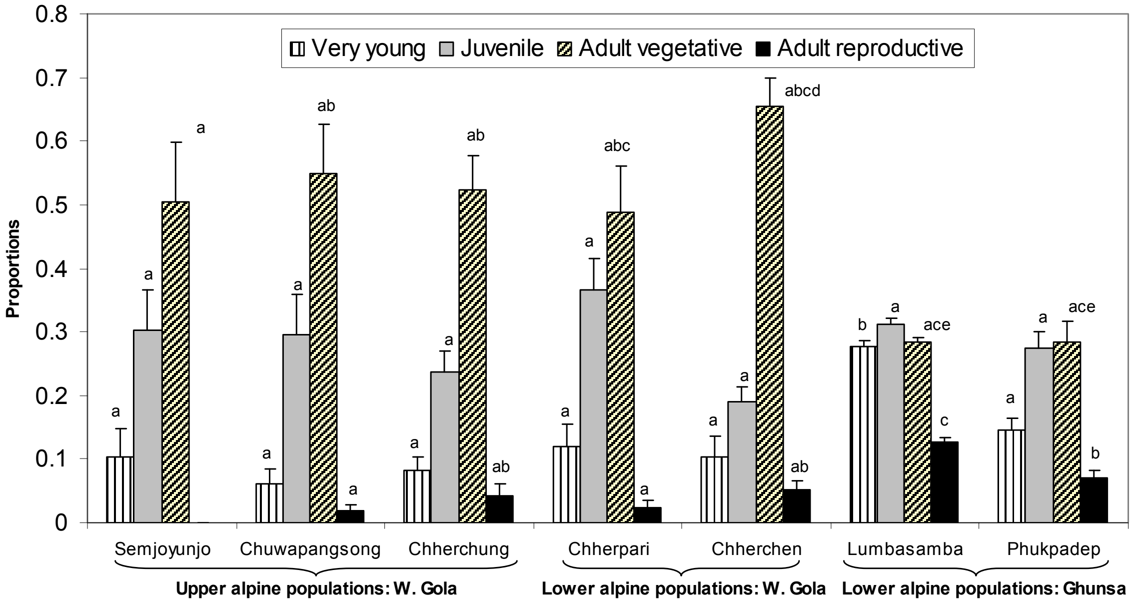

Fig. 4.8. Proportion of the total number of ramets of different size classes among seven populations of *N. scrophulariiflora* from two sectors [W. Gola (Walangchung Gola) and Ghunsa] and two altitude levels. Bars with different letters at the top indicate significant difference in overall mean proportion of individuals of a particular size classes among populations at $p = 0.05$ level based on one-way ANOVA (seedling proportion, $F_{1, 135} = 27.05$, $p < 0.001$; juvenile proportion, $F_{1, 135} = 0.79$, $p = 0.377$; adult vegetative proportion, $F_{1, 135} = 18.37$, $p < 0.001$; adult reproductive proportion, $F_{1, 135} = 23.45$, $p < 0.001$).

Like ramet population structure, the genet population structure also strongly varied between two sectors (Fig. 4.9 and 4.10). In either of the two sectors, altitudinal trend was not observed in genet population structure. Although the genet size (mean and range) was very high among the populations from Walangchung Gola sector (e.g., 1-217 in Chherchen population), they showed higher proportion of genet of smaller size (Fig. 4.9). Proportion of genet with single ramet was mostly represented in almost all of the populations from Walangchung Gola sector. On the other hand, the populations from Ghunsa sector (in which overall genet size was significantly smaller than in the former sector) showed higher proportion of medium-sized genet (Fig. 4.10).

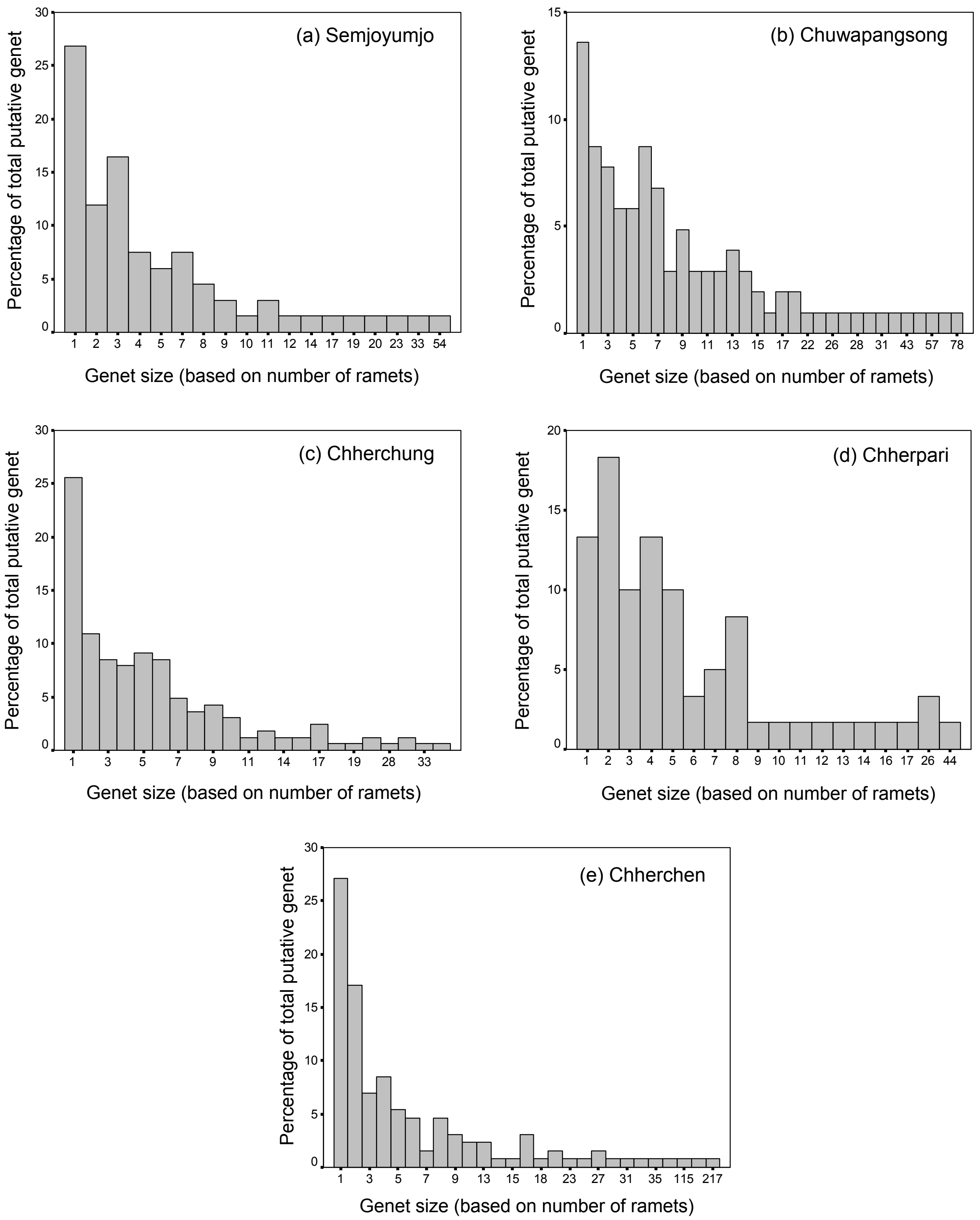

Fig. 4.9. Genet population structure in five populations (a-e) of *N. scrophulariiflora* from Walangchung Gola sector [populations a, b and c from upper alpine level (>4500 m), and populations d and e from lower alpine level (<4500 m)]. Data shown are percentage of putative genets of different sizes (classified based on number of interconnected ramets).

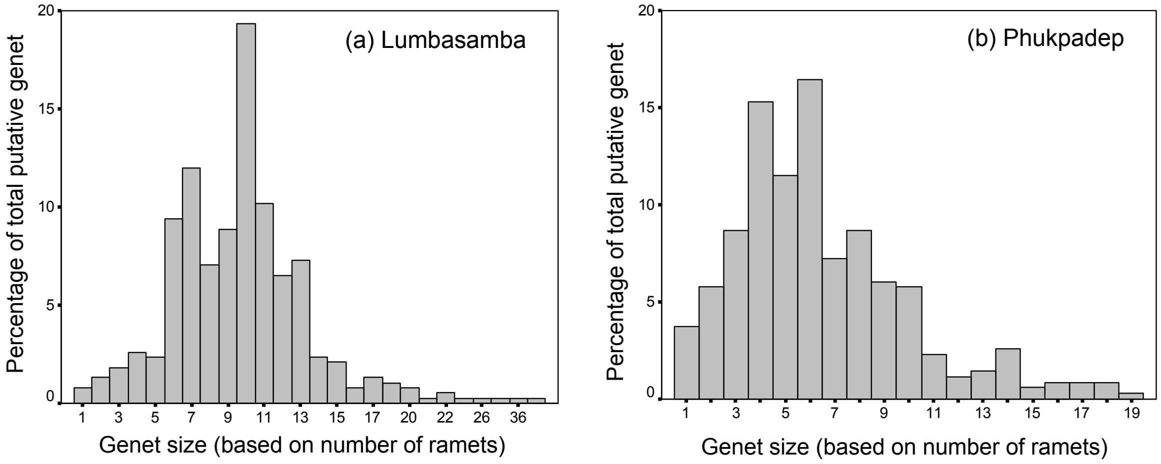

Fig. 4.10. Genet population structure in two populations (a and b) of *N. scrophulariiflora* from Ghunsa sector.

4.5 Relationships Between Population Structure and Environments

Results of univariate regression analysis (based on linear or quadratic models) of the relationship of population density, genet composition and ramet population structure of *N. scrophulariiflora* with different environmental variables are given in Table 4.7 (also in Fig. 4.11-4.14). Regression analysis of the relationship of population density, genet composition and ramet population structure of *N. scrophulariiflora* with different environmental variables (Table 4.7) showed that combined soil variable (extracted as PCA axis score) and altitude were the major components affecting the population attributes of *N. scrophulariiflora*. Although combined soil variable (Soil-PCA) showed the most significant positive linear relationships with almost all of the population parameters studied, the relationship was restricted only for the populations of Walangchung Gola sector (since soil data were not collected from the populations of Ghunsa sector). Altitude explained greater variation in number of flowering ramet per genet (28.6%), reproductive adult proportion (21.6%), reproductive adult density (21.3%), young ramet density (13.9%), and young ramet proportion (11.8%). There was significant negative linear relationship between altitude and young ramet density and its proportion (Fig. 4.13a, d). But, altitude showed unimodel relationships with reproductive adult density, its proportion and with number of flowering ramet per genet (Fig. 4.13b, c, f). On the other hand, altitude showed significant positive linear relationship with the proportion of vegetative adult ramets (Table 4.7; Fig. 4.13e). The next physical factor showing greater influence on the population parameters was RRI, which explained greater variation in genet density (14.4%), total ramet density (8.6%), and densities of juvenile (9.3%), vegetative adult (6.0%) and reproductive adult (each 6.0%)

ramets. RRI showed unimodel relationships with these population variables (Table 4.7). Besides these, other physical variables had negligible influence in explaining the variations in populations attributes.

Among the vegetation variables, grass cover had the highest explanatory power. It explained greater variations in number of flowering ramet per genet (27.9%), densities of reproductive (22.0%) and young (17.8%) ramets, and proportions of reproductive adult (21.3%), vegetative adult (16.7%), and young (15.9%) ramets. There was significant negative linear relationship between grass cover and most of these population attributes, except vegetative adult ramet proportion, in the later case grass cover had unimodel relationship. Total ramet density, and the density of young, juvenile and adult reproductive ramets and their proportions (except vegetative adult ramet proportion) decreased with the increasing value of grass cover (Table 4.7). Tall shrub cover explained only about 2.2-7.6% variations in density of ramet at different size classes and about 8.8% variations in genet composition (e.g., number of flowering ramet per genet). Tall shrub cover showed negative linear relationship with these population parameters.

Disturbance did not show significant relationship with any of the population attributes in the entire data set. Thus an attempt was made to analyze separately the population data from Walangchung Gola sector; since in this sector the enforcement of forest management rules and regulations is weak thus it is less protected than Ghunsa sector from illegal activities. In the subset of population data from Walangchung Gola sector, disturbance showed significant negative effect on adult vegetative and adult reproductive ramet densities, adult reproductive ramet proportions and number of flowering ramets per genet (Fig. 4.14).

Table 4.7. Univariate [linear (L) or quadratic (Q)] regression analysis of the relationship of population density, genet composition and ramet population structure of *N. scrophulariiflora* with different environmental variables. Only significant ($p<0.05$) explanatory variables are given in the table. R^2 is the adjusted coefficient of determination; Beta is the standardized regression coefficient. Abbreviations are as in Appendix 3.

	Population attributes	Environment variables	Regression model	df	Ad.R^2	Beta	t	p
Genet composition	**Total number of ramet per genet**	Soil-PCA*	L	42	0.223	0.491	3.658	0.0007
		TWcov	L	128	-0.032	-0.199	-2.299	0.0231
	Number of flowering ramet per genet	Soil-PCA*	L	42	0.371	0.621	5.132	0.0000
		Alt	Q	123	0.286	8.732	3.409	0.0009
		GRcov	L	128	0.279	-0.533	-7.131	0.0000
		TScov	L	128	0.088	-0.308	-3.667	0.0004
		TWcov	L	128	0.087	-0.306	-3.643	0.0004
		Asp	L	134	0.0611	0.260	3.124	0.0022
		RKcov	L	128	0.039	-0.217	-2.521	0.0129
		NVcov	L	128	0.038	-0.213	-2.466	0.015
		RRI	L	134	0.031	-0.177	-2.078	0.0000
Plant density	**Genet density**	Soil-PCA*	L	42	0.326	0.585	4.674	0.0000
		RRI	Q	128	0.144	-0.328	-4.112	0.0001
		GRcov	L	128	0.073	-0.283	-3.337	0.0011
		Alt	L	123	0.073	-0.267	-3.106	0.0024
	Total ramet density	Soil-PCA*	L	42	0.501	0.719	6.709	0.0000
		RRI	Q	128	0.086	-0.242	-2.933	0.0040
		GRcov	L	128	0.047	-0.232	-2.705	0.0078
		Alt	L	123	0.031	-0.197	-2.23	0.0275
		TScov	L	128	0.022	-0.172	-1.982	0.0496
	Young ramet density	Soil-PCA*	L	42	0.249	0.516	3.903	0.0003
		GRcov	L	128	0.178	-0.430	-5.383	0.0000
		Alt	L	123	0.139	-0.382	-4.584	0.0000
		RKcov	L	128	0.053	-0.246	-2.875	0.0047
		Asp	L	134	0.047	0.232	2.757	0.0066
		TWcov	L	128	0.040	-0.218	-2.528	0.0127
		TScov	L	128	0.030	-0.194	-2.236	0.0271
	Juvenile ramet density	Soil-PCA*	L	42	0.373	0.623	5.159	0.0000
		RRI	Q	134	0.093	-0.266	-3.236	0.0015
		GRcov	L	128	0.076	-0.289	-3.412	0.0009
		Alt	L	123	0.041	-0.221	-2.51	0.0134
		RKcov	L	128	0.025	-0.179	-2.063	0.0411
	Vegetative adult ramet	Soil-PCA*	L	42	0.49	0.709	6.510	0.0000

	Population attributes	Environment variables	Regression model	df	Ad.R^2	Beta	*t*	*p*
	density	RRI	Q	134	0.060	-0.172	-2.056	0.0417
	Reproductive adult ramet density	Soil-PCA*	L	42	0.575	0.765	7.698	0.0000
		GRcov	L	128	0.220	-0.476	-6.117	0.0000
		Alt	Q	123	0.213	9.391	3.491	0.0007
		TWcov	L	128	0.087	-0.307	-3.657	0.0004
		TScov	L	128	0.076	-0.289	-3.417	0.0008
		RRI	Q	134	0.060	-0.214	-2.555	0.0117
		Asp	L	134	0.042	0.222	2.639	0.0093
		RKcov	L	128	0.028	-0.190	-2.190	0.0304
		NVcov	L	128	0.023	-0.174	-2.000	0.0477
Population structure	**Young ramet proportion**	GRcov	L	128	0.159	-0.407	-5.043	0.0000
		Alt	L	123	0.118	-0.353	-4.188	0.0001
		RKcov	L	128	0.058	-0.256	-2.994	0.0033
		Asp	L	134	0.034	0.202	2.387	0.0184
	Vegetative adult ramet proportion	GRcov	Q	128	0.167	0.960	5.018	0.0000
		Alt	L	123	0.074	0.285	3.297	0.0013
		Asp	L	134	0.062	-0.264	-3.164	0.0019
		Slp	L	134	0.024	0.176	2.068	0.0406
	Reproductive adult ramet proportion	Soil-PCA*	L	42	0.305	0.566	4.455	0.0001
		Alt	Q	123	0.216	8.887	3.311	0.0012
		GRcov	L	128	0.213	-0.468	-5.996	0.0000
		TWcov	L	128	0.092	-0.315	-3.758	0.0003
		TScov	L	128	0.062	-0.264	-3.103	0.0024
		Asp	L	134	0.037	0.210	2.493	0.0139

Abbreviations: Alt: Altitude, Slp: Slope, Asp: Aspect, RRI: Relative radiation index, Soil-PCA: Combined soil variable, NVcov: Non-vascular plant cover, TScov: Tall shrub cover, DScov: Dwarf shrub/shrublet cover, TWcov: Total woody vegetation cover, HBcov: Herb cover, GRcov: Grass cover, RKcov: Rock cover,

*all the soil variables (moisture and depth) and all the disturbance variables (harvesting, grazing, trampling, fire) were separately combined together to obtain combine measures based on Principle Component Analysis (PCA). **Population structure data were arcsine transformed before analysis.

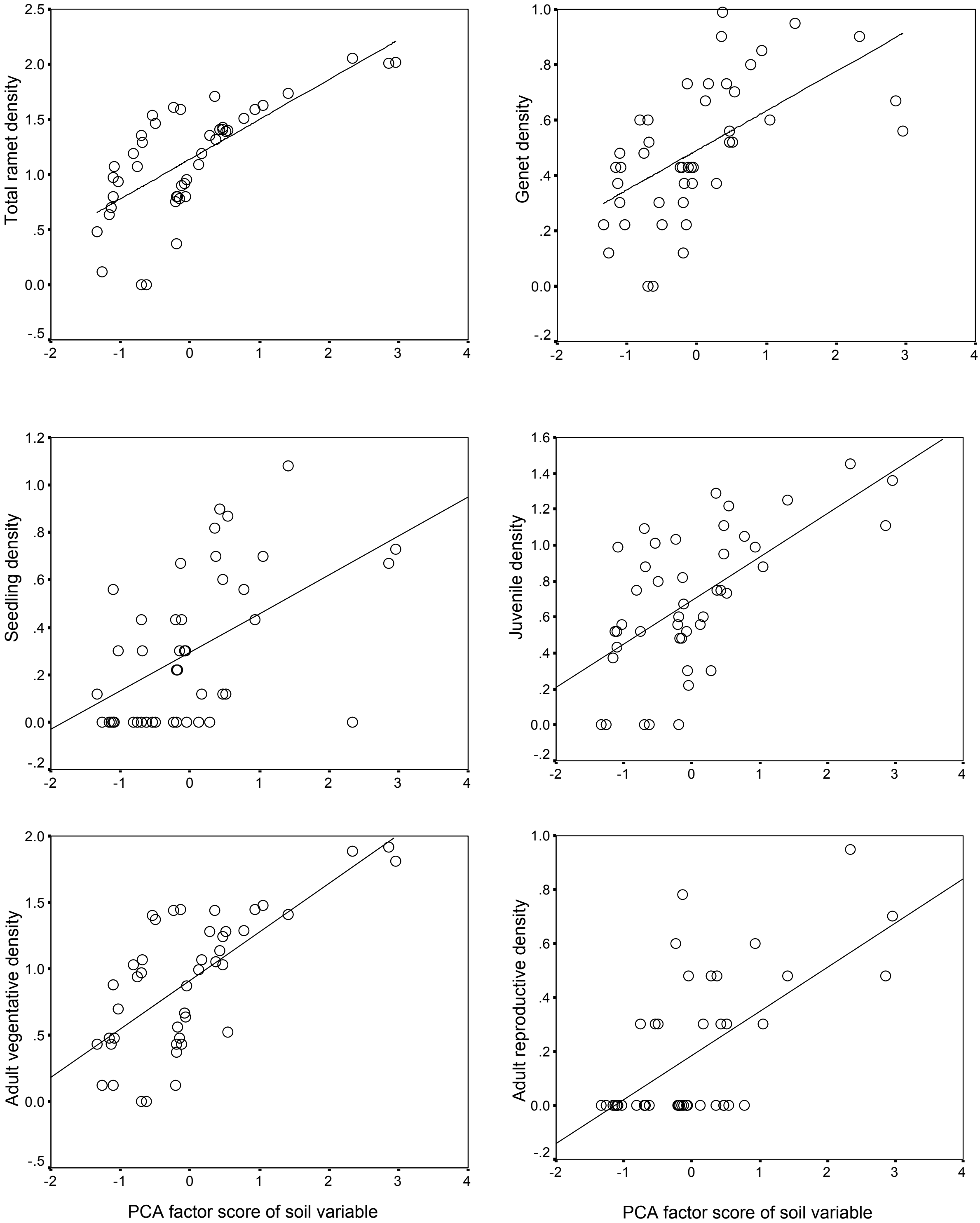

Fig. 4.11. Relationships between combined soil variable (Soil-PCA; derived as PCA factor scores) and population densities based on linear regression analysis. Values in Y axes were log transformed before analysis.

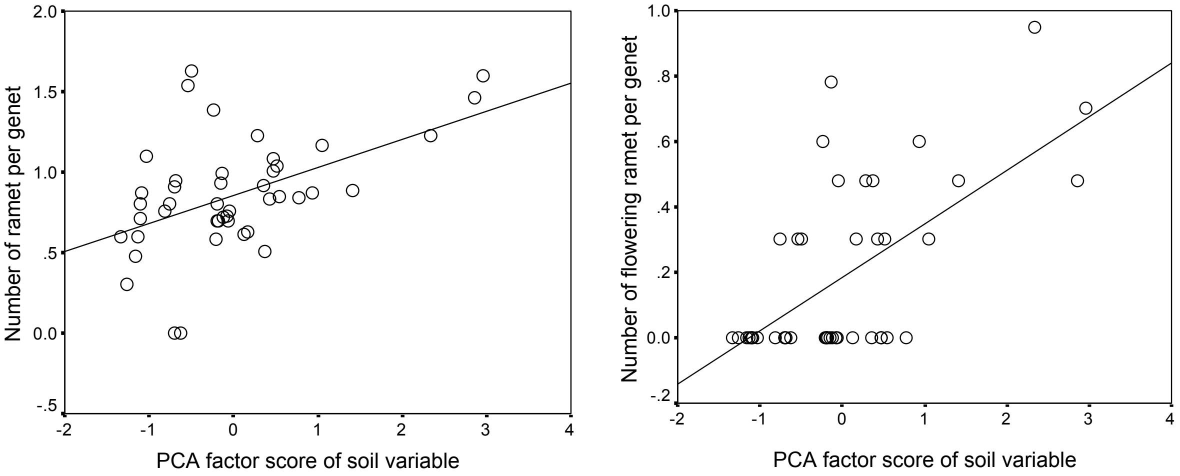

Fig. 4.12. Relationships between combined soil variable (Soil-PCA; derived as PCA factor scores) and genet composition based on linear regression analysis. Values in Y axes were log transformed before analysis.

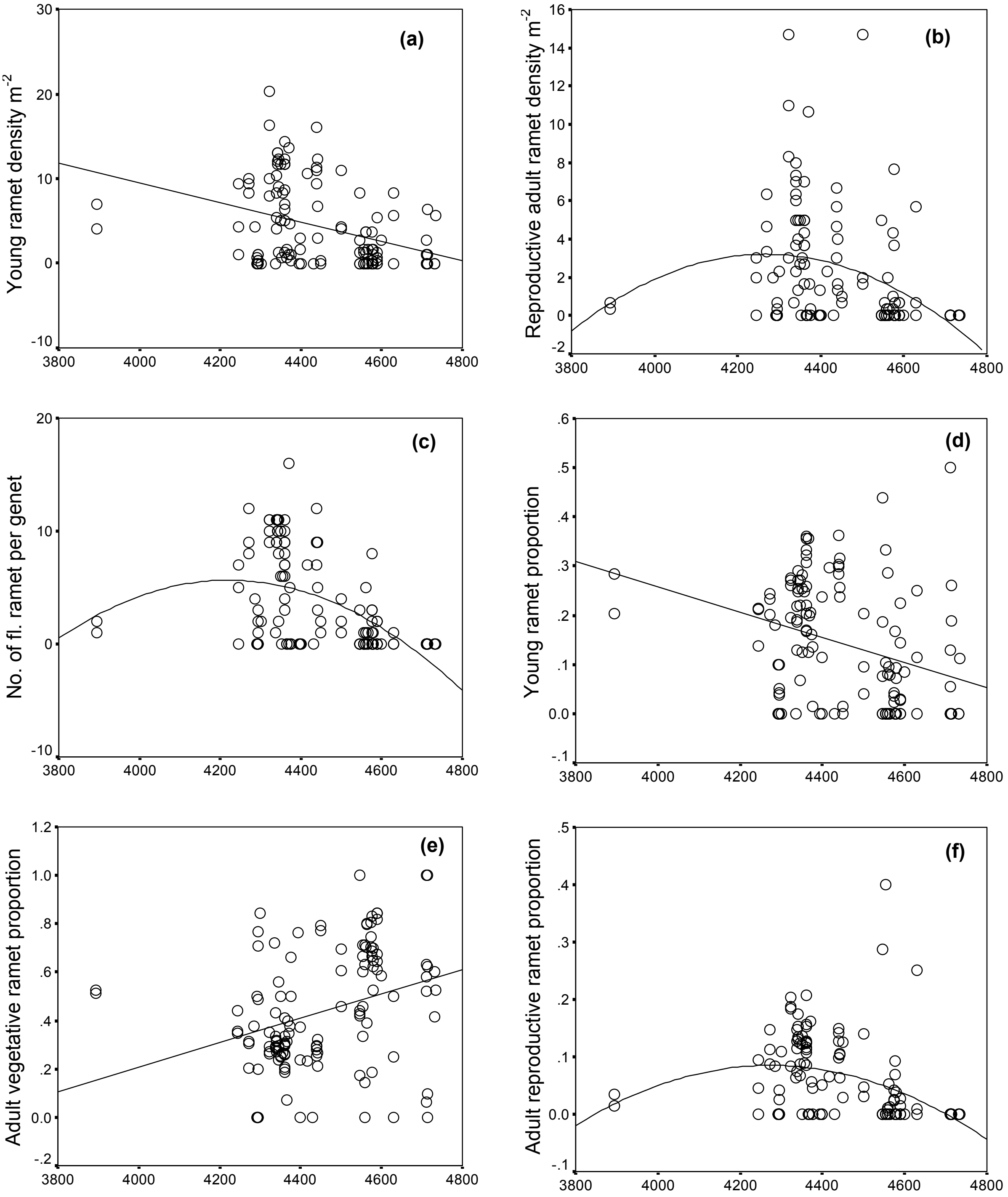

Fig. 4.13. Relationships between altitude and population attributes based on linear regression analysis.

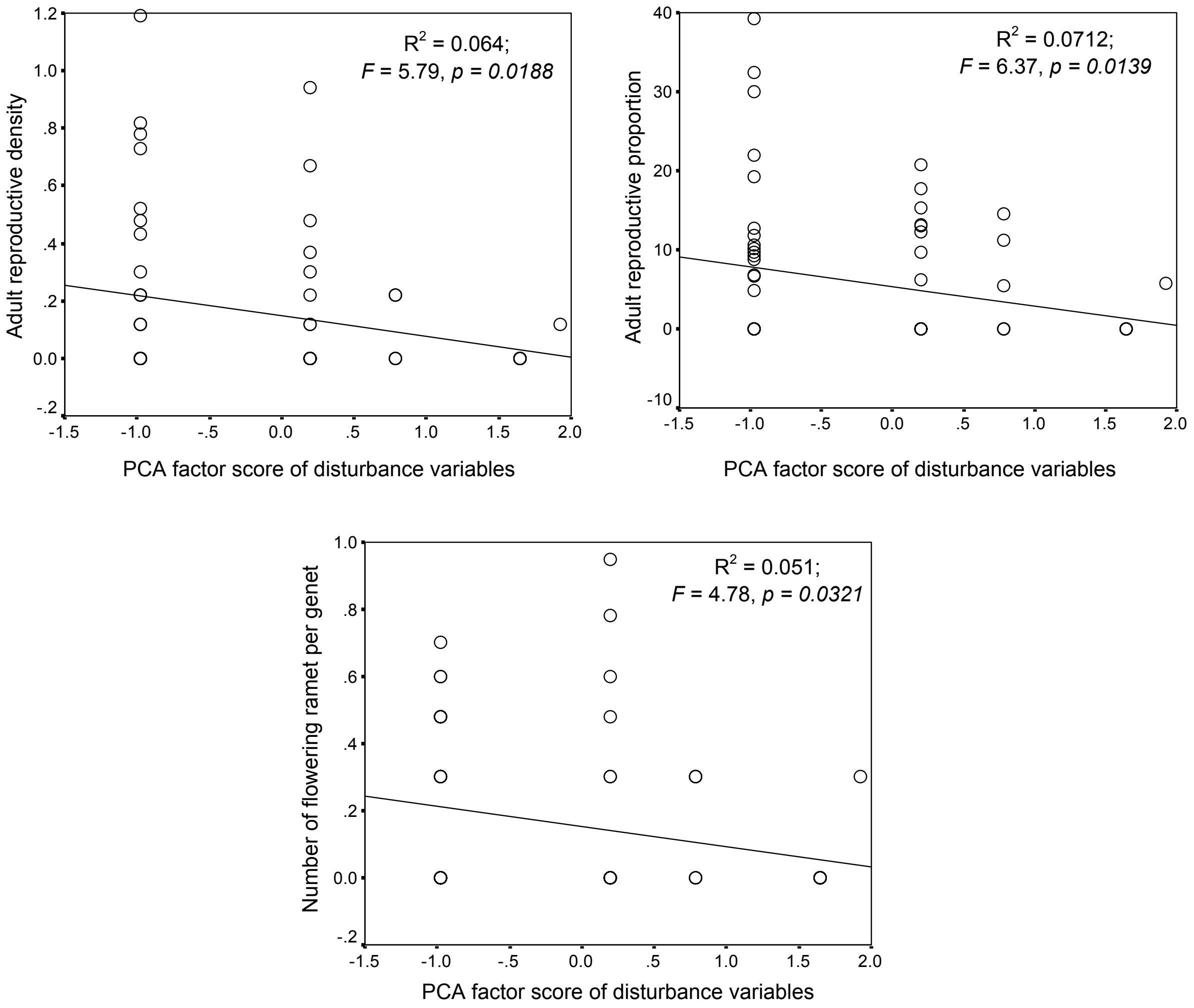

Fig. 4.14. Relationships between combined disturbance variable (Dis-PCA; derived as PCA factor scores) and different population attributes in the subset of data from Walangchung Gola sector based on linear regression analysis. Values in Y axes were log or arcsin transformed before analysis.

Chapter 5

DISCUSSION

5.1 Architecture and Growth Patterns: Enabling Adaptation and Persistence in Harsh Climate

Perenniality is the most essential attribute for the majority of plant species growing at harsh high-altitude habitats, where plants suffer from progressive cold and aridity and are affected by very short growing period (Körner and Renhardt 1987; Wang *et al*. 2002; Klimeš 2003; Körner 2003). *N. scrophulariiflora* is a perennial herb with long life span (> 10 years) (Ghimire *et al*. 2005). It exhibits perenniality by clonal growth of its rhizome. Rhizomes of *N. scrophulariiflora* are long-lived and grow horizontally just (few centimeters) below the ground with regular production of nodes and giving buds apically and laterally.

The clonal growth pattern of *N. scrophulariiflora* has been described as 'guerrilla strategy', where the widely spaced ramets rapidly spread into new areas (Ghimire *et al*. 2005). Such growth strategy enables the plant in exploiting open space and searching of nutrition efficiently (Lovett Doust 1981; de Kroon and Schieving 1990; Bazzaz 1991; de Kroon and Hutchings 1995). Guerrilla species are also better adapted to compete effectively with other species (Schmid and Harper 1985; David Humphrey and Pyke 1998). *N. scrophulariiflora* is a moisture-dependent species (IUCN Nepal 2004; Shrestha and Jha 2009), plentiful supply of water makes the north facing slope a suitable habitat for this plant. The horizontal extension of rhizome of *N. scrophulariiflora* seems particularly well suited to the marginal invasion in the moist habitat. The specific rhizome arrangements also permit plants of other habitats to exploit the space available to them in most efficient way (cf. Bell and Tomlinson 1980). The rhizome of *N. scrophulariiflora* produces new branch at an average angle of 33.76^0 ± 11.21 (mean ± SD), which can be considered as an efficient geometric shape. Greater the branch angle higher will be the chance to exploit available space to the full without crowding themselves (Smith and Palmer 1976).

The architectural and vegetative growth characters of *N. scrophulariiflora*, like horizontal extension of rhizome, sympodial type of branching with apical buds, lateral branching and plagiotropic part of the stem bearing scales, remnants of old leaf bases, etc. are some of the important features for the adaptation and long-term existence in the high altitude habitat.

Particularly, the sympodial type of branching with apical buds, each capable of producing new branch of plagiotropic rhizome, may be the efficient means of occupying space and searching nutrition. The plagiotropic part of the stem bears scales, bracts, remnants of old leaf bases, and a few roots at nodes. Dead leaves coating stems of giant rosettes efficiently prevent night time freezing of the xylem (Körner 2003) under moist situations.

5.2 Variation in Plant Growth and Reproductive Traits

In clonal plants, like *N. scrophulariiflora*, sexual reproduction is poor and the plant invests more on vegetative growth (MacArthur and Wilson 1967). However, there were strong sectorial and altitudinal differences in the relative contribution of sexual versus vegetative growth strategies in population persistence. As compared to Ghunsa sector (moist sites), populations from Walangchung Gola sector (drier sites) produced large-sized genets (in terms of total number of ramets per genet; except in highly disturbed site such as Semjoyunjo). Ghunsa populations on the otherhand showed significantly large number of flowering ramets per genet than did by Walangchung Gola populations. This shows that at high altitudes and at drier sites (such as those of Walangchung Gola sector), plants tend to invest less in sexual fecundity and more in traits ensuring persistence of vegetative offshoots (or production of new ones, although this seems to be very slow in the high altitude environment (Ghimire *et al*. 2008). Similarly, the higher sexual allocation (biomass of inflorescence) and higher sexual reproductive effort (inflorescence biomass/total biomass) of *N. scrophulariiflora* in lower alpine populations (e.g., Phukpadep) supports the hypothesis that sexual reproduction in clonal plant is favored by favorable site conditions (e.g., Loehle 1987; Kanno and Seiwa 2004). However, the lowest sexual and vegetative allocations in Semjoyunjo population, however, were not only related to the environmental stress determined by higher altitude. Semjoyunjo population lies at high altitude and receives highest level of disturbance (see Fig 4.2). Thus in addition to being affected by environmental stress, anthropogenic disturbance (mainly harvesting) had significant impact on the underground biomass of *N. scrophulariiflora* at high altitudes. In KCA, the high altitude sites are more disturbed due to high encroachment by the people from Tibet or Sikkim. Further studies are needed to differentiate the relative impact of anthropogenic factors from natural factors on the biomass allocation pattern in *N. scrophulariiflora*.

The main limitation on the biomass study was related to the selection of few (only three) populations from the two sectors. Time constraints did not permit selection of multiple populations from each sector. Although detail assessment of sexual reproduction of *N. scrophulariiflora* in relation to habitat condition is lacking, the present study has unveiled that altitude and climatic factors at the broad spatial level, and soil moisture status, vegetation variables and disturbance at the micro-(site) level are related to the variation in vegetative and sexual reproductive efforts. In environments with limited resources (e.g., high altitude and nutrition-poor drier sites of Walangchung Gola sector), vegetative propagation has been found to be more advantageous than sexual reproduction for maintenance of populations. Seed production and sexual recruitment in *N. scrophulariiflora* is also limited by other biotic and abiotic aspects of the environment at very high altitudes (Ghimire *et al.* 2005). For example, the probability of flowering and seed production in such plants declines on increasing density and competition (Newell and Tramer 1978; Eriksson 1989), the cover value of associated herbaceous dicot and graminoid species being high in higher alpine sites compared to lower alpine sites indicating greater competitive stress in the former sites (see below).

Altitude is the most important factor also related to the interpopulation variation in other plant performance traits (mainly growth-related phenotypes) of *N. scrophulariiflora*. The populations growing at lower altitudes (<4500 masl) tended to have longer rhizomes and produced significantly larger inflorescences than did the populations growing at higher altitudes (>4500 masl). The lower altitude areas are warmer than higher altitude areas. Furthermore, altitude showed negative relationship with soil moisture (Appendix 3a), thus high altitude habitats are drier as compared to lower altitude habitats. The early melting of ice in lower altitude habitats provides longer time for growth and development of plant; the higher altitude habitats are snow protected for longer periods. Severe frosts in almost snowless winters at high altitude create enormous mechanical tensions for plant growth during freeze-thaw cycles (Körner 2003).

At upper alpine sites, *N. scrophulariiflora* grows either on open slopes with scattered thickets of shrubs or on scree and rocky habitats, on northwest- or northeast-facing slopes, where soil nutrient level was comparatively low. High cover values of graminoids (grasses), dicotyledons herbs and thickets of dwarf shrubs in upper alpine sites indicate that the target species faces greater competitive stresses in such habitats. At the lower alpine sites it grows mainly under the canopy of tall shrubs (mainly comprising *Rhododendron* *anthopogon*), where the habitat is moist and soil is rich in terms of nitrogen, phosphorus and organic matter content (see Appendix 2b). In habitats dominated by tall shrubs, low light availability due to high canopy closure by shrubs (e.g., *Rhododendron* spp. and *Salix* spp.) prevent occupation by light-demanding herbaceous species, therefore overall abundance of herbaceous species in such habitat is low (Ghimire *et al*. 2006), thus decreasing the chance of competition with limited resources. This could be the reason why the growth-related traits (such as rhizome length and girth, spacer length, etc), sexual allocation (biomass of inflorescense) and sexual efforts (biomass of inflorescense/total biomass) of *N. scrophulariiflora* showed higher values in lower alpine habitats. On the otherhand, vegetative (clonal) effort (in terms of belowground biomass/total biomass) was high in drier higher alpine habitats of Walangchung Gola sector (Fig. 4.7).

Guerrilla species are better adapted to compete effectively with other species (Schmid and Harper 1985; David Humphrey and Pyke 1998) and have an advantage in exploiting open space (Lovett Doust 1981; de Kroon and Schieving 1990; Bazzaz 1991; de Kroon and Hutchings 1995). Thus, the biomass of clonal plants, that bear ‘guerrilla’ strategy, increases more rapidly when more space is available (David Humphrey and Pyke 1998). This is true for the plants growing at lower alpine level where competition with other associated species is very low. On the other hand the populations growing at upper alpine level have been facing greater competition with associated graminoid and herbaceous species. But most of the populations (except highly disturbed Semjoyunjo population) from upper alpine level showed higher number of vegetative ramets per genet regardless of competition with associated graminoid and herbaceous dicot species. In general, populations from Walangchung Gola sector showed large-sized genets than did by the populations from Ghunsa sector. Ghunsa population with high sexual fecundity and small-sized genet may reflect the nature of a dynamic population, where production of new ramets through sexual means and growth and death of vegetative offshoots undergo more or less simultaneously. On the otherhand, large genet size but with low sexual fecundity in Walangchung Gola population may reflect very static nature of the population where very large genet (probably of very old age) may have been persiting since long. In large genets, interconnected ramets are less affected by intraspecific competition at high density than are individual shoots grown from seeds (Harnett and Bazzaz 1985). Survival of established genet, in harsh climate (such as those of Walangchung Gola sector) is most important determinant of population growth (cf. Eriksson 1992). Clonal plants are likely to have evolved the ability to maintain genet survival in the face of competition (David Humphrey and Pyke 1998).

Canonical Discriminant Analysis revealed that the *N. scrophulariiflora* populations from Walangchung Gola and Ghunsa sectors were distinct from one another. The first discriminant function separated the population of Ghunsa sector from that of Walangchung Gola sector. Populations of these two sectors mostly differed with respect to total length of inflorescence and total length of rhizome branch, the values of which were observed highest in Ghunsa sector. Ghunsa sector lies towards the far eastern corner of Nepal which can be assumed to receive generally higher precipitation. Walangchung Gola sector receives relatively lower precipitation as most of its high altitude areas are sheltered from monsoonal precipitation by high mountain ranges that lie towards the east.

Some traits appear consistently in some of the populations, like longest inflorescence and rhizome branches separated Phukpadep population from other. Similarly, Chherchen population had thickest rhizome and Semjoyunjo population had shortest inflorescence among the six populations studied. These are some of the parameters which may better characterize the individual population.

5.3 Variation in Population Density and Structure

The reduced cold, richer soil and longer growing period (Wang *et al*. 2002; Klimeš 2003) in lower altitude has affected positively to ramet density as well as on number of flowering ramet per genet, thus all the lower altitude populations (except Chherpari) showed higher density of ramets and genets as well as higher number of flowering ramets per genet. At the same altitude level, populations from Walangchung Gola and Ghunsa sectors did not differ significantly in terms of ramet and genet densities, although these values tended to be high in populations from Ghunsa sector. However, there was strong sectorial difference in genet size (in terms of total number of ramets per genet) and number of flowering ramets per genet. The sectorial variation in genet size and number of flowering ramets per genet is mainly related to climatic factor and disturbance (harvesting pressure, see above).

Sectorial variation was also observed in ramet population structure. The analysis of the population structure in perennial plant species may give valuable insights into population processes and may help to assess the status of populations (Oostermeijer *et al.* 1994).

Higher proportions of juvenile and vegetative adult ramets, with poor representation of very young and reproductive adult (flowering) ramets in the populations from Walangchung Gola sector reflect a condition of loss of reproductives. The climatic (overall dryness of the area), edaphic (poor soil nutrients) and vegetation (high grass cover) variables are responsible for the reduce seedling recruitment and loss of reproductives in these populations. It has been shown for several other species of meadow habitat that competition by the established vegetation is an important factor affecting seedling establishment (Krenova and Leps 1996; Spackova *et al*. 1998; Kotorova and Leps 1999). In highly disturbed populations from Walangchung Gola sector (such as Semjoyunjo), proportion of reproductive adult (flowering) was even greatly reduced indicating that the harvest limits are too high in these populations, changes may be needed to existing harvesting practices. Although long life spans in *N. scrophulariiflora* allow its populations to withstand long periods of unfavorable environmental conditions, they are strongly affected by human use as recovery in such species can be slow due to recruitment limitations (Schemske *et al*. 1994; Eriksson 1996; Ghimire *et al*. 2008); the reduced sexual recruitment may have strong negative effect for the long-term survival of the populations subjected to human harvest. On the other hand, the populations from Ghunsa sector showed almost equal proportions of young, juvenile and vegetative adult ramets. Proportion of adult reproductive ramets was also significantly high in the populations from Ghunsa sector than in the populations from Walangchung Gola sector. This shows that the populations of Ghunsa sector are stable in density and are self-replacing.

Univariate linear regression analysis, however, showed that combine soil variable, altitude and RRI were the principal components having greater influence on population density and structure. Among these, soil variable was the most important factors with greater influence on population size of *N. scrophulariiflora*. In high altitude areas, soil fertility (particularly in terms of humus content) has been found to be positively correlated with various parameters related to plant growth (Körner 2003). Colling *et al.* (2002) and Colling and Matthies (2004) reported site productivity and soil moisture to be the principal factors contrastingly affecting densities of genets and ramets in clonal plants. For example, genet density has been reported to be decreased but ramet density increased with increasing productivity, thus indicating that if a site is fertilized, recruitment of new genets and survival of genets is reduced, but growth of surviving genets is increased (Colling *et al.* 2002). But such relationship has not been observed in the present study. In the present study, the relationship between soil variable and plant density is restricted only for the populations of Walangchung Gola sector (since soil data were not collected from the populations of Ghunsa sector). Therefore, due to limited information overall relationship between soil variable and population attributes in the entire data set is difficult to generalize.

Altitude showed significant positive linear relationship with the proportion of vegetative adult ramets and negative linear relationship with plant density. However, with reproductive adult density, its proportion and with number of flowering ramet per genet, altitude showed unimodel relationships, indicating higher values of these parameters at mid-altitude (4300-4400 masl; which still falls under lower alpine level). This also supports above finding that the lower alpine sites (particularly falling between 4300-4400 masl) provide more favorable habitat conditions for greater reproductive efforts of the plant. At higher altitude in upper alpine level plants may take longer time to flower. Flowering in one season is seen nearly impossible in higher altitudes because the plant does not get sufficient time due to delay snow melting and soon snow covering (Tilman 1993; Foster and Gross 1998; Colling *et al.* 2002; Körner 2003).

Similarly, moderate RRI favored genet density, total ramet density, and densities of juvenile, vegetative adult and reproductive adult. Among the vegetation variables grass cover had the highest explanatory power. The significant negative linear relationship between grass cover and reproductive plant number and densities (number of flowering ramet per genet, and density of reproductive adult ramets) supports the hypothesis that the the probability of flowering and seed production in clonal plants declines on increasing density and competition (Newell and Tramer 1978; Eriksson 1989). Unlike reproductive adults, vegetative adults were less affected by grass cover. However, higher proportion of vegetative adults at moderate value of grass cover reflects that very high competition indeed has negative effect on vegetative adult. Surprisingly, shrub cover did not influence much on the studied population parameters. Tall shrub cover showed negative linear relationship with density of ramet at different size classes and genet composition (e.g., number of flowering ramet per genet). *N. scrophulariiflora* is most commonly found in the shrubland dominated by *Rhododendron anthopogon*. The low influence of shrub cover in explaining variations in population attributes is related to almost similar abundance of shrubs in most of the studied populations.

In the subset of population data from Walangchung Gola sector, disturbance showed significant negative effect on adult vegetative and adult reproductive ramet densities, adult reproductive ramet proportions and number of flowering ramets per genet. In the Walangchung Gola sector, the enforcement of forest management rules and regulations is weak thus it is less protected than Ghunsa sector from illegal activities. Even in the Walangchung Gola sector, populations from higher alpine level received greater impact (livestock grazing, trampling and plant harvesting) than the populations from lower alpine level. Higher human pressure at high altitude in Walangchung Gola Sector was due to the fact that Tibetan MP collectors and traders used to cross high pass, harvest MPs for their need as well as for trade (Sherpa 2001). In addition, herders from Walangchung Gola also used to move their livestock to high Himalayan pass for rotational grazing in summer. Particulatly those populations of *N. scrophulariiflora* were highly disturbed where the transhumance activities are high because herders used to collect the plant near to their 'Goth' (temporary settlements in the high pasture). Mainly trampling [*N. scrophulariiflora* is unpalatable and grazing tolerant (Lama *et al.* 2001; Ghimire *et al.* 2004)] and high level of harvesting has reduced ramet and genet densities as also reported for other species (Ghimire *et al.* 2005, 2008). These practices mainly break the plagiotropic rhizome branches and reduce the number of ramet per genet, thus in very highly disturbed sites (such as Semjoyunjo) proportions of single ramets was greatly increased with the decrease in the proportion of large genets and the number of adult reproductive ramets per genet. Because of long creeping rhizomes of *N. scrophulariiflora*, some parts of rhizome can easily be left in the ground while harvesting and new plants could sprout from these small fragments through vegetative means. This increases the number of fragments of the same genet but may decrease the overall genetic diversity. Sexual reproduction is the major tool for the genetic vatiation. Due to the reduced number of flowering ramets genetic variation could be very low in the harvested and fragmented populations (Oostermeijer *et al.* 2000, 2003). Among the studied populations, genet size was largest in Chherchen population of Walangchung Gola sector, where grazing was not observed and was least disturbed by harvesting; whereas smallest genets were recorded in Phukpadep population of Ghunsa sector, where harvesting was high.

The strong relationship between environmental conditions and the population size and structure of *N. scrophulariiflora* has important implications for the conservation of the species. In conclusion, our results suggest that an analysis of the population size and structure of long-lived perennials like *N. scrophulariiflora* can give valuable insights into population processes and, in combination with an analysis of the vegetation structure and the environmental conditions may indicate suitable management measures to preserve populations.

5.4 Patterns of Utilization and Existing Management Practices

Plant part harvested is the important factor in which effects of harvesting on population dynamics depend on (Tiktin 2004; Cunningham 2001; Ghimire *et al.* 2005; Ghimire and Aumeeruddy-Thomas 2005). In KCA, underground rhizomes of *N. scrophulariiflora* are harvested for medicinal and trade purposes. Harvesting of root and underground rhizome may lead to a serious threat to existing population. Ideally, collection of *N. scrophulariiflora* is sustainable when it is collected during late September to October/November (Edwards 1995). Collection at this time permits seed dispersal and rhizome may be larger and may also yield more active ingredients per unit weight. Well grown and mature rhizomes are more potent with higher medical efficacy (Lama *et al.* 2004; Ghimire *et al.* 2004, 2005). But local people of KCA generally collect rhizome of *N. scrophulariiflora* in June-July which is the peak flowering period and its fruiting time is from late July to September. The pre-mature collection of *N. scrophulariiflora* not only reduces the quality of the collected rhizome but also reduces the chance of sexual fecundity. Local harvesters are forced to collect early because they have to compete for the scarce resource not only among themselves but also with the people illegally coming from Tibet. Harvesting in June-July is also easy due to moist substrate. People also harvest prematurely to exchange MPs with food and other accessories in Tibet during trans-Himalayan trade, which commence before the snow fall. This practice is believed to be serious as the species are declining from the area once they flourished and people have found it more difficult to collect the products.

Harvesting of *N. scrophulariiflora* rhizome was generally carried out during transhumance activity. Most of the collectors are untrained and employ destructive harvest method (collecting whole clump irrespective of size class and maturity stage of the plant), which is responsible for declining the populations of *N. scrophulariiflora* from high altitude areas. However, collection of high altitude medicinal plants, including *N. scrophulariiflora*, for health care use by local healers (e.g. *amchis*: herbal healers trained in Tibetan medicine) appears to be sustainable as such collectors have good knowledge of habitat, life history traits and regeneration of medicinal plants they collect (Ghimire *et al*. 2005). Recent declaration of Conservation Community Forests (CCF) and handing over forest management to the local community in KCA may fulfill the expectation of public awareness towards developing sustainable harvesting regimes and management rules of *N. scrophulariiflora* and other MPs (Ghimire and Nepal 2007).

5.5 Implication of Results for Conservation and Sustainable Management

The major problem for conservation of any species is the lack of knowledge about the species' biology and population status. In addition conservation strategies required for clonal taxa may differ from nonclonal sexual species. Here *N. scrophulariiflora* is a clonal species where sexual reproduction is seemed to be less effective than vegetative clonal propagation. Because of its long creeping rhizomes, the recruitment rate of ramet in *N. scrophulariiflora* through vegetative mean is high (Ghimire *et al*. 2005). High adult survival is common method of population persistence in plants exhibiting such type growth strategy rather than recruitment of new seedlings (Harper and Bell 1979; Lovett-Doust 1981; Cook 1985; Silvertown 1987). However, there were strong sectorial and altitudinal differences in the relative contribution of sexual versus vegetative growth strategies in population persistence. The lower performance of plants (in terms of growth-related vegetative traits) and low sexual reproductive efforts in the population from higher alpine sites (especially those from Walangchung Gola sector) indicate that these populations need immediate action to protect them from illegal and premature harvesting. Thus long-term plan with a strong measure for sustainable utilization system (including sustainable rate and frequency of harvesting, and appropriate time and method of harvesting) is needed to manage the plant for the long run simultaneously respecting traditional access rights of the local users. Ghimire *et al.* (2005) stated that harvesting period, amounts harvested and methods applied are the important factors that differentially affect the population ecology of high-altitude clonal species including *N. scrophulariiflora*.

In higher Himalayas, in fragmented landscape, plant populations become both smaller and more isolated from each other. Smaller populations are more prone to demographic, environmental and genetic stochasticity. Sexual reproduction is the major tool for the maintenance of genetic vatiation in plant populations (Oostermeijer *et al*. 2000, 2003). Highly reduced sexual fecundity in some populations of *N. scrophulariiflora* particularly from high altitude areas of Walangchung Gola sector suggests low genetic variation. In such populations management should focus on increasing seedling recruitment and reducing damage to the reproductive adults.

Species with predicted low level of genetic variation are supposed to have low ability to colonize new environments. However, clonal growth strategy is also an advantage for rapid growth of *N. scrophulariiflora* observed in shrubland habitat in the present study as well as in other studies (Ghimire *et al*. 2005). The rapid rate of ramet recruitment in shrubland habitat has been reported to favor fast recovery of population of *N. scrophulariiflora* which can withstand certain level of harvesting (Ghimire *et al*. 2005). But, extremely low seedling recruitment makes this species highly vulnerable if harvesting is applied at fairly higer level, because in such condition population genetic diversity can be expected to be very low (Ghimire *et al*. 2005). Thus applying low level of harvesting with farly long rotation is a good strategy for population persistence. Level of harvesting should be even low in harsh higher alpine habitats of Walangchung Gola sector.

To develop sustainable conservation strategy of *N. scrophulariiflora*, following measures are suggested:

1. Conservation by commercialization

N. scrophulariiflora is a commercially traded and threatened species in this area. Local people depend mostly on the trade of such MPs for their livelihood. Because of scarce lands for cultivation, *in situ* management applying sustainable practice should be started at the local level by involving local users as the main steward for the management of the resource. For this, collectors should be well organized as a cooperative to negotiate with the outside traders, monitor the resource from illegal activities and to ensure that the sustainable practices are implemented and respected. This will generate considerable employment opportunities in local level which may reduce unscientific harvesting practices and habitat destruction. Sustainable harvesting guidelines should be developed and peoples should be made aware about the threats and conservation issues concerning the target species.

2. Developemt of long term plan

Prematured harvesting without applying proper harvesting methods is one of the serious threats for the long-term survival of *N. scrophulariiflora* populations. Therefore, a long-term plan with a strong measure for sustainable utilization system (including sustainable rate and frequency of harvesting, and appropriate time and method of harvesting) should be developed separately for the two sectors. Appropriate rotational harvesting technique should be followed to harvest sustainable amount in appropriate harvesting season and the plants should be in harvestable age and size. The level of harvesting should be even low in harsh higher alpine habitats of Walangchung Gola sector. Besides, management should also focus on increasing seedling recruitment and reducing damage to the reproductive adults.

3. Enrichment planting

Being a clonal species, *N. scrophulariiflora* has great capacity of ramet recruitment through vegetative means. In an enrichment planting experiment, Ghimire *et al*. (2005) found survival of 58-70% of replanted young rhizome fragments (these fragments are medicinally less important and collectors usually discard them). Thus *in situ* management of *N. scrophulariiflora* should involve a combination of selective collection of matured rhizomes and replanting of the younger ones (proximal end, with few roots).

4. Public awareness

Public awareness program should be lunched at local level to aware the people about the importance of the species conservation and opportunities for future use. People should be aware about the vulnerability of harvesting of higer alpine populations of *N. scrophulariiflora* from Walangchung Gola sector and appropriate method for maintaing long-term survival of these populations.

Chapter 6

SUMMARY AND CONCLUSION

Variation in growth strategy, plant performance, and population size and structure was studied in a threatened long-lived clonal medicinal herb [(*Neopicrorhiza scrophulariiflora* (Pennell) D.Y. Hong)] in relation to altitude and other environmental variables. Seven distinct populations of *N. scrophulariiflora* were selected from lower alpine (mean altitude <4500 masl) and upper alpine (mean altitude >4500 masl) habitats in Walangchung Gola and Ghunsa sectors within Kangchenjunga Conservation Area, Taplejung, East Nepal. The two sectors differ in terms of degree of protection at the landscape level (Ghunsa sector being more protected than Walangchung Gola sector from illegal activities) and level of precipitation (higher precipitation in Ghunsa sector). Variation in growth strategy and plant performance was studied in five populations. In each population, 15 matured clumps or putative genets of *N. scrophulariiflora* were excavated and a variety of plant performance traits (in terms of rhizome size and its branching pattern, number of vegetative and reproductive ramets, size of inflorescence and seed output) were measured. Biomass allocation pattern was assessed in three populations to know indirectly the relative contribution of vegetative and sexual reproduction in population persistence. Population density and structure were studied at the ramet and genet level in all the seven populations using stratified sampling method. In total, 136 plots (consisting of 408 sub-plots of 1 m x 1 m size) were studied in 7 populations. Physical, topographical and human management variables were recorded for each sampling plot.

Strong habitat differences between upper alpine and a lower alpine site was detected in terms of stand structure and vegetation composition. The habitat of *N. scrophulariiflora* ranged from shrubland to open rocky/scree or grassy slopes on increasing altitude. Dwarf shrubs or shrublets contributed much in the community composition at the upper alpine sites, whereas tall shrubs had high contribution at lower alpine sites. The upper alpine sites also showed high cover values for dicotyledonous herb, grass and rock. Lower alpine sites were rich in terms of soil nutrients than upper alpine sites. Intensity of human disturbance showed positive relationship with altitude, indicating higher human pressure at higher altitude and lower pressure at lower altitudes. As compared to Ghunsa sector, populations from Walangchung Gola sector received higher harvesting pressure.

There were strong sectorial and altitudinal differences in the relative contribution of sexual versus vegetative growth strategies in population persistence. Altitude was the most important factor also related to the inter-population variation in other plant performance traits (mainly growth-related phenotypes) of *N. scrophulariiflora*. Canonical Discriminant Analysis revealed that *N. scrophulariiflora* populations from Walangchung Gola and Ghunsa sectors were distinct from one another mainly with respect to size of inflorescence and rhizome branch, the values of which were observed highest in Ghunsa sector. As compared to Ghunsa sector (moist sites); populations from Walangchung Gola sector (drier sites) produced large-sized genets (except in highly disturbed site, such as Semjoyunjo). Ghunsa populations on the other hand showed significantly large number of flowering ramets per genet than did by Walangchung Gola populations. This shows that at high altitudes and at drier sites (such as those of Walangchung Gola sector); plants tend to invest less in sexual fecundity and more in traits ensuring persistence of vegetative offshoots. Similarly, sexual allocation (biomass of inflorescence) and sexual reproductive effort (inflorescence biomass/total biomass) were high in lower alpine populations supporting the hypothesis that sexual reproduction in clonal plant is favored by favorable site conditions. Although detail assessment of sexual reproduction of *N. scrophulariiflora* in relation to habitat condition is lacking, the present study has unveiled that altitude and climatic factors at the broad spatial level, and soil moisture status, vegetation variables and disturbance at the micro-(site) level are related to the variation in vegetative and sexual reproductive efforts. In environments with limited resources (e.g., high altitude and nutrition-poor drier sites of Walangchung Gola sector), vegetative propagation has been found to be more advantageous than sexual reproduction for maintenance of populations.

Increased temperature, richer soil and longer growing period in lower altitude also affected positively to ramet and genet densities. Univariate linear regression analysis, however, showed that combine soil variable, altitude and relative radiation index (RRI) were the principal components having greater influence on population density and structure. At the same altitude level, populations from Walangchung Gola and Ghunsa sectors did not differ significantly in terms of ramet and genet densities, although these values tended to be high in populations from Ghunsa sector. However, there was strong sectorial difference in ramet population structure. Higher proportions of juvenile and vegetative adult ramets, with poor representation of very young and reproductive adult (flowering) ramets in the populations from Walangchung Gola sector reflect a condition of loss of reproductives. The climatic (overall dryness of the area), edaphic (poor soil nutrients) and vegetation (high grass cover) variables are responsible for the reduced seedling recruitment and loss of reproductives in these populations. In highly disturbed populations from Walangchung Gola sector (such as Semjoyunjo), proportion of reproductive adult was even greatly reduced indicating highest level of harvesting, thus changes may be needed to existing harvesting practices. Although long life spans in *N. scrophulariiflora* allow its populations to withstand long periods of unfavorable environmental conditions, they are strongly affected by human use as recovery in such species can be slow due to recruitment limitations; the reduced sexual recruitment may have strong negative effect for the long-term survival of the populations subjected to human harvest. On the other hand, the populations from Ghunsa sector showed almost equal proportions of young, juvenile and vegetative adult ramets. Proportion of adult reproductive ramets was also significantly high in the populations from Ghunsa sector than in the populations from Walangchung Gola sector. This showed that the populations of Ghunsa sector were stable in density and self-replacing.

The strong relationship between environmental conditions and the population size and structure of *N. scrophulariiflora* has important implications for the conservation of the species. In conclusion, our results suggest that an analysis of the population structure of long-lived perennials like *N. scrophulariiflora* can give valuable insights into population processes and, in combination with an analysis of the vegetation structure and the environmental conditions may indicate suitable management measures to preserve populations. The lower performance of plants (in terms of growth-related vegetative traits) and low sexual reproductive efforts in the population from higher alpine sites (especially those from Walangchung Gola sector) indicate that these populations need immediate action to protect them from illegal and premature harvesting. In such populations management should focus on increasing seedling recruitment and reducing damage to the reproductive adults. Thus long-term plan with a strong measure for sustainable utilization system (including sustainable rate and frequency of harvesting, and appropriate time and method of harvesting) is needed to manage the target species population for the long run simultaneously respecting traditional access rights of the local users. Extremely low seedling recruitment makes this species highly vulnerable if harvesting is applied at fairly higher level, because in such condition population genetic diversity can be expected to be very low. Thus applying low level of harvesting with fairly long rotation is a good strategy for population persistence. Level of harvesting should be even low in harsh higher alpine habitats of Walangchung Gola sector.

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Appendix 1: Threat status of medicinal plants found in KCA assigned under different categories.

SN	**Plant species**	**Family**	**Nepali name**	**Local names (W = Walung, L = Limbu)**	**Threat category**			**Protection category (according to Forest Act)**	**Prioritization by Government of Nepal**	**Local availability**	**Local status**
					CAMP	IUCN	CITES				
1	*Abies spectabilis* (D. Don) Mirb.	Pinaceae	Gobresalla,	Cherukpa (W)				3		A	NT
2	*Aconitum bisma* (Buch-Ham.)Rapaics	Ranunculaceae	Bikh	Bhungna (W)	DD					R	T^{r}
3	*Aconitum ferox* Wall.ex Seringe	Ranunculaceae	Seto bikh	Kumakla (L)	DD	T				R	T^{r}
4	*Aconitum gammiei* Stapf	Ranunculaceae	Bikh	Ning (L)		T				R	T^{r}
5	*Aconitum spicatum* (Bruhl.) Stapf	Ranunculaceae	Bikh	Chenduk (W)	V	T		4		NK	T
6	*Arisaema costatum* (Wall.) Mart.ex Schott	Araceae	Sarpako makai	Thwa (W)	LC					NK	NT
7	*Asparagus racemosus* Willd.	Liliaceae	Satawari, Kurilo		V			1, 4		R	T^{r}
8	*Bergenia ciliata* (Haw.) Stern	Saxifragaceae	Paakhandbed	Kopsyokpa (W)		T		4		C	T^{ht}
9	*Ceropegia pubescens* Wall	Asclepiadaceae					II			R	T^{r}
10	*Corydalis megacalyx* Ludlow	Papaveraceae			EN					R	T^{r}
11	*Dactylorhiza hatagirea* (D. Don.) Soo	Orchidaceae	Atis	Ongulakpa (W)	EN			1, 2, 4		R	T^{ht}
12	*Daphne bholua* Buch.-Ham. ex D. Don	Thymelaeaceae	Seto lokta	Da mendok(W)				6		C	T^{hr}
13	*Daphne papyracea* Wall. ex Steud	Thymelaeaceae	Kalo lokta	Dangma (W)				6		C	T^{hr}
14	*Delphinium himalayai* Munz	Ranunculaceae			V					R	$T^{r,ht}$
15	*Dioscorea deltoidea* Wall.	Dioscoreaceae	Bhyakur	Bhyakur (W)	EN	T	II	4		R	T
16	*Fritillaria cirrhosa* D. Don	Liliaceae	Kaakoli		V					C	NT
17	*Heracleum* nepalense D.Don	Apiaceae	Chimbing	Chimbing (W)	EN					C	C
18	*Juglans regia* L.	Juglandaceae	Okhar	Tarka (W)				2		R	T^{hr}
19	*Meconopsis grandis* Prain	Papaveraceaae	Upamendok	Upagnono (W)				5		R	T^{hr}
20	*Michelia champaca* L.	Magnoliaceae	Champ		CR			6		R	T^{hr}
21	*Nardostachys grandiflora* Dc.	Valerianaceae	Jatamansi	Pangboe (W)	V	V		1, 3, 4		R	T^{hr}
22	*Neopicrorhiza scrophulariiflora* (Pennell) Hong	Scrophulariaceae	Kutki	Hungle	V			2, 4		C	$T^{r,ht}$
23	*Oroxylum indicum* (L.) Kurz	Bigniniaceae	Tatelo		EN					R	T

SN	Plant species	Family	Nepali name	Local names (W = Walung, L = Limbu)	Threat category			Protection category (according to Forest Act)	Prioritization by Government of Nepal	Local availability	Local status
					CAMP	IUCN	CITES				
24	*Panax pseudo-ginseng* Wall.	Araliaceae	Mangan		V					R	T^{hr}
25	*Paris polyphyla* Smith	Liliaceae	Satuwa,Tintale banko		V	V				R	T^{hr}
26	*Podophylum hexandrum* Royle	Berberidaceae	Laghupatra	Ramasisi	V	V	II	4		R	T^{hr}
27	*Rheum australe* D.Don.	Polygonaceae	Padamchal	Chhucha	V	V		4		R	T
28	*Rheum nobile* Hook.f.&Thoms.	Polygonaceae	Amalbetas	Chhulama	V	R				R	T
29	*Rubia manjith* Roxb.ex Fleming	Rubiaceae	Majitho		V			4		C	NT
30	*Saussurea gossipiphora* D. Don	Asteraceae	Maikopila	Yazembawa				5		R	T^{r}
31	*Saussurea topkegolensis* H. Ohba & S. Akiyama	Asteraceae	Maikopila	Yazembawa				6		R	T^{ht}
32	*Saussurea tridactyla* Sch. Bip. ex Hook.f.	Asteraceae	Maikopila	Yazembawa				6		R	T^{ht}
33	*Swertia angustifolia* Buch.- Ham.ex D. Don.	Gentianaceae	Bhale chirayito		EN					R	T^{ht}
34	*Swertia chirayita* (Roxb.ex Fleming) Krstrn	Gentianaceae	Chirayito	Tikta	V	V		1, 4		C	T^{ht}
35	*Swertia multicaulis* D. Don.	Gentianaceae	Sarmaguru	Sepugunduk	DD					R	T^{ht}
36	*Tinospora sinensis* (Lour) Merr	Menispermaceae	Gurjo		V					R	T
37	*Taxus wallichiana* Zucc	Taxaceae	Lauth salla	Chende (S)			II	1, 4		R	T
38	*Valeriana jatamansii* Jones	Valerianaceae	Sugandhawal	Jaboe	V			1, 3, 4		R	T

A=abundant, C=common, CT=commercially threatened, CR=critical, DD=data deficient, E=endangered, NK= not known, NT=not threatened, R=rare, T= threatened, V=vulnerable, 1=prioritized for agro-technology development, 2=banned for collection and trade, 3=banned for export outside the country without processing, 4=prioritized for research and development, 5= threatened by trade, 6= threatened by anthropogenic activities.The letters in the superscript denotes: T^{r}= threatened due to rarity, T^{hr}= threatened due to harvesting, T^{ht}= threatened due to harvesting and trade (Source: HMG/N, 2006; Sherpa, 2001).

Appendix 2: Environmental Variables

(2.a) Physiographic characteristics of the study sites (for altitude, slope and aspect, the values given are range and mean in the parentheses).

Sites/populations of *N. scrophulariiflora*	**Valleys**	**Latitude**	**Longitude**	**Altitude (m)**	**Slope(°)**	**Aspect(°)**
Semjoyunjo	Singjema, Walangchung Gola	27°46'3.0" N- 27°46'12.8"N	87°47'6.0" E - 87°47'12.3" E	4712-4734 (4718)	27-28 (27.5)	87-350 (175) SE-NW
Chuwapangsong	Syamdo, Walangchung Gola	37°43'17.1"N - 27°43'21.5"N	87°46'10.7" E - 87°46'25.9" E	4560-4600 (4570)	18-50 (32)	10-350 (64) N-NW
Chherchung	Syamdo, Walangchung Gola	27°43'20.7"N - 27°43'40.3"N	87°45'18.7" E - 87°45'34.8" E	4336-4630 (4554)	30-60 (47)	14-330 (55) NE-NW
Chherpari	Singjema, Walangchung Gola	27°45'45.5"N - 27°46'48.3"N	87°45'25.2" E - 87°45'27.7" E	4352-4450 (4390)	38-50 (41)	309-332 (317) NW
Chherchen	Syamdo, Walangchung Gola	27°43'42.8"N - 27°43'53.9"N	87°46'18.2" E - 87°46'35.3" E	3993-4475 (4368)	38-45 (40)	300-330 (314) NW
Lumbasamba	Yamatari, Ghunsa	27°37'45.1" N - 27°37'49.8" N	87°58'35.6" E - 87°58'41.2" E	4322-4441 (4373)	22-40 (28)	160-360 (218) SE- NW
Phukpadep	Yamatari, Ghunsa	27°37'41.9" N - 27°38'42.5" N	87°58'11.4" E - 87°58'42.9" E	4245-4373 (4316)	22-40 (35)	320-360 (341) NW

(2.b) Soil characteristics of the study sites (values represent mean ± SE).

Population	**pH**	**Nitrogen (%)**	**Phosphorus (kg/ha)**	**Potassium (kg/ha)**	**OM (%)**	**Moisture (%)**	**Depth (cm)**
Semjoyunjo		-	-	-	-	21.63 ± 2.57	23.83 ± 2.80
Chuwapangsong	4.40	0.62	7.00	498.00	12.50	26.58 ± 4.95	25.84 ± 4.83
Chherchung	4.50 ± 0.29	0.47 ± 0.17	39.00 ± 32.0	574.33 ± 137.39	9.333 ± 3.47	24.05 ± 4.60	11.62 ± 5.53
Chherpari	4.20 ± 0.13	0.85 ± 0.22	86.50 ± 36.07	706.50 ± 104.23	17.05 ± 4.40	30.96 ± 1.27	34.25 ± 1.398
Chherchen	4.33 ± 0.03	0.87 ± 0.22	56.33 ± 27.74	774.00 ± 71.02	17.37 ± 4.42	46.92 ± 5.06	51.50 ± 5.67
Overall mean	*4.36 ± 0.11*	*0.73 ± 0.11*	*58.09 ± 17.40*	*669.91 ± 57.90*	*14.62 ± 2.25*	*29.84 ± 2.05*	*31.19 ± 2.43*
F	0.45	0.75	0.61	0.81	0.75	7.05	8.71
p	0.72	0.55	0.63	0.53	0.56	0.00	0.00

Appendix 3: Spearman rank correlations between major environmental variables (physical and disturbance) and vegetation variables.

Environmental variables	Alt	Slp	Asp	RRI	Smois	Sdept	PH	Soil-PCA[¥]	NVcov	Tscov	DScov	TWcov	HBcov	GRcov	RKcov	ASR
Altitude (Alt)	1															
Slope (Slp)	ns	1														
Aspect (Asp)	-0.576**	ns	1													
Relative radiation index (RRI)	ns	ns	ns	1												
Soil moisture (Smois)	-0.312*	ns	0.479**	ns	1											
Soil depth (cm) (Sdept)	ns	0.414**	0.493**	ns	0.949**	1										
Soil pH	ns	-0.581*	-0.686**	ns	ns	ns	1									
Combined soil variable (Soil-PCA)[¥]	-0.302*	0.421**	0.456**	ns	0.978**	0.993**	ns	1								
Non-vascular plant cover (NVcov)	ns	0.194*	0.182*	ns	0.372*	0.391**	ns	0.322*	1							
Tall shrub cover (TScov)	ns	0.180*	ns	ns	0.287*	0.316*	ns	ns	0.576**	1						
Dwarf shrub/shrublet cover (DScov)	ns	-0.184*	ns	ns	ns	ns	ns	ns	ns	ns	1					
Total woody vegetation cover (TWcov)	ns	ns	ns	ns	0.339*	0.318*	ns	0.302*	0.597**	0.887**	0.263*	1				
Herb cover (HBcov)	ns	0.219*	-0.304**	ns	ns	ns	ns	ns	-0.295*	-0.264*	ns	-0.267*	1			
Grass cover (GRcov)	0.618**	ns	-0.430**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	1		
Rock cover (RKcov)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.192*	1	
Asssociated species richness (ASR)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.224*	ns	0.529**	ns	ns	1
Combined disturbance variable (Dis-PCA)[¥]	0.222*	ns	ns	ns	-0.571**	-0.370*	ns	-0.611**	-0.244**	-0.211*	ns	-0.229**	ns	ns	-0.191*	-0.331**

[¥] Using principal component analysis (PCA), all the soil variables were combined to an overall measure of soil variable (Soil-PCA); similarly all the disturbance variables were combined to an overall measure of disturbance (Dis-PCA).

**Correlation is significant at 0.01 level (two- tailed); *Correlation is significant at the 0.05 level (two- tailed). ns = not significant, all other coefficients are significant ($p<0.05$).

Appendix 4: Species found in association with *Neopicrorhiza scrophulariiflora* and their cover value (mean per plot) in different populations

Sn	Species[†]	Local name	Habit[‡]	Semjoyumjo	Chhuwapangson	Chherchung	Chherpari	Chherchen	Lumbasamba	Phukpadep
1.	*Anaphalis monocephala*	buki phool	H		2.67			0.03	5.28	3.76
2.	*Berginea purpuracens*		H			9.89	3.33			
3.	*Bistorta emodi*	rambu	H	1.89	4.33			0.44	3.75	3.33
4.	*Cassiope fastigiata*		DS		5.43	12.48		3.30		
5.	*Corydalis cashmeriana*		H			1.67			2.90	0.33
6.	*Crementhodium nepalense*		H							5.00
7.	*Gaultheria trichophyla*		DS		10.86	14.86		8.60		
8.	*Pedicularis* sp.		H		0.03	0.67		0.08	2.50	
9.	*Polygonum* sp.		H			13.29				3.33
10.	*Potentilla peduncularis*		H		8.33					
11.	*Primula* sp.		H		0.17					
12.	*Rheum acuminatum*	kenjo	H				1.83			
13.	*Rheum nobile*	khokkim	H		1.67				1.67	5.50
14.	*Rhododendon anthopogon*	sunpati	TS	33.06	28.08	37.79	44.55	50.00	20.42	27.37
15.	*Rhododendon campanulatum*	chimal	TS							15.00
16.	*Rhododendron lepidotum*	sulu	TS			3.89			14.42	24.65
17.	*Rhododendron setosum*		TS		4.11	10.00	10.58	7.50		
18.	*Salix calyculata*	langma	DS		3.33	9.46		2.83	5.39	15.80
19.	*Salix lindleana*	langma	DS	9.58	16.15	8.97	3.33	3.13		
20.	*Swertia multicaulis*	sarmaguru	H	4.44	0.07			0.42	2.67	
21.	*Saussurea gossipiphora*	maikopila	H			1.67		0.03		6.67
22.	*Saussurea* sp.	maikopila	H		1.83			0.10		
23.	*Saxifraga* sp.		H				3.33			
24.	*Sedum ewersii*		H			1.67	0.33			
25.	*Selinium* sp.		H		0.17					
26.	*Sibbaldia cuneata*		H					0.83		
27.	*Thalictrum alpinum*		H			1.67				
28.	*Viola* sp.		H		1.17					
29.	Unknown 1 (?*Dicentra* sp.)		H				0.33			
30.	Unknown 2	hubuphula	H						1.00	
31.	Unknown 3	hungu phula	H						1.67	
32.	Unknown 4	penji	H						6.22	8.33
33.	Unknown fern		H					1.67		

[†] Only angiosperms (dicotyledons) were considered. [‡] Plant habit = H = perennial herb, DS = dwarf shrub/shrublet, TS = tall sh