# POPULATION ECOLOGY OF THREATENED ETHNO-MEDICINAL PLANT SPECIES IN RELATION TO FOREST FRAGMENTATION IN KANCHENDZONGA BIOSPHERE RESERVE, SIKKIM

BY

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NORTH-EASTERN HILL UNIVERSITY SHILLONG – 793 022, INDIA 2011 Forest fragmentation was identified as the most important factor reducing the plant diversity of Kanchendzonga Biosphere Reserve (KBR) located between 27°06' and 28°05' North latitudes, and 88°02' and 88°47' East longitudes in the Eastern Himalayan state of Sikkim in north-eastern India. In addition to fragmentation, several other anthropogenic and natural factors have brought about extreme pressure on the persistence of threatened species in KBR. Several ethno-medicinally important plant species inhabiting the forests of KBR are also under threat due to these factors. In addition, over-exploitation of their populations for their medicinal value has put additional pressure on these plant species, many of which have reached on the brink of extinction. Empirical assessment of their populations is a prerequisite for developing any effective in-situ conservation strategy. Therefore, the present research work was undertaken to prepare a list of ethno-medicinal plants, and their conservation status, and uses under traditional medicine system. The research aimed to assess the population ecology of threatened and ethno-medicinal plant species in relation to forest fragmentation. The habitat distribution modeling of the three study species viz., Swertia chirayita, Paris polyphylla and Panax bipinnatifidus was undertaken to characterize the habitats of the species and map their distribution within KBR. The spatial and temporal dynamics of their populations in forest fragments (FF) and continuous forest (CF) were studied to assess the impact of forest fragmentation on population ecology of these species. Their metapopulation status was ascertained, characterized and Population Viability Analysis (PVA) was performed. The extinction probability analysis was also undertaken to evaluate their extinction risk. Generation of these empirical data and analyses are expected to help in preparing an efficient management plan for the threatened ethno-medicinally important plant species of KBR.

### **Ethno-medicinal Plants Species of KBR**

A detail inventory of ethno-medicinal plants species present in KBR was prepared both through primary and secondary survey. Primary data was collected through a questionnaire survey among the forty traditional healers residing in 36 villages located around KBR. Prior Informed Consent (PIC) was taken before interviewing the traditional healers. The available literature was also consulted on medicinal uses of the plant species reported by the traditional healers. The plants so documented were classified based on their uses, ecologic distribution, taxonomic classification and threat status.

A total of 105 ethno-medicinal plant species were recorded belonging to 64 families. Dominant families were Ericaceae and Zingeberaceae with five species each. Fabaceae and Araceae had four species each, while 12 families viz., Valerianaceae, Solanaceae, Saxifragaceae, Rubiaceae, Rosaceae, Ranunculaceae, Poaceae, Orchidaceae, Liliaceae, Gentianaceae, Asteraceae and Apiaceae were represented by three species each and contributing 18% of the total plant species. On the other hand, about 67% of the families were represented by only one species. Araliaceae, Scrophulariaceae, Convovulaceae and Polygonaceae were represented by four species each and contributed 6% to the total species. On the other hand, 18% species belonged to Valerianaceae, Solanaceae, Saxifragaceae, Rubiaceae, Rosaceae, Ranunculaceae, Poaceae, Orchidaceae, Liliaceae, Gentianaceae, Asteraceae and Apiaceae families which were represented by three species each. Araceae and Fabaceae had two species each, and Ericaceae and Zingeberaceae had maximum representation with five species each. The ailments treated were grouped into 52 types. Common ailments such as cough, fever, headache and other gastrointestinal disorders were being treated by a number of species. 27% of ailments were being treated by only one species, 23% by two species, 15% by three species, 12% by four species, 6% by five species, and 4% by six and seven species each. Another 10% of the common ailments were being treated by more than eight species. In terms of medicinal value of the plant species, most species were used for a few ailments *i.e.*, one to four only, while three species were used for treatment of a variety of ailment types. Valeriana jatamansii was used for treatment of five ailments, Costus speciosus was used for six ailments and Centella asiatica was used for a maximum of eight different ailments. From threat perspective, 75 species were not evaluated

and a total of 30 species were found to be threatened. Of these 30 species, three (3%) were critically endangered, seven (7%) were endangered, 19 were vulnerable (18%) and one species (1%) *i.e.*, *Abies densa* was near threatened.

### **The Study Species**

Three species were selected from the documented ethno-medicinal plants for detailed population studies in relation to forest fragmentation. These were: *Swertia chirayita* Roxb. ex Flem. (Gentianaceae), *Paris polyphylla* Smith (Melanthiaceae) and *Panax bipinnatifidus* Seem (Araliaceae).

To study the life history and phenology, twenty five matured individuals of each of the selected species were randomly selected at three sites. Different morphological parameters were measured during the peak flowering and fruiting season. The parameters were: individuals per clump, plant height, leaf number of leaves and flowers per plant, flower length and diameter, and number of fruits and seeds produced per plant. Phenological cycles from emergence period to death of the species were closely observed over a period of one annual cycle from January 2005 to December 2005.

Although the habit of *Swertia chirayita* had been a subject of debate, in this study it was established as a biennial species. The life cycle was distinguished into four demographic stages *viz.*, small rosette, large rosette, vegetative aerials and flowering. Seedlings emergence (small rosette) occurred during spring with the onset of rain from early May to late June. The species attained its rosette form during the first year as two distinct stages *i.e.*, small and large rosettes. The large rosette reappeared after dormancy during spring in the second year and initiation of flowering or reproductive stage developed during late monsoon period and completed during autumn. Fruiting commenced simultaneously around the same period and seed setting started before the onset of winter.

*Paris polyphylla* is a polycarpic perennial plant with a short, thick non-clonal rhizome bearing several irregular constrictions which is formed annually. Life stages of the species are represented by distinct types of annual shoots. Three stages were recognized *viz.*,

seedling, adult and reproductive individuals. Seedling recruitment took place during spring about a month after the emergence of stem from perennating rhizome. Germinated seeds gave rise to radicle which later formed the rhizome system, and followed by plumule which formed the stem. The growth of seedlings occurred during spring and continued till the end of rainy season (September). Seedlings did not attain maturity during the first year during which it grew up to a maximum of 10 cm with 2-4 leaves. Much of the growth however, took place in the underground rhizome component. Dormancy period continued throughout the winter season. Spring season marked the emergence of adult plants which grew up to 20-50 cm height with a terminal whorl of 4-12 leaves. Adult plant produced flower bud during July and peak flowering was observed in August. Fruiting occurred from late August to September, and seed setting started in mid-September to October.

Panax bipinnatifidus is a non-clonal perennial herb. The stem is erect that arose singularly from a primary storage rhizome root joined to a chain of nodes which are added annually. The species is distinguished into five stages viz., seedling, and four stages based on the number of leaves i.e., 1-4 leaved stages. Seedlings were recruited during spring and they did not develop into higher stages *i.e.*, 2-4 leaved stages within the first year. Seedlings are identified by the growth of one leaf and remained one-leaved during the first year. Shoot withered prior to the onset of winter and persisted only as rhizome which reappeared in the following year as annual shoots. This pattern of death of annual shoots was also characteristic of 2 to 4-leaved stages. Reproductive individuals were all 3-leaved and 4leaved stages. Some 3-leaved individuals produced flowers but failed to fruit, while all 4leaved stage plants did bear fruits every year during the study period. Berries were borne terminally on short or long peduncle. Fruits were not observed to mature synchronously, rather maturity and dehiscence occurred at different times, and usually the central fruits matured earlier. Each individual produced about 10 to 30 seeds during early spring (Late March to April) while the re-emergence of annual shoots of seedlings and mature individuals took place during mid-march. Seedling establishment and shoot growth took place during pre-monsoon and monsoon season. Flowering occurred during early June and fruiting occurred during August. Fruit setting took during late August to September. The plants withered during mid-September to October.

#### Habitat Characteristics of the Study Species

Habitat characteristics of each species such as vegetation type, species association, spatial occurrence, slope, aspect, and altitudinal range were recorded. Vegetation sampling was conducted through belt transects. The detailed information on species association was obtained from 40 1 m x 1 m randomly-placed quadrats at each site. Species association was estimated through chi-square pair-wise association of attributes. Cole's coefficient of association was used to quantify the strength of association. Microenvironment and soil characteristics studied were, light intensity, air temperature and relative humidity, which were measured 1.0 m above the forest floor on a particular day. The measurements were taken at four hourly intervals at ten random points in each site on the day of microclimate measurement.

Swertia chirayita occurred along the buffer zone of KBR from 1,700 m to 3,000 m altitude in forests, shrublands and patchily on the margins of cultivated land adjacent to buffer zone of KBR. It grows typically on sandy to sandy-loam soil texture with a range of pH from basic to weak acid condition. It flourished along the south-western slopes at 20°-40°. Due to its tolerance to drier environment, it also flourished in shrub lands, open ground and along the forest edges in the buffer zone of KBR.

*Paris polyphylla* thrives in cool temperate (2,400-2,800 m a.s.l. elevation) forests in moderate to deep shade condition and is found most commonly in soft humid and humus rich sandy loam and weak acid soil and on moderate slopes of  $10^{\circ}-30^{\circ}$ .

*Panax bipinnatifidus* occurred at an elevational range of 3,300 m to 3,700 m a.s.l. It is a typically shade-preferring plant whose normal growth and development is ensured exclusively under forest shade and is found in  $5^{\circ}-25^{\circ}$  slope. It preferred well drained although sufficiently moist weak-acid soils rich in mould. In KBR, *Panax bipinnatifidus* 

thrived in the *Abies densa* dominated coniferous forests and was found in cool microclimate conditions, and on litter- rich/moss-matted well drained soils. The habitat distribution model successfully predicted the potential habitat distribution of the three study species. This was evident from the high AUC output of 0.91, 0.99 and 0.97 for *Swertia chirayita, Paris polyphylla* and *Panax bipinnatifidus*, respectively. The contribution of vegetation to the overall habitat model prediction (as seen by EVI values) was maximum in all the three species. It was 89.3% in *Swertia chirayita*, 68% in *Paris polyphylla* and highest proportion of 94.9% in *Panax bipinnatifidus* habitat models. Among different EVI layers, maximum contribution was evidently by EVI 145 representing spring season with 37.5%, followed by EVI 289 which corresponds to autumn season. For *Panax bipinnatifidus*, EVI 097 corresponding to early spring had maximum contribution (20.6%) to the predictive distribution pattern. Pre-monsoon vegetation index (EVI 145) with 18% contribution and monsoon data (EVI 209) with 18.4% contribution had equal importance to the model prediction. These were followed by other fewer contributors such as early monsoon EVI 161 (13.6%) and post monsoon EVI 273 (13.2%).

The contribution of physiographic parameters (elevation slope and aspect) as predicted by DEM was much lower in all cases with 10%, 32% and 5.1% in *Swertia chirayita, Paris polyphylla* and *Panax bipinnatifidus*, respectively. Among physiographic variables, slope (7.4%) was a strong determinant for the distribution of *Swertia chirayita* followed by elevation with 2.8% and least by aspect with 0.5%. On the other hand, elevation had high importance in the distribution of *Paris polyphylla* with 27.3%, followed by aspect (3.6%) and slope (1.1%). Elevation also had more importance (4.6%) in *Panax bipinnatifidus*, whereas, slope and aspect contributed only 0.2% and 0.3%, respectively. Overall, the potential habitats of all the three species were confined towards the southern part of KBR. For *Swertia chirayita*, the distribution of potential habitats was more patchy and dense, while for *Paris polyphylla* and *Panax bipinnatifidus*, it showed a restricted range. For all the species, the occurrence of potential habitats in the northern KBR was relatively less.

#### **Population Dynamics**

For studying population dynamics, the populations of the three species were systematically surveyed by walking 50 transects of 50 m x100 m dimension along the accessible trekking path. All the populations encountered were mapped. A population was defined as an individual patch separated by a distance of at least 100 m from the nearest neighbor patch. Patch isolation was measured as the mean distance between the two nearest populations. To estimate the area occupied by the population, the outermost plants of a population were marked on a map, and the area of the convex polygon defined by these plants was determined using Geographical Information System (GIS) software ArcMap 9.3. The sub-populations were categorized according to the habitats of occurrence. *Swertia chirayita* populations occurred in four habitats or group type (henceforth, referred to as group) *viz.*, continuous forest (CF), forest fragments (FF) and shrubland populations (SH). Populations of *Paris polyphylla* and *Panax bipinnatifidus* occurred in two groups *i.e.*, CF and FF.

Population size of the study species was estimated during peak flowering season. Matured individuals were counted in the case of *Swertia chirayita*. For the two perennials *Paris polyphylla* and *Panax bipinnatifidus*, the population size was determined by counting the total number of individuals including all stages in a life cycle. This was chosen because unlike *Swertia chirayita*, all the life cycle stages of these two species grow simultaneously during the growing season. For populations, estimates were based on the total number of plants was counted, while for larger populations, estimates were based on the total number of reproductive individuals per unit area. This was determined through laying 3-10 replicated quadrats of 1 m x 1 m size depending on the population size and area of occupancy. The population size of each patch was extrapolated by multiplying the area of each patch with the number of reproductive individuals per unit area of that population.

The population growth rate was calculated using the formula:  $\lambda = N_{(t+1)}/Nt$ ; where N<sub>t</sub> is the population size at time t and N<sub>(t+1)</sub> is its size after time t. Populations with  $\lambda > 1$  indicates an increase in population size;  $\lambda = 1.0$  shows no growth whereas,  $\lambda < 1.0$  signifies a decline in

growth. To initiate demographic studies, the life cycle of the three species was divided into Jiscrete life-stages based on preliminary survey prior to conducting detailed demographic studies. Demographic studies on the three species were conducted for four years beginning 2005. For *Swertia chirayita*, the complete demographic data pertaining to three generations were studied (2005, 2006 and 2007). While for long-lived *Paris polyphylla* and *Panax bipinnatifidus*, a 4-years demographic census was completed during 2005 and 2008. The study was performed in permanent plots which were established in each population (n= 72 plots for *Swertia chirayita*, n= 44 plots for *Paris polyphylla* and n= plots 28 *Panax bipinnatifidus*). For *Swertia chirayita*, the plot size was 1 m x 1 m and each plot was demarcated by putting boundary structures made of bamboo. For *Paris polyphylla* and *Panax bipinnatifidus*, the plot size was 2 m x 2 m, and the plants were identified, tagged and counted during successive censuses. The populations of the two latter species were very sparse *i.e.*, 0.39  $\pm$ 0.03 plants/m<sup>2</sup> for *Paris polyphylla* and 0.22 plants/m<sup>2</sup> for *Panax bipinnatifidus*. Demographic census therefore, was based on the number of individuals that was recorded in each of the plots.

Micro-environment and soil factors were measured during pre-monsoon, monsoon and post monsoon period. Climatic variables such as light intensity, relative humidity and air temperature were recorded at 1m above ground level at each population of the three species at five hourly intervals during the day of measurement. Each parameter was measured at ten random points adjacent to the demographic plots of all populations. Light intensity was measured by a Digital Luxmeter (TES-1332A), while air temperature and relative humidity were measured by a Thermo hygrometer TH–103 (Mex–therm). The mean values for the microclimatic parameters were used for comparing the variables among the populations of each of the three species.

Soil samples were collected on a seasonal basis for two annual cycles during 2006 and 2007. One composite sample for each population patch was prepared by mixing soil samples collected from the five replicate points in each patch. Soil temperature was measured using a digital portable soil thermometer (Multi–Thermometer). Soil pH was estimated by a digital portable pH meter. Edaphic variables *viz.*, soil moisture content (SMC), water holding capacity (WHC), soil organic carbon (SOC), total Kjeldahl nitrogen (TKN), available phosphorus (P) and exchangeable potassium (K) were estimated in the laboratory.

The metapopulation of Swertia chirayita consisted of 18 populations. These were classified into 4 demographic groups based on their similarity of habitat of occurrence viz., subtropical continuous forest (CS), temperate continuous forest (CFT), sub-tropical forest fragment (FF) and shrubland (SH). Seven populations occurred in CFS, 3 each in CFT and FF, and 5 were located in the SH. The mean patch size of populations was 2.57 ha in CFS, 1.55 ha in CFT, 0.47 ha in FF and 1.32 ha in SH. The total patch areas were 17.95 ha in CFS, 466 ha in CFT, 1.4 ha in FF and 6.6 ha in SH. The mean plant density was 37/m<sup>2</sup>, 14/m<sup>2</sup>, 5/m<sup>2</sup> and 25/m<sup>2</sup> in CFS, CFT, FF and SH, respectively. Average patch isolation of populations in each group was  $0.29 \pm 0.13$  km in CFS,  $0.54 \pm 0.05$  km in CFT,  $0.28 \pm .22$  km in FF and 0.31  $\pm$ 0.16 km in SH. All the three cohorts of the populations exhibited concave survivorship curve. A notable decline in population was observed during the transition from seedling stage to vegetative-rosette stage, and the individuals exhibited higher survival during latter stage *i.e.*, from vegetative-aerial to reproductive stage. Overall, the populations in CFS and CFT had better survivorship than those in FF and SH. However, significant difference was observed only in CFS and FF (survivorship test; log-rank test  $\chi^2$ = 4.24, p= 0.039; Gehan-Breslow-Wilcoxon test = not significant).

For *Paris polyphylla*, the metapopulation consisted of 11 populations of which 7 were in CF and 4 in FF. The mean size of the population patches was 2.85 ha in CF populations and 1.06 ha in FF. The total patch size of the populations in CF was 19.95 ha and that in FF it was 4.24 ha. The average density of plants was  $3/m^2$  in CF while in FF it was only  $1.4/m^2$ . The mean patch isolation was  $0.75 \pm 0.25$  km in CF populations and  $0.44 \pm 0.17$  km in FF populations. The survival of seedlings exhibited an identical pattern in all the populations where the annual mortality was more or less uniform except in two populations *i.e.*, YCF and

ANCF population of CF where all seedlings died in 2007. Similarly, seedling survivorship in FF populations also had similar pattern. Five out of 7 CF populations had seedlings survival .ill 2008 while in FF, 2 out of 4 populations had seedlings that survived till 2008. However, the overall seedling survival till 2008 in CF was 3.9% while in FF it was 5.9%. Survival of adult individuals depicted a sharp decline during the first year, followed by low mortality in subsequent years and for BCF1 population, it was constant till the year 2008. ANCF had no adult survival and so was in FFF population. There was however, no significant difference in the survivorship between CF and FF populations.

Panax bipinnatifidus metapopulation was represented by only 7 populations which included 4 in CF and 3 in FF. The mean patch size of the population was 3.72 ha in CF and 1.2 ha in FF. There was a total of 14.88 ha patch size in CF populations and 3.59 ha in FF populations. The mean plant density was  $017/m^2$  and  $0.06/m^2$  in CF and FF, respectively. The mean patch isolation of CF population was  $0.32 \pm 0.01$  km and that of FF populations it was  $1.26 \pm 0.7$ km. The survivorship curve for seedlings was concave and consistent for all populations. The one-leaved and three-leaved stages had better survivorship in all populations, and there was approximately only a linear increase in mortality during the study period. Two-leaved stage on the other hand, represented a transient stage with fewer individuals, and was encountered in only one population of CF i.e., DCF1 and only 4.7% survived till the year 2008. The occurrence of four-leaved stage was also very rare and found only in DCF2 and KCF1 populations of CF. 100% mortality was observed in KCF1 during the first year of census *i.e.*, 2006 while in DCF2, mortality was observed in the first year, after which, it remained constant till the third year, and declined in the fourth year with 16.7% survival. There was significant difference in the survivorship curves between CF and FF population groups (log-rank test  $\chi^2$ = 3.859, p= 0.04; Gehan-Breslow-Wilcoxon test  $\chi^2$ = 4.277, p= 0.04).

### **Effect of Micro-environment**

Different micro-environmental factors were correlated with different stages of the populations of the three species using multiple regression analyses. For *Swertia chirayita*,

large rosettes density was significantly correlated with three factors *viz.*, soil moisture content, TKN and soil temperature. Vegetative aerial density was significantly correlated with soil moisture content, while reproductive plant density was correlated to soil pH and available phosphorus. On the other hand, the annual growth rate was significantly correlated with soil moisture content and TKN.

In *Paris polyphylla*, seedling density was correlated with pH, TKN, available phosphorus and light intensity while adult plant density was significantly correlated with pH and light intensity. Reproductive plants density was significantly correlated with soil temperature, and annual growth rate of populations with relative humidity.

In *Panax bipinnatifidus*, seedling density was significantly correlated only with soil exchangeable potassium while annual growth rate of population was significantly correlated with soil water holding capacity and pH.

### **Population Viability Analysis**

The viability analysis used two different model scenarios *i.e.*, M1 base model and M2 alternate model to simulate the future of the species based on demography data of the populations of the three study species. The PVA presented the demographically structured models. The individual populations of each of the study species were classified into distinct groups called population groups, based on their habitat of occurrence wherein they share similar micro-environmental conditions and hence had similar demography. The PVA for the three species involved two basic analyses (i) Deterministic analysis which included two important measures *viz.*, finite rate of increase and elasticity analysis and, (ii) Stochastic analysis which included metapopulation occupancy and extinction risk analysis. The threat status of the three species was assessed as per IUCN criteria and management options were explored to suggest conservation measure of the species.

The projection matrix model was used for metapopulation analyses of the three species. Field demographic data was used to estimate the vital rates for each stage. Vital rates considered in this study were survival, and fecundity rates. Stage abundance (column vector of the matrix,  $n_t$ ) was defined as the total population size in each population at each life cycle

stage. This was enumerated by determining density per unit area in each population through sampling in 50 1 m x 1 m quadrats for each life cycle stage. The density so obtained was multiplied by the respective patch sizes of each population to obtain the total stage-specific population size. Vital rates for stage elements were calculated as the average changes over a period of six months for *Swertia chirayita*, and one year for *Paris polyphylla* and *Panax bipinnatifidus*.

Survival rate is the proportion of individuals that survived from a previous stage to the next. Therefore, survival rate for stage i (S<sub>i</sub>) was calculated as:  $S_i = N_j/N_i$  where  $N_i$  and  $N_j$  are the number of individuals at stage i and j, respectively. Fecundity (F) was calculated using the average number of seedling per flowering or reproductive plant in each population. Fecundity was the only entry in the matrix that did not represent a probability, rather, it represented the average number of seedlings a single flowering plant produced in a given year; Fecundity (F) =  $S_{(t+1)}/A_{t_i}$  where,  $A_t$  was the number of adults at time t and  $S_{t+1}$  was the total number of seedlings recruited at time t+1. Stage-based transition matrices were built from field data collected over 4 years period *i.e.*, 2005, 2006, 2007 and 2008.

Projection was carried out by a series of multiplication of the matrix (A) until a stable configuration was reached. The finite rate of increase ( $\lambda$ ) was calculated as: Finite rate of increase ( $\lambda$ ) =  $n_{t+1}/n_t$ . Sensitivity determines how much various life-history stage transitions affect the population dynamics by examining how changes in a particular stage affect the magnitude of the leading eigen value. Sensitivity ( $S_{ij}$ ) =  $w_i/W$  where, wi was the population vector at the i<sup>th</sup> generation and W was the sum of all population vector. Elasticity was a measure of proportional effect, *i.e.*, the effect that a change in a given matrix element had as a proportion to the change in that element; Elasticity (Eij) = ( $aij/\lambda$ ) Sij where, Eij was the elasticity value and represented the proportion of  $\lambda$  due to transition  $a_{ij}$ .

Demographic data was used to construct a stage structured projection matrix model (basemodel) for each group. Two different matrices designated as M1 (base-model) and M2 matrices (alternate model was used to account for biotic interference) were used separately for each population groups. The logic of using the two model matrices was to compare the population performance in two contrasting scenarios *i.e.*, with disturbance in natural conditions (M1) and without disturbance (M2). The stochastic population dynamics was modelled using Monte Carlo based software RAMAS Metapop version 5.0 (Akçakaya, 2005). Parameters included in the Model were vital rates, demographic and environmental stochasticity, and initial population structure/stage abundance. Since no catastrophic event disturbed the populations during the 4 years of study period, changes in population size most likely reflected true environmental and demographic stochasticity. Environmental stochasticity was modelled through introducing random fluctuations in stage-specific fecundities and survivals. Demographic stochasticity was modelled using binomial distribution. In the first set, all simulation was run with 1000 replications until time to extinction was achieved. In the second set, a threshold population size (N<sub>e</sub>) was set for *guantification* of quasi-extinction. A threshold size of Ne= 1000 was set for *Swertia chirayita*. While for *Paris polyphylla* and *Panax bipinnatifidus*, the threshold size was set at Ne= 100 each.

#### Deterministic Analysis

The asymptotic growth rate ( $\lambda$ ) of all the three species fell below 1.0 depicting a general decline in population. For *Swertia chirayita*, the population groups in the descending order of  $\lambda$  was CFS> SH> CFT> FF. For *Paris polyphylla*, it was greater in CF than FF by 32%. The CF populations of *Panax bipinnatifidus* on the other hand, had marginally greater  $\lambda$  (1% higher) than that of FF. The trend of  $\lambda$  in yearly matrix differed among the three species. For *Swertia chirayita*, the overall growth pattern in the base model (M1) was better in the transition matrix of second year for all populations, except for CFT group which although had lower growth rate than other groups, had a relatively steady increase in  $\lambda$  over the three years period. In *Paris polyphylla* too, the  $\lambda$  value in CF increased in the second year matrix and declined in the third year. Nevertheless, both the second and third year matrices yielded a growth in population with  $\lambda$ > 1.0. The FF matrices had gradual decline in  $\lambda$  in the second year matrix. *Panax bipinnatifidus* had

constant  $\lambda$  in CF populations but differed marginally among the yearly matrices of FF and showed a small increase with the years. The M2 yearly matrices had the similar trend with M1 matrices for all the three species and with an observed increase in  $\lambda$  as expected for all groups. This was caused by the reduced vital rates in one of the matrix elements due to increased in the number of survivors in the preceding stages. There was significant variation in  $\lambda$  between the groups of *Swertia chirayita* in both M1 and M2 matrix (F = 75.26, p = 0.00 in M1 and F = 32.78, p = 0.00 in M2).

ANOVA and the confidence interval (CI) of mean  $\lambda$  showed that there was significant difference in  $\lambda$  between the groups of *Swertia chirayita* and it was due to the difference of growth rate in CFS and SH from CFT and FF. For *Paris polyphylla*, significant variation in  $\lambda$ between CF and FF was observed only in M1 matrix model (F= 12.91, p= 0.02) although partial overlapping in confidence interval was seen in M1, and total overlapping in M2 was observed. *Panax bipinnatifidus* showed total overlapping of confidence intervals between  $\lambda$ of CF and FF populations, hence there was no significant variation. Three demographic processes contributed to the finite rate of increase of *Paris polyphylla* and *Panax bipinnatifidus i.e.*, fecundity (F) growth (G) and survival (L). For *Swertia chirayita*, the contribution of growth was more in all the habitat groups both in M1 and M2 matrices since growth elasticities were higher in all groups compared to fecundity. *Paris polyphylla* exhibited a slight variation in the elasticities values. Survival had high elasticity values in all populations, but highest values were noted in FF populations in both M1 and M2 models. While in CF populations, growth had more elasticity in both the demographic scenarios.

#### Stochastic Risk Analyses

The model predicted a continuous decline in the course of time, both in terms of metapopulation size and number of occupied habitat patches for *Swertia chirayita* and *Panax bipinnatifidus*. On the other hand, *Paris polyphylla* metapopulation exhibited a stochastic trend with some growth at least during a few time steps in the future, usually a characteristic feature of a stable population. A very large decline was expected in *Swertia chirayita* and

*Panax bipinnatifidus* during the next few years both in M1 and M2 scenarios. The trajectories for *Swertia chirayita* and *Panax bipinnatifidus* stabilized after the sharp decline, and although in a stochastic simulation the projection tends to lead to an almost deterministic decline in the future in both M1 and M2 scenarios. For *Paris polyphylla* however, the M1 predicted the similar trend in the initial time steps of the simulation but became more undulating as metapopulation size stabilized. M2 model of *Paris polyphylla* typically had an unpredictable trajectory with growth and decline in most time steps.

In *Swertia chirayita*, the persistence of all 18 populations was projected to maintain for the next 4 years. However, the curve depicted a sharp decline in the number of patches, and only 50% of which would remain extant within 12 years and most of which would remain are populations of CFS habitat. The metapopulation occupancy of *Paris polyphylla* also showed similar trend and approximately 50% of the population would be extant by 25<sup>th</sup> year, while in M2 model, the reduction by half the number of populations would not occur before 40 years and some patches may remain till 100 years. In the case of *Panax bipinnatifidus*, the decline in number of patches occurred at regular pace in both M1 and M2 unlike *Swertia chirayita* and *Paris polyphylla*. Model prediction showed that approximately 50% of the population would remain extant till 12<sup>th</sup> and 13<sup>th</sup> year in M1 and M2 scenarios. In *Swertia chirayita*, maximum time that a population would remain extant was 56 time step or 27 years in M1, and 81 time steps or 40 years in M2 (T-FE2 population of CFS habitat) while lowest time a population would persist was 7 years (T-FF of FF habitat).

In *Paris polyphylla*, the average time the population would persist through time was predicted at 30 ( $\pm$ SD 15.6) years in M1 and 45 ( $\pm$ SD 25.5) years in M2 scenarios. The CF populations had 41 ( $\pm$ SD 8.9) years in M1 and 62 ( $\pm$ SD 12) years in M2 models. The FF populations had persistence time of 13 ( $\pm$ SD 2.4) years in M1 and 15 ( $\pm$ SD 2.9) years in M2 models. The sub-population BCF1 of CF had the most persistence time with 52 and 77 years in M1 and M2 models, respectively. While BFF of FF had the least persistence time with 11 and 13 years in M1 and M2 models, respectively.



The average persistence of populations of *Panax bipinnatifidus* on the other hand, was 12 ( $\pm$ SD 25.5) years in M1 and 13 ( $\pm$ SD 3.4) years in M2. Habitat-wise, the persistence of CF populations was 14 ( $\pm$ SD 2.4) years in M1 and 15 ( $\pm$ SD 2.5) years in M2, and that of FF populations was 9 ( $\pm$ SD 1.6) years in M1 and 10 ( $\pm$ SD 1.8) years in M2 models. Among all populations, DCF had highest persistence time of 18 years in M1 and 19 years in M2 scenarios. Whereas, PFF of FF was projected to have 7 and 8 years persistence in M1 and M2 models, respectively.

The probability of extinction of *Swertia chirayita* was different for different groups. The extinction probability curves revealed a more or less identical shape for FF, CFT and SH population groups in both M1 and M2 models. However, the CFS and metapopulation extinction curves were relatively smoother in M2 model than in M1 model. CFT and FF were predicted to have the shortest quasi-extinction (Q<sub>E</sub>) and extinction (E) times. CFS had significantly longer persistence and a relatively extended range of predicted extinction. In M1 model, the quasi-extinction time was 25 years, and in M2 model it was 54 years while times to extinction were 48 and 88 years, respectively. The overall metapopulation had a quasi-extinction time of 25 years in M1 model while in M2 projection it was 46 years. The trend revealed an approximate doubling of persistence time in M2 scenarios in comparison to M1 scenarios in CFS and SH groups and metapopulation. Thus, groups with lower risk *i.e.*, SH and CFS had a greater persistence time range *i.e.*, up to 90 years, whereas, high risk groups *i.e.*, CFT and FF were predicted to be extinct within 20 years. Therefore, across the scenarios and population groups, the species is facing a projected extinction within 100 years.

The risk curves for *Paris polyphylla* exhibited a wide disparity in isolated scenario between FF populations and that of CF where the latter was highly skewed while the former was relatively smooth. However, the risk of CF populations and metapopulation was almost uniform throughout. This was seen both in M1 and M2 scenarios. Additionally, the prediction between M1 and M2 model showed wide differences although the pattern was

similar. The risk in M1 of CF was 0.86 while in the metapopulation scenario it was 0.97. In M2 the CF was predicted to have 0.43 risk probability and 0.42 for the entire metapopulation. The range of extinction was only shown for FF populations as it had probabilities of quasi-extinction and time to extinction well within 100 years. In the isolated scenario it had a time to extinction range from  $Q_E$ = 20 to E= 36 years and in the M2 from  $Q_E$ = 33 to E= 46 years.

*Panax bipinnatifidus* had similar pattern of prediction with skewed curves in the isolated scenarios as well as metapopulation scenarios. Contrastingly, the risk curves in both M1 and M2 model did not show much variation as that of the other two species. In the isolated scenario the FF populations had an extinction risk ranging from Ne= 10 to E= 25 in M1 model, and Ne= 11 to E= 28 in M2 model. CF had  $Q_E$ = 16 to E= 33 in M1, and  $Q_E$ = 22 to E= 39 years in M2. Metapopulation in M1 had extinction risk that ranged from  $Q_E$ = 17 to 38 years and  $Q_E$ = 23 to E= 42 years in M1 and M2 models, respectively.

Threat status of the study species was determined. *Swertia chirayita* and *Paris polyphylla* were vulnerable, while *Panax bipinnatifidus* was endangered. Management options of the two perennial species *i.e.*, *Paris polyphylla* and *Panax bipinnatifidus* was explored and introduction of approximately 10,000 plants for 3 years is required to bring down the risk of *Panax bipinnatifidus* from endangered to vulnerable category. While for *Paris polyphylla*, introduction of approximately 75,000 plants for three years is expected to bring down the species to lower risk category and approximately 2,00,000 individuals of *Paris polyphylla* is required to be introduced to reach its Minimum Viable Population (MVP) size.

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# **NORTH-EASTERN HILL UNIVERSITY**

# DEPARTMENT OF BOTANY



## **DECLARATION**

I, Mr Mark Kordor Lyngdoh, hereby declare that the subject matter of this thesis entitled, "Population ecology of threatened ethno-medicinal plant species in relation to forest fragmentation in Kanchendzonga Biosphere Reserve, Sikkim" is the record of work done by me. I declare that the contents of this thesis did not form basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

The thesis is being submitted to the North-Eastern Hill University for the degree of Doctor of Philosophy in Botany.

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(Mark Kordor Lyngdoh)

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## AND TEAMS - ADDINOX & WALL BURN - ADDINOX

Appendix 4.1 Ethno-medicinal plant species with family and their uses

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# CHAPTER 1 INTRODUCTION

Forest fragmentation is the process by which formerly continuous forest area turns into forest patches of different sizes, isolated from each other by non-forested land (Haila, 1999). Kelt (2000) defined habitat fragmentation as the breaking up of previously continuous habitat as well as a shift in landscape composition from late successional to early successional communities. Fragmentation at landscape level often leads to change in physiognomy and species composition of the constituent plant communities (Dunning et al., 1992). Fragmentation frequently causes habitat loss. The process is initiated by change of one type of habitat into another as a result of which the virgin forests get converted into a mosaic of forest fragments, pasture and degraded habitats. Forest fragmentation affects habitats in three major ways, all of which may have distinctive effects on plant populations: (1) Complete loss of habitats eliminating all the species that occurred in these habitats, (2) reduction in habitat size, and (3) increasing isolation of the remnant fragments. (Andren, 1996; Jacquemyn et al., 2002). Over the years fragmentation is increasingly becoming one of the most destructive manifestations of cultural disturbances. Intensive agriculture and development activities have contributed much to the changes in land use resulting in fragmented landscape with modified ecosystems. These have many biological consequences some of which are short-term and others are long-term (Saunders et al., 1991). Fragmentation is considered as a major threat to biodiversity (Saunders et al., 1991; Collinge, 2000; Simberloff, 2000). Forests all over the world are becoming more and more fragmented resulting in a mosaic landscape where forests are being interspersed with nonforested land (Jacquemyn et al., 2001).

Most impacts of forest fragmentation are related to the increased susceptibility of forest remnants to edge effects. Such impacts are, changes in microclimate, forest structure, biotic composition and ecological function that occur along forest edge exposed to non forested habitats such as agricultural fields, clear cuts etc (Lovejoy *et al.*, 1986; Laurence, 1997). At

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the forest edge, the habitats experience more dry conditions than the interior of the forest patch due to their exposure to higher wind velocity and solar radiation. Forest edges are also more accessible to predators, parasites and grazing by cattle than the forest interior. Forest fragments are also prone to invasions by successional species adapted to recurring disturbances (Janzen, 1983; Laurence, 1997). Marvier et al. (2004) concluded that invasive species are symptoms of habitat destruction, disturbance and fragmentation. Forest fragmentation causes isolation of forested land and divides continuous forests into numerous small patches which are isolated from each other by environment which more often is detrimental to the survival of the species inhabiting the dense forests. As a consequence of the decreased patch area, species richness decreases (Whitcomb et al., 1981, Ambuel and Temple, 1983, Howe, 1984, Lynch and Whigham, 1984, Opdam et al., 1985, Robbins et al., 1989, Piessens et al., 2004, Aparicio et al., 2008). At the scale of individual forest patch, several factors affect its value as habitat for plants and wildlife. In general, larger patches support more species. This is because larger forest patches have more habitat types than the smaller patches and support larger populations that are less vulnerable to extinction. Moreover, only larger patches are likely to contain enough space to support species that require large habitat. Fragmentation reduces the area suitable to such species and their continuous populations are broken down creating isolated subpopulations occupying the remaining forest fragments. Such isolation often disrupts the exchange of individuals and prevents gene flow among the populations (Lacy and Lindenmayer, 1995). In small patches, habitat quality may deteriorate (Oostermeijer et al., 1994) and isolated populations within each habitat have a decreased population fitness which for a number of reasons are expected to face high extinction than large populations because they will be particularly vulnerable to the environmental, genetic and demographic stochasticity (Shaffer, 1981; Menges, 1991). In the absence of external influences, a population's fate is determined by a balance between the collective fecundity and mortality of the constituent individuals, *i.e.*, demographic stochasticity. In a large population with a stable age structure and sex ratio, future population

size is predictable from life table and fecundity schedule. When populations fall to critically low levels, rates of population change become progressively dependent on the fate of the small number of surviving individuals. Imbalances in the age structure and sex ratio of the remaining members of the population and chance variation in their reproductive success will produce unpredictable oscillations in population growth. This stochasticity increases as population size declines, thus increasing the probability that the populations will go extinct purely by chance. It is important to realize that a small population can slide to extinction in this random way, even when all individuals are perfectly healthy and there are no adverse external influences. In other words, an uneven age structure or sex ratio in a population can precipitate its decline to extinction, while a population with a balanced birth and death rates would ensure rapid growth (Spellerberg, 1996).

As a habitat becomes fragmented, the species inhabiting the fragmented habitats are exposed to unpredictable changes in environmental conditions, *i.e.*, environmental stochasticity. Although all populations are subjected to environmental stochasticity, extreme variations caused by forest fragmentation may have a direct effect on the survival of species with specific habitat requirement, such as dense canopy cover. The most important characteristic of environmental stochasticity is that its effects are largely independent of population size. Thus, forest fragmentation results in deterioration of habitats which in turn causes the decrease and isolation of natural populations of plant and animal species.

Since population size has important conservation implications, characterization of a population is fundamental to species conservation. Because of this, biologists have broadened the concept of population biology from local level research and experimentation to a regional perspective. Traditional population ecology has been concerned with the understanding of how populations of plants, animals and other organisms change in time and space, and how these populations interact with their environment. This basic understanding is useful to forecast a population size or distribution and to estimate the chance that a population will increase or decrease. The advancement of technology in recent years has

markedly improved our understanding of population ecology. Metapopulation and extinction risk analyses are being carried out using powerful tools such as metapopulation modelling often integrated with GIS. Thus, population ecology today has evolved as a powerful quantitative discipline with popularization and elevated importance of metapopulation studies of plant and animal species.

Studies in metapopulation dynamics and the application of metapopulation models to species-specific problems in conservation have contributed significantly to the discipline of conservation biology (Brito and Fernandez, 2002; Yuttham *et al.*, 2003) This has inspired many field studies for collecting key data on demography and movement of several plant and animal populations, particularly those facing the risk of continuous decline (Holyoak and Ray, 1999) Metapopulation concept has also prompted renewed research interest concerning dispersal capacities that is extremely valuable in understanding population structure (Yuttham *et al.*, 2003). The term metapopulation was first introduced by Levins (1969). Studies using metapopulation approach generally subdivide the general population into a series of local populations with a balance between extinctions and re-colonization of local populations that facilitates long-term persistence of the metapopulation. The key process is the interpatch connection function by migration (Hanski and Gilpin, 1997). Metapopulation models have been widely used in the biological field including in population ecology, conservation biology, and pest control (Harrison, 1994; Wu, 1994; Hanski and Gilpin, 1997; Takagi, 1999; Fagan *et al.*, 2002).

Extinction and metapopulation theories emphasize that stochastic fluctuations in local populations cause extinction and that local extinctions generate empty habitat patches that are then available for re-colonization. Metapopulation persistence depends on the balance of extinction and colonization in a static environment. For many rare and declining species, Thomas (1994) argued that (i) extinction is usually the deterministic consequence of the local environment becoming unsuitable e.g., through habitat loss or modification, introduction of a predator, etc., (ii) the local environment usually remains unsuitable

following local extinction, so extinctions only rarely generate empty patches of suitable habitat, and (iii) colonization usually follows improvement of the local environment for a particular species (or long-distance transfer by humans). Thus, persistence depends predominantly on whether organisms are able to track the shifting spatial mosaic of suitable environmental conditions or on maintenance of good conditions locally.

Mathematical modelling has become an important tool in population and conservation biology (Cappuccino and Price, 1995; Haefner, 1996; Hanski and Gilpin, 1997; Hilborn and Mangel, 1997; Roughgarden, 1998; Shugart, 1998). Many ecological theories today are represented in mathematical terms because mathematics provide the most precise language to describe complex ecological systems and is also an ideal tool for prediction in ecological systems (Tilman *et al.*, 1994; Jansen, 1995; Gyllenberg and Hanski, 1997; Hanski and Ovaskainen, 2000; Keymer *et al.*, 2000; Casagrandi and Gatto, 2002). However, mathematical formulations have limitation in that they usually force ecologists to make clear and unambiguous assumptions. Metapopulation models are always represented as analytical or simulation models.

There are three types of modelling approaches used in metapopulation studies assuming many habitat patches and local populations (Hanski and Gilpin, 1997): (i) spatially implicit approaches, often based on a critical simplification of what at first appears as a complex problem, in which the habitat patches and local populations are discrete (and are generally assumed to have independent dynamics) but are assumed to be all equally connected to each other, (ii) spatially explicit approaches in which it is assumed that local populations are arranged as cells on a regular grid (lattice), with population sizes modeled as either discrete or continuous variables and where local populations are assumed to interact only with local populations in the nearby cells, and (iii) spatially realistic approaches in which the models allow one to include in the model the specific geometry of particular patch networks, such as how many patches are there in the network, how large they are, and where exactly they are located.

With the recent development of metapopulation theory (Hanski, 1989), it has been recognized that regional-scale processes are also important for long-term survival of species in the landscape (Carroll *et al.*, 2003; du Toit *et al.*, 2004). In such cases, population viability analysis has to be performed at regional scale and should take into account both the present distribution of the species in the landscape and the number, and distribution of patches that are potentially available for re-colonization. Management of threatened populations will be necessary to guarantee long-term survival of threatened species and ecosystems (Beissinger and Westphal, 1998; Reed *et al.*, 2002). Population viability analysis (PVA) is becoming an ever more central tool in conservation biology (Beissinger and McCullough, 2002; Morris and Doak, 2002). In PVA, biological and landscape data are used to parameterize a population model which projects the dynamics, abundance and metapopulation structure of a focal threatened species into the future. From these projections, viability is estimated and compared under different scenarios of landscape or population management to design effective conservation guidelines.

One of the widely used approaches to PVA is the demographic method which is extensively used during the last decade (Menges, 1990; Hiag *et al.*, 1993; McCarthy *et al.*, 1994). While this approach may not be feasible for species which are critically endangered because of their population being scanty in nature, it however has been proved to be one of the most effective of all PVAs if the dynamics of all subpopulations is accounted for. Therefore, demographic study continues to be the most important and fundamental aspect of population biology. Several workers have highlighted the importance of demography in landscape models to study the invasive spread of species (Moody and Mack, 1988; Lavorel *et al.*, 1995; With, 2002, 2004). Other recent works have demonstrated that demographic rates might in fact be more important than dispersal ability of populations to persist in a fragmented landscape (South, 1999; With and King, 1999). The status of populations whether declining, increasing or stable can be established from accurate demographic data (Maschinski *et al.*, 1996). Vital rates which are the main component in demography do not differ much in

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pattern for any given population under natural stochastic environment and these determine the growth or decline of that population. In the presence of external factors however (*e.g.*, anthropogenic interference), these underlying factors may vary within a metapopulation depending upon severity of external influence, and could adversely affect the persistence of a species. Since PVAs are also useful for comparing effects of different factors or management options (Beissinger and Westphal, 1998; Menges, 2000), stochastic simulation can therefore be used to test and compare how different populations or groups would respond under these different scenarios (Gracia, 2003) where threats (*e.g.*, predation, diseases, inbreeding, population reduction) and management regimes are suspected to affect population growth rate (Groenendael and Slim, 1988; Ehrlen, 1995; Nantel *et al.*, 1996; Menges, 1997; Menges and Dolan, 1998; Pfab and Witkowski, 2000; Lennartsson and Oostermeijer, 2001).

Concrete assessment of the threat status of declining plant species based on their population size and distribution is a prerequisite for any conservation action. The parameters such as metapopulation dynamics, rate of decline, environmental stochasticity and external perturbations are important determinants of species perpetuation and need to be quantified in respect of a species for correct threat categorization. Metapopulation studies therefore include a wide range of habitat inventory so that the regional cover of the species is known. Therefore, the first step towards this endeavor is scientific baseline information on the habitat and distribution of these species prior to any in-depth research on their populations. Modelling the habitat and potential distribution of species is therefore an important component of conservation biology. The outputs of which would help detailed population sampling and study. For this purpose, an inventory for acquiring maximum presence localities is a pre-requisite.

Seventeen Biosphere Reserves have been established in India for the purpose of conservation of ecosystems including the natural habitats of plants and animals with a focus on balanced relationship between the biosphere and humans. In the Eastern Himalayan zone, the Kanchendzonga Biosphere Reserve (KBR) is one of the most important BR and is located in the Sikkim Himalaya. It is home to about 140 endemic plant species spread over 41 families (Sharma et al., 2001). KBR is exposed to various kinds of disturbances both natural and anthropogenic, leading to forest fragmentation and disturbance of natural vegetation. From ethno-botanical point of view, the biosphere reserve is home to a number of ethno-medicinal plants which are subjectively considered as threatened. The particular significance of ethnomedicinal plants in conservation stems from the major cultural, livelihood or economic roles that they play in lives of the local people. There is a need to categorize threatened ethnomedicinal species of KBR more carefully using the data on their population abundance and demography, for prioritizing and initiating the conservation action. Therefore, population studies on these species need to be conducted particularly since these species are reportedly under threat from forest fragmentation although their actual status is not known. Some of these threatened species with their widely placed populations and observed inter-population movement of their propagules provided opportunity to apply metapopulation approach to conservation. Beside, an understanding of the effects of forest fragmentation on the population ecology of some of these species and predicting their fate in the biosphere reserve is important for their conservation. Therefore, the present research was aimed to study the population ecology of a few threatened and ethno-medicinal plants in the KBR. The main objectives of the study are as follows:

### **OBJECTIVES**

- 1. To document ethno-medicinal plants of KBR and their uses.
- 2. To identify the habitat types and occupancy pattern of herbaceous threatened and ethnomedicinal plant species of KBR.
- 3. To study the spatial and temporal changes in the populations of selected species in forest fragments and in continuous forests.
- 4. To study the factors affecting the population dynamics of the selected species in the forest fragments.
- 5. To analyse the metapopulation dynamics and to predict extinction probabilities.
# CHAPTER 2 REVIEW OF LITERATURE

Theoretically, the population fitness of species should be significantly affected by the process of forest fragmentation (Nason and Hamrick, 1997). However, empirical evidences to this assumption are still inadequate (Kramer *et al.*, 2007). Progression of population research from local scale to broader regional scale including the dynamics of metapopulation has taken place during the past two decades (Husband and Barrett, 2006). This has led to the more recent simulation approach of risk analysis of several metapopulations (Lindenmayer *et al.*, 1995). Population research on ethno-medicinal plant species however is very rare. Considering their conservation importance, particularly those belonging to threatened category, need of population studies of such species has been highlighted by several workers (USGS, 2005; Liedner and Neel, 2011).

Traditional medicine has been an important component of health care systems in Asian countries and includes Ayurveda, Yunani, Siddha, Homeopathy and folk medicine systems. These systems have been in practice in India since time immemorial. Even today, hundreds of millions of people, mostly in developing countries, derive a significant part of their health care and subsistence needs, and income from medicinal plant and animal products gathered from the wild (Iqbal, 1993; Walter, 2001). Demand for a wide variety of ethno-medicinal species is increasing particularly those with potential for commercial trade. With the increased realization that some of these species are being over-exploited, agencies such as WHO, IUCN and WWF are recommending that wild species be brought into cultivation systems (Lambert *et al.*, 1997). Cultivation can also have conservation impacts, which need to be better understood. Production of ethno-medicinal plants through cultivation, for example, can reduce the extent to which wild populations are harvested. However, it may lead to loss of genetic diversity as well as loss of incentives to conserve wild populations (Anon, 2002).

The Himalayan region is known for its diverse medicinal plants and Sikkim Himalaya is a home to a variety of them. Using wild plants for medicinal purposes are quite common particularly in remote areas. Some of these plant species are over-exploited, thereby threatening their populations in the wild (Rai and Sharma, 1994). Most of these species are used only in folk medicine at the local level. Among the widely used plants, quite a number of them have not been assessed in the context of their population status in the natural habitats. Considerable efforts have been made by various institutions to document the usage and exploitation of ethno-medicinal plant species. However, there is no empirical research on their actual status in the wild.

The history of plant population biology dates back to Nageli's work in 1874, which was the first significant published work on the subject. Thereafter, Tansley (1917), Sukatschew (1928) and Clements et al. (1929) made pioneering studies on different aspects of population biology. Prof. J.L. Harper and his group (Harper and White, 1971; Sarukhan and Harper, 1973; Hawthorn and Cavers, 1976; Harper, 1977; Watkinson and Harper, 1978) gave a new thrust to the population studies in plants. This was a departure from the traditional way of studying the population biology, which was largely based on mere counting and describing the populations. In addition to the natural dynamics, population studies included the dispersal and dormancy of plant propagules, recruitment of seedlings and the effects of neighbouring plants and predators on plant populations. The demographic approach for population analysis of weeds was introduced by Sagar and Mortimer (1976) and a complete review of the subject is found in Cousens et al. (1987). Several population studies were conducted on a range of species. Studies on the population dynamics of several perennial grasses and herbs have been carried out by Williams (1970), Antonovics (1972) Sarukhan and harper (1973), Hawthron and Cavers (1976), Bishop et al. (1978), Tripathi and Dwivedi (1978), Kushwaha et al. (1981), Yadav and Tripathi (1981), Auld and Myerscough (1986) and Kataoka et al. (1989). Klemow and Raynal (1981) and Kelly (1989a and b) studied the population dynamics of biennial species.

In most of these studies, the dynamics of populations were represented by cohort survivorship curves, which were treated as the most important predictor of population growth and mortality risk in the life of the populations. Pattern of survivorship was studied in detail for many weed species by several workers (William, 1970; Rai and Tripathi, 1984; Pandey and Dubey, 1989). Survival of plants was also studied with reference to the influence of a range of climatic, edaphic and biotic variables of the environment (Harper, 1977; Silvertown and Dickie, 1981; Burton and Muller-Dombois, 1984; Fenner, 1985). Of late, population studies of some threatened plants have been undertaken owing to their alarming rate of depletion of their populations to wide-scale ecological disturbance and habitat fragmentation. Kolb and Diekmann (2004) studied the effects of forest fragmentation on life history traits and Kolb (2005) on reduced reproductive success and offspring survival of Phyteuma spicatum. Bruna and Kress (2002), and Bruna and Oli (2005) studied the demographic structure of Amazonian herb Heliconia acuminata as influenced by forest fragmentation. Falk et al. (1996) provided significant insights into the conservation of threatened species and emphasized the need for restoring diversity and developing strategies for reintroduction and conservation of endangered species particularly in the face of rapid forest fragmentation. Similarly Marlin and Whelan (1994) highlighted the need for restoration of dwindling populations of numerous plants and animals species because of human-caused environmental degradation and habitat fragmentation.

The study of species populations at the level of landscape is an increasingly popular research area for conservation of species in patchy or fragmented environment (McCullough, 1996; Marsh and Trenham, 2001). There are different approaches towards undertaking population studies at a regional scale. Several workers such as Johnson *et al.* (1992a and b), Weins *et al.* (1993), Ims (1995), With and Crist (1995, 1996) and Turchin (1998) emphasized the importance of temporal habitat configuration in predicting the population dynamics of a species in fragmented landscape. Gruber and Henle (2004) studied the habitat structure and orientation in an arboreal *Gehyra variegata* and their findings suggested that species

configuration lowers predation risk of the population. Such study suggested that determining the habitats of the species and their distribution is a pre-requisite of metapopulation research. Most studies of metapopulation consider the dynamics of populations divided into a number of sub-populations that may to some extent exchange migrants and that may be subjected to local extinction and recolonizations (Hanski and Simberloff, 1997).

Large-scale assessment of the distribution of species populations prior to 1990 was difficult due to lack of robust methods for summarizing or predicting species' geographic distribution. Although conceptualized much earlier by Grinnell (1917) and Hutchinson (1957), MacArthur (1972) described the role of ecological niche in ecological community and explained it as the quantity that governs the limits of geographic distribution of species. Computer aided algorithm of habitat suitability and ecological niche modelling had since then become an interesting area of ecological research. However, it was Stockwel and Peters (1999) who introduced a robust system of distribution modelling called the Genetic-Algorithm for Rule-set Prediction (GARP) which is widely being used in species distribution studies. Another modelling tool is MAXENT (Maximum Entropy) developed by Phillips et al. (2006) which generates a continuous binomial probability distribution representing habitat suitability of the species. The habitat suitability map can be calculated in a number of different ways, including statistical analyses e.g., logistic regression that find the relationship between the occurrence or density of the species and independent variables that describe its habitat requirements. The relationship can be statistically validated by estimating the function from half of the available data, and predicting the habitat suitability of known locations in the other half (Akçakaya and Atwood, 1997). The habitat suitability map is then used to calculate the spatial structure of the metapopulation. This is done by identifying cluster of cells in a raster map that are suitable *i.e.*, above a threshold value of habitat suitability. This patch recognition is based on species-specific characteristics such as the home range size, dispersal distance and minimum habitat suitability for reproduction. The demographic parameters such as carrying capacity and average vital rates of the population

inhabiting each habitat patch can be determined as functions of patch-specific characteristics, such as the total habitat suitability in the patch (Akçakaya, 1998). This provides a link between the spatial and demographic components of the model, and makes it easier to parameterize models with large number of populations, based on limited data.

Inferential procedures that provide robust and reliable predictions of species geographic and ecological distribution are thus critical to biodiversity analyses. This approach has recently been explored under the domain of "Ecological Niche Modelling" (ENM) (Soberon and Peterson, 2005), and refers to reconstruction of ecological requirements of species that are analogous to the Grinnellian ecological niche (Grinnell, 1917). ENM can provide diverse insights into the ecological and geographic extents of species distribution (Soberon and Peterson, 2004). Predictions from niche-based models of species distribution (Guisan and Zimmermann, 2000) are promising tools in this respect (Cote and Reynolds, 2002; Edwards *et al.*, 2005) as a way to improve the sampling of species of conservation interest. Although population viability analyses have long been used in management of the rare species (Brook *et al.*, 2000), spatially explicit, predictive, habitat distribution models have only recently been used in conservation biology (Vaughan and Ormerod, 2003; Rushton *et al.*, 2004).

The majority of predictive models published in the literature were developed for common plant and animal species. Very few successful applications of this approach have been published for rare and endangered plant species (Miller, 1986; Elith and Burgman, 2002; Engler *et al.*, 2004), although reliable spatial predictions are essential for these species which are of great conservation interest. Paucity of data, spatial inaccuracy, and lack of valid absences are the main reasons identified for this shortcoming (Engler *et al.*, 2004).

Conservation strategies have largely focused on patterns of diversity, specifically how to maximize the number of species that can be protected within a particular geographic region. Species inventory data are fundamental to the development of conservation plans or "portfolios," which represent the full array and diversity of native species, communities and ecosystems within an area (TNC, 2004). Conservation of rare species must be guided by the

biological attributes of the taxon, yet the lack of basic biological data has been implicated in the failure of many recovery plans, especially for plants (Pavlik, 1994; Schemske *et al.*, 1994; Schultz and Gerber, 2002).

By combining reliable locational data with technological and analytical tools, however, we can learn more about species distribution. The development of high-speed computers and GIS software now allows us to model the distribution of a particular species by analyzing the environmental characteristics of its known localities (Guisan and Zimmermann, 2000; Elith and Burgman, 2003; Guisan and Thuiller, 2005). These mathematically defined models can then be combined with known constraints based on the species life history to predict where else on the landscape the species might occur. A variety of environmental data are used as the basis for these mathematical models, some of which have only recently become widely available. These include EVI, NDVI, digital elevation models (and other descriptions of topography such as terrain, slope, and aspect that can be derived from these data); current vegetation cover based on analysis of satellite imagery, and digital data layers providing estimates of precipitation, temperature, and other climatic conditions.

Species distribution models generated in this quantitative fashion are much more detailed than the familiar polygon depictions of species ranges found in field guides. Another benefit is that they control somewhat for the bias that most collectors work near cities or along roads and rivers (Nelson *et al.*, 1990). If one simply examines localities from where a particular plant was collected, you might find that it is restricted to roadsides only, where the collectors had easy access. Species distribution models identify remote natural areas where a species is likely to occur because of shared characteristics with sites where collectors have worked. Through the use of these models, we hope to improve our knowledge of the distribution of plant and animal species endemic to an area. Analyses of these data help pinpoint areas of population patch occupancy for different kinds of organisms as well as identify concentrations of endemic species that occur outside the existing protected areas system (Young, 2007).

Following a known occupancy and distribution of species populations, it become feasible for an in-depth metapopulation research. Theoretical metapopulation research can be traced back to 1970 when Levins coined the term and described a simple model for metapopulation dynamics (Levins, 1970). A history of research in metapopulation biology has been narrated by Hanski and Simberloff (1997) and Hanski (1999). To date, there are over 1000 citations to the key word metapopulation from 1970-2001 (BIOSIS database, Hanski and Gaggiotti, 2004). The core mathematical model in metapopulation theory is the Stochastic Patch Occupancy Models (SPOM). SPOMs assume a network of habitat patches, which have only two possible states, occupied by the focal species or empty. Ecologists working with conservation tend to prefer individual based metapopulation model (Akcakaya and Ferson, 1992; Lacy, 1993, 2000; Akcakaya, 2000) or population based (Sjogren-Gulve and Ray, 1996) or stage-structured (Caswell, 2001; Morris and Doak, 2005; Akcakaya, 1998) simulation models. The advantage of these models is that any process and mechanism that the researcher may wish to add to the model can be added readily. The best use of these models, as perhaps of any population models, for conservation and management is to contrast alternative scenarios that differ in only a small number of factors (Hanski, 1997; Ralls and Taylor, 1997; Beissinger and Wesphal, 1998; Akcakaya and Sjogren-Gulve, 2000). Research on metapopulation primarily stemmed from rapid extinction and colonization processes and several theoretical models have been developed to assess species survival probabilities at the landscape level (Lahaye et al., 1994; Gustafson and Gardner, 1996; With et al., 1997; Hanski and Ovaskainen, 2000; Casagrandi and Gatto, 2002; Dreschler et al., 2003). Generally only two types of metapopulations are possible for a given species: (i) Discrete metapopulation- characteristic feature of a true metapopulation (Freckleton and Watkinson, 2002) and (ii) Patchy metapopulation- there are populations exhibiting a rather more continuous structure which are arbitrarily defined using grid-based as suggested by Thomas and Kunin (1999) or threshold distance criteria as followed by Kolb (2002, 2004) and Jacquemyn et al. (2005) for demarcating species populations. Hence spatial structure is

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an important determinant of metapopulation. Spatial structure refers to the location of individuals, which are grouped into sub- or local populations. Different sub-populations may have different demographic characteristics, such as population size (abundance), carrying capacity, and vital rates. Hanski (1997) outlined four conditions that, if satisfied, suggest that a Levins metapopulation approach will help explain regional persistence of a species: (i) suitable habitat occurs in discrete patches; (ii) all local populations have a substantial risk of extinction; (iii) habitat patches must not be too isolated to prevent recolonizations; and (iv) local populations do not have completely synchronous dynamics. These were offered as general criteria, not necessarily tailored to plant systems. Eriksson (1996) had argued the need to conceptually extend the metapopulation approach to plants due to the difficulties outlined above. Rather than extending the metapopulation studies, classified large scale spatial dynamics in plants to restrict the definition of metapopulation to more closely match Hanski's criteria. They then identified additional categories, distinct from metapopulations, based on the relative dominance of regional versus local processes.

It is therefore seen that most of these models assume that the current distribution of species in habitat patches is the result of equilibrium between the two processes *i.e.*, colonization and extinction (Lahaye *et al.*, 1994). However, the population turnover in disturbed landscape is disrupted when the change in habitat characteristics goes beyond the adaptability of species and the demographic rates get affected. Disturbance is not only a factor that has important effects on the demography of plants, but also one that can often be manipulated to increase population growth rates and population sizes. For example, increased rates of canopy disturbance promoted population growth of the understorey shrub *Lindera benzoin* (Cipollini *et al.*, 1994). Population growth rates for an annual grass *Andropogon brevifolius* were four fold higher in burned than in unburned savanna, owing to higher fecundity in burned areas (Canales *et al.*, 1994). Many plant species do not vary randomly, but instead vary cyclically. The effects of periodic disturbance and of subsequent recovery can be modeled by a combination of information about the disturbance cycle, the frequency and intensity of disturbance, and the demographic response to disturbance. These can be handled by a megamatrix approach. Megamatrices have been used to show that open, post-hurricane environments supported the highest population growth rates for the tropical understorey shrub *Ardisia escallonioides* (Pascarella and Horvitz, 1998) and to model dynamics of the tropical forest gap specialist *Cecropia obtusifolia* in a shifting mosaic of canopy gaps in tropical rainforest (Alvarez-Buylla, 1994). In *Primula vulgaris*, complex matrices were used to examine the impact of increased forest disturbance rates on metapopulation growth rates (Valverde and Silvertown, 1997). Simulations were carried out on varying disturbance frequency and plant demography in relation to the time since the last disturbance, to predict optimal sod-cutting intervals for *Gentiana pneumonanthe* (Oostermeijer *et al.*, 1996) and to predict optimal fire return intervals for *Banksia attenuata* (Enright *et al.*, 1998).

Plant species adapted to particular disturbance regimes that impose frequent population turnover, fit metapopulation approaches. For example, many prairie plants are adapted to frequent small-scale soil disturbance created by gophers (Thomson *et al.*, 1996; Wolfe-Bellin and Moloney, 2000). Similarly, Gawler *et al.* (1988) found that populations of the riverbank endemic *Pedicularis furbishiae* were often destroyed in the short term by ice scouring and flooding, but relied on these disturbances to create suitable habitat for longterm persistence. In the xeric scrublands of central Florida, plant species are adapted to fire, the dominant type of disturbance. While some species persist in the landscape via resprouting, others rely on seed dispersal and recruitment to recover from fire and are most likely to show measurable metapopulation dynamics. These differing responses to fire, combined with microhabitat diversity and differences in between fire competitive abilities interact to structure the heterogeneous plant communities in Florida scrub (Menges and Hawkes, 1998). Besides external influences and disturbances, internal demographic properties like births and deaths interact with both population size and life history in their

effect on population persistence time and extinction risk. Stochastic demographic variability increases the risk of extinction of small populations (Shaffer, 1987). Further, Kokko and Ebenhard (1996) showed that life history characteristics and the demographic effective population size were more important than total population size determining the strength of demographic stochasticity. Variation in life history characters like seed bank and clonal propagation may also affect the risk of population extinction due to environmental stochasticity (Eriksson, 1996). In a theoretical study, Lande (1993) showed that the average life time of a population subjected to environmental stochasticity or random catastrophes is more dependent on its long run population growth rate than on initial population size.

In practice, there are various ways of adding spatial structure to a model to account for the spatial factors. At one extreme as discussed above are simple occupancy models that are based on the number of occupied and unoccupied habitat patches, ignoring their location (Sjögren-Gulve and Hanski, 2000). At the other extreme are individual-based models that describe the spatial structure with the location of each individual in the population, or the location of territories or home ranges. Stage-structured population models which is employed in the present research, fundamentally used the projection matrix model which is the matrix form of the basic model ( $N_{t+1} = N_t + B - D + I - E$ ). Leslie (1945) developed the age-structure model form of the matrix which Lefkovitch (1987) described the stage-structured form. Recent analytical methods allow the estimation of age-based parameters from stage-based matrices (Cochrane and Ellner, 1992), which is likely to lead to more plant studies integrating stage and age approaches (Morris and Doak, 1998).

Many structured models concentrate on a single sex *i.e.*, usually only the females are modeled. In species where one male can mate with several females, the number of males may not affect the total fecundity very much, and only females should be modeled. For other species, it is possible to develop models that include both sexes, but this requires additional information about reproduction. In particular, the contribution of males to fecundity (which is likely to be frequency-dependent) must be known. In models of monogamous species with

only one breeding stage, fecundity can be expressed simply as number of offspring per breeding pair, multiplied by the minimum of the number of males and the number of females in the breeding stage. If more than one age class is reproductive, or if breeding is not monogamous, the frequency dependence will be more complicated.

Simulations of stage-based populations can also be used to estimate age-related parameters, such as life span (Damman and Cain, 1998). A comprehensive study and description however was provided by Caswell (2001). Caswell (2001) gives a comprehensive account of the mathematical development of the topic, which includes stochastic extensions (demographic and environmental variation), and asymptotic analyses of growth rates and age and stage class distributions for deterministic and stochastic matrix models. Matrix population models are used to assess the viability of structured populations (Morris and Doak, 2002). Repeated iterations of the matrix model result in the projection of a population's equilibrium growth rate and extinction risk, providing a measure of the overall performance of populations. Moreover, sensitivity and elasticity analyses of matrix models can identify the life-history stages most critical for the persistence of a species. The results of matrix model analysis and simulation are often used to assess the vulnerability of a population to extinction and to evaluate different management options (Freckleton *et al.*, 2003; Beverly and Martell, 2004; Garcia, 2004; van Mantgem *et al.*, 2004).

Between these are spatially explicit metapopulation models that describe the dynamics of each population with structured demographic models, and incorporate spatial dynamics by modelling dispersal and temporal correlation among populations. Both dispersal and correlation between each pair of populations depend on the location of the populations, making these models spatially explicit. One type of spatially explicit metapopulation model is based on a regular grid, each cell of which is modeled as a subpopulation of a metapopulation (Price and Gilpin, 1996). Another approach expands spatially explicit metapopulation models by incorporating information about habitat relationships of the species and the characteristics of the landscape in which the metapopulation exists

(Akçakaya and Atwood, 1997). This method uses a habitat suitability map to determine the spatial structure of the metapopulation (number and location of habitat patches in which subpopulations of the metapopulation live) and population-specific parameters. Many plant population studies are presented as sets of deterministic models developed from data for specific years (Shaffer, 1981; Bierzychudek, 1982; Kalisz and McPeek, 1992). In contrast, very few have calculated a stochastic population growth rate (Caswell, 1989; Nakaoka, 1996). Efforts to introduce stochasticity into these models have often taken the form of choosing a sequence of matrices obtained for different years according to a random sampling scheme or a scheme designed to address theoretical questions (Bierzychudek, 1982; Kalisz and McPeek, 1993). More commonly, environmental stochasticity or various types of disturbance or catastrophe have been considered in Population Viability Analyses (PVAs). Of the 21 studies using stochastic modelling to predict extinction, 19 reported extinction probabilities; others reported times to extinction or both.

The time periods used for projecting extinction risk vary from 25 to 1000 years (many authors used several different periods), with 50, 100 and 200 years being the most frequently used time periods; thus, comparison of extinction risk is difficult. A few studies did present risk analyses with full distribution of times to extinction. These full-time distributions are recommended because they are less misleading than single results (Beissinger and Westphal, 1998). As far as deterministic models are concerned, many other parameters have been calculated, with elasticities (Benton and Grant, 1999) as the most common. However, because elasticities within species vary across space and time (Horvitz and Schemkse, 1995; Oostermeijer *et al.*, 1996; Silvertown *et al.*, 1996) interpretations of elasticities need to be made with caution. In particular, elasticities of declining populations differ from those of increasing populations. Stochastic modelling and other complex approaches have been less commonly used in plant PVAs. Demographic stochasticity (Damman and Cain, 1998; Menges, 1998) is not considered as great a threat to population viability as systematic factors (such as continuing habitat loss) or other stochastic factors.

Metapopulation viability is also drastically and negatively affected by the strength of the environmental stochasticity; temporal variation in population growth rate may lead several local populations and even the whole metapopulation to extinction. Doak et al. (2002) showed that, in the absence of good information on seed demographic rates, model predictions based on assumptions about a persistent seed bank can vary widely depending on the amount of variation in vital rates for the reproductive phases of the plant life cycle. This points to the need for realistic assessment of environmental variation and its impact at all life history stages. Extinction of populations is of prime evolutionary and conservation interest and stochasticity is a decisive factor in the survival or extinction of populations (Goel and Richter-Dyn, 1974; Goodman, 1987a, b). Not surprisingly, theoretical research on stochastic extinction of populations and the application of stochastic models to population viability analysis (PVA) have become very popular (Leigh, 1981; Soulé, 1987; Lande and Orzack, 1988; Hanski and Gilpin, 1991; Mace and Lande, 1991; Burgman et al., 1993; Wissel et al., 1994; Settele et al., 1996; Drechsler et al., 1998; Amler et al., 1999). The popularity of the PVA approach in conservation biology is also reflected in the availability of several reviews (Goel and Richter-Dyn, 1974; Akçakaya and Ferson, 1990; Boyce, 1992; Lacy, 1993; Lindenmayer et al., 1995; Oostermeijer et al., 1996; Reich and Grimm, 1996; Groom and Pascual, 1997; Beissinger and Westphal, 1998).

Alternatively, measured among-year variation in vital rates can be examined statistically to generate means, variances and correlation structures that can be incorporated into the modelling procedure (Morris and Doak, 2002). This allows vital rates to vary randomly within the constraints of defined statistical distributions, resulting in greater realism than that obtained by selecting entire matrices randomly from an array of matrices. Another approach for incorporating stochastic variation into a population model is to use documented long-term variation in environmental variables as drivers of among-year variation in demographic rates. This approach starts with regression of measured vital rates for a series of years on measured value for environmental driver variables for those years. These equations are then

used to parameterize the model, so that the variance structure of the driver variables controls the variance structure of corresponding vital rates. This results in a correlation structure among vital rates that is mediated by their dependence upon common driver variables. This approach has the important advantage of incorporating realistic levels of environmental variation into the model and can be helpful in extrapolating demographic data collected over relatively short time spans (Fieberg and Ellner, 2001). It may be most appropriate in environments where abiotic factors such as precipitation are the main determinants of demographic performance.

Metapopulation approaches might be particularly relevant to understand persistence in plants because many plant species have patchy distribution and occur on specialized, identifiable sites that can be censused for occupancy. Some additional patchiness is created by disturbances and by disturbance-specialized or disturbance avoiding species. The dispersal of many species is limited so that suitable patches can remain unoccupied. The concepts of minimum viable metapopulation and minimum available suitable habitats (Hanski *et al.*, 1996) are likely to be applicable to many plant species.

Data on species presence or absence in suitable habitat patches have been used to infer metapopulation dynamics in 80 species of Florida scrub plants. For 25 species, with occupancy related to patch size, isolation or fire regime, an incidence-based metapopulation model was used to infer colonization and extinction rates (Quintana-Ascencio and Menges, 1996). This work suggests that, as patch size decreases, herbs are more sensitive than woody plants to increased extinction risks. Natural variability in population dynamics is compounded by uncertainty in the population parameters due to lack of perfect information. Therefore, development of models in population studies have become an integral part for better and clear understanding of the population behaviour. Besides, the predictive capacities of the models help in species conservation. The consequent difficulty of making precise predictions has shaped the language of PVA (Shaffer, 1990). The conservation-related problems and questions that PVA addresses are usually phrased in terms of probabilities. For

example, we may want to assess the probability of extinction or the chance of recovery from a population bottleneck. The earliest plant PVA was calculated for age-structured data derived from a *Pinus sylvestris* forest (Usher, 1969). The classic study of *Ranunculus repens* brought matrix methods to the attention of plant ecologists (Sarukhán and Gadgil, 1974). Some other notable plant PVAs are those of Werner and Caswell (1977), Bierzychudek (1982), Fiedler (1987), van Groenendael and Slim (1988), Menges (1990), Burgman and Lamont (1992), Cochrane and Ellner (1992), Kalisz and McPeek (1992), Alvarez-Buylla (1994), Bullock *et al.* (1994), Erikkson (1994), Ehrlén (1995), Nantel *et al.* (1996), Valverde and Silvertown (1997), Bradstock *et al.* (1998), Damman and Cain (1998), Enright *et al.* (1998), Gross *et al.* (1998), Menges and Dolan (1998), Pascarella and Horvitz (1998) and Oostermeijer (1999).

Most plant PVAs have been carried out based on short duration data. PVAs have been performed on a single species and have considered only a few populations. The mean, median and modal length of a PVA is about four years (Fiedler, 1998). Most studies also consider very few populations (mean 3.4, median 2.0 and mode 1.0), but because populations within species vary widely in demographic parameters, studies based on only a few populations would seem incomplete. Demographic variation over time (environmental stochasticity) is only weakly correlated among populations (Horvitz and Schemkse, 1995; Crone and Gehring, 1998), which suggests that multiple populations need to be followed for several years. Some of the earliest PVAs used a single parameter which is time to extinction (Brigham and Schwartz, 2003).

PVAs can be used to define, given an assumption of the maximum risk to be tolerated (*e.g.*, less than 5% risk of extinction in 100 years), a Minimum Viable Population (MVP) that will forestall extinction. Deterministic and stochastic analyses, which incorporated harvesting pressure on wild ginseng (*Panax quinquefolium*) and wild leek (*Allium tricoccum*) in Quebec, Canada, were used to formulate MVPs (Nantel *et al.*, 1996). While there are a large number of both short-term and long-term studies focusing on almost all aspects of plant

population biology in the temperate zone (Tamm, 1972; Whigham, 1984; Calvo, 1990; Leeson *et al.*, 1991; Primack and Stacey, 1998; Brzosko and Wroblewska, 2003; Wotavova' *et al.*, 2004), the plant demography of the much more species-rich tropical zone has been little studied. Such demographic studies are essential for a better understanding of the relationship between these plant communities.

Population viability models were used for *Banksia cuneata* considering fire frequency effects, environmental stochasticity and potential inbreeding (Burgman and Lamont, 1992). The fire frequency that maximizes population size does not minimize extinction, because it exposes vulnerable seedlings to the risk of catastrophic mortality during droughts. Minimizing extinction and maximizing population size do not always require the same conditions.

The above review of literature clearly reveals that plant population analysis following metapopulation approach has not been undertaken in India for any of its species. This provided an important basis and impetus for the present research which aims to undertake an extensive population research of KBR landscape for selected threatened and ethno-medicinal plant species. Viability analysis of species population was also undertaken to demonstrate a method that can fill-up the much needed data for successful conservation of several threatened species in the country.

# CHAPTER 3 STUDY SITES

### Location

The study area is situated in Kanchendzonga Biosphere Reserve (KBR) of Sikkim (27°06'-28°05'N, 88°02'-88°47'E) in the Eastern Himalayan state of Sikkim in north-eastern India (Plate 3.1).

Kanchendzonga landscape was notified by the Ministry of Environment and Forests, Government of India as a Biosphere Reserve (BR) vide notification No.J-22016/76/91-BR on 7<sup>th</sup> February, 2000. KBR encompasses a total area of 2,619.92 km<sup>2</sup> with 1,784 km<sup>2</sup> comprising of the core zone and 835.92 km<sup>2</sup> of the buffer zone. The entire BR is a part of Eastern Himalayas, often called as Sikkim Himalayas being in the state of Sikkim. The BR covers two districts, *viz.*, North and West Sikkim districts.

The BR lies along the Sikkim-Nepal border and occupies about 40% of the state's geographical area. It falls in the elevation range of 1,220 m to >8,000 m a.s.l. Yambung-Singalila range forms the transboundary corridor with Nepal in the West. In the North, KBR is bound by Lungnak La (5,537 m a.s.l) ridge and the Teesta river forms the eastern boundary. In the south, KBR boundary touches various reserved forests of the South and West Forest Division of Sikkim. It also touches a short stretch of International boundary with the Tibet Autonomous Region (TAR) of China in the North West of the State (Plate 3.1).



Plate 3.1. Map of Sikkim showing the location of Kanchendzonga Biosphere Reserve (KBR) in India

#### Climate

The altitudinal and topographical variation of the BR resulted in a wide variety of climatic conditions. The climatic conditions vary from subtropical in the southern part of the BR to cold desert and permafrost areas in the northern and western parts bordering Nepal. The rainfall pattern is also influenced greatly by the elevation. Sikkim is the most humid place in the whole of the Himalayan range because of its proximity to the Bay of Bengal and direct exposure to the moisture laden southwest monsoon. Three seasons are distinguishable in a year *viz.*, winter (October-March), summer (March-May) and monsoon (June-September) seasons.

Continuous climatic data for the BR are not available because the two closest meteorological stations had data for limited periods and parameters. The Geyzing meteorological station (1,533 m a.s.l) is close to the BR (7-10 km distance) from the western direction while Chungthang meteorological station (1,606 m a.s.l) is close to the BR (1-5 km distance) from northern direction. The total annual rainfall recorded at Geyzing (lower montane) during September, 2004-August, 2006 was 7,861.5 mm, 70 % of which was received during April-September and a maximum of 2,051.6 mm rainfall was received during the month of August (Figure 3.1). The maximum average daily temperature of 24.8 °C was recorded during April and the minimum 5.7 °C was recorded in January at Geyzing. The rainfall data for the four rainy months in each year during 2006-2009 at Chungthang (montane) revealed that the maximum rainfall occurred during the months of July and August (Figure 3.1). The continuous data for the upper montane forests were not available.



Figure 3.1: Climatic data for Geyzing (lower montane) (a) and Chungthang (montane) (b) recorded during the study period at the nearest two meteorological stations from Kanchendzonga Biosphere Reserve

#### Geology, Geomorphology and Drainage

The state of Sikkim falls in the upper part of the Teesta basin. The BR landscape owes much to the drainage network of the river Teesta. The structural slope of the land is from North to South. Hence all the rivers and the streams in the BR have southern flow. The north-western part of the BR reaches an elevation of >8,000 m a.s.l and therefore remains under snow cover almost throughout the year. The resultant topography is that of the typical glaciated one, characterized by cirques, aretes, glacial trough, and morainic deposits. Besides, there are numerous glaciers such as Zemu and Talung glacier in the North, and Rathong glacier in the West, which get frozen during the winter. Geologically, the BR constitutes hard massive gneissose rocks capable of resisting denudation. The main ridges *viz.*, the Singalila and the Chola ridges within the BR run in a north-south direction. Another north-south ridge runs

through the central portion of the BR separating the Rangit from the Teesta valley. The Rangit and the Teesta which form the main channels of drainage, run nearly north-south. Teesta originates from a glacial lake Chho Lhamo located at the north-eastern corner of the BR.

### **General Soil Characteristics**

The soils of BR were in general acidic in reaction due to heavy rainfall and leaching of bases from surface soil to low horizons. They were excessively drained and sandy-loam in texture. According to Harmonized World Soil database, the soil of KBR consists of three main dominant soil types *i.e.*, Cambisols, Leptosols and Glaciers.

# Land Use

Forest is the dominant land use in KBR. The analysis of imagery supervised classification of Indian Institute of Remote Sensing (IIRS), LISS III imageries of February, 2002 pertaining to the year 2002 revealed that more than 43.4% of the total geographical area of the BR was under forest cover or scrub (Plate 3.2). The forest cover/and scrub of the BR is 1,115.4 km<sup>2</sup>, followed by barren land (23.1%), glaciers (12%), meadow (9.3%) and snow cover (9.8%). As such, forestry is the major land use in Sikkim and nearly 84% of the total geographical area of the state is under the administrative control of the forest department. The forest cover of the state is 3,129 km<sup>2</sup>, which is 42% of the total geographical area, followed by barren land 25.4%, pasture and grazing land 17.0%, and the net sown area is 8.9%.



Plate 3.2: Land use of Kanchendzonga Biosphere Reserve as analyzed from the imagery of 2002

#### **Biodiversity**

The Eastern Himalayan region is a biodiversity hotspot of the Indian subcontinent that harbours more than 3,000 endemic species (McGinley, 2008). The great varieties of environmental conditions found in Himalayas have resulted in diverse ecosystems types, which are rich in species and genetic diversity. Therefore, the Eastern Himalayan region is one of the richest zones of biological diversity in the world. Takhtajan (1969) had considered this region as the "*Cradle of flowering Plants*". *The flora of Sikkim* was first described by Sir J.D. Hooker in 1872-97 in the form of seven voluminous books entitled "*The flora of British India*". Besides Sir J.D. Hooker, G. King and his colleagues explored the flora of Sikkim latter (King and Pantling, 1898).

A part of Kanchendzonga BR falls within the biogeographic province of Trans-Himalaya-Tibetan plateau biogeographic zone with biota of Palaearctic affinity (Rodgers *et al.*, 2002). The rests of the BR is a part of Indo-Malayan Biogeographic region. The BR has the richest biodiversity in the Himalayan region (Sharma *et al.*, 2001). The topography, elevational variation, high peaks, glacial lakes, and forest wilderness in BR has enriched the KBR's biodiversity. Singh and Chauhan (1997, 1998) reported the presence of 16 species of gymnosperms belonging to 12 genera under seven families from BR. Maity (2004) reported 11 species under 9 genera belonging to five families. Maity (2004) also reported the presence of 1,463 species of angiosperms belonging to 138 families. However, the taxa of higher elevations still remain to be explored.

BR is also equally gifted with high faunal diversity. Ali (1960) reported as many as 430 bird species. Chettri (2000) compiled a list of rare and endangered birds under different schedules of Wildlife Protection Act, 1972. Some of these birds are *Lophophorus impejanus* (state bird), *Budo nepalensis, Ithagenus cruentus* etc. About 150 species of mammals belonging to 28 families have been recorded from BR. Some of the important ones are *Uncia uncia, Canis lupus, Pseudois nayaur* and *Ailurus fulgens*.

# **Forest Types**

Because of wide elevational variation, the BR has diverse forest types ranging from lower montane subtropical to Alpine scrubs. Among the forest types described by Lepcha (1998), broad-leaved dense forests occupy the maximum area of 478.2 km<sup>2</sup> in the BR (Table 3.1).

Table 3.1: Area (kn	<sup>2</sup> ) statistics of Kanchendzong	a Biosphere Reserve
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Forest type	Area
Mixed dense forest	478.24
Mixed open forest	180.84
Mixed degraded	172.32
Dense conifer forest	135.81
Open conifer forest	228.12
Degraded conifer forest	140.83
Oak-Rhododendron forest	62.81
Scrubs	28.59
Forest blanks	84.28
Alpine scrubs	244.79
Alpine pastures	20.62
Alpine barren	216.78
Snow	480.93
Glaciers	152.68
Lakes	4.28
River/major streams	13.42
Dry river bed	7.98
Totai	2,653.32

# **Location of Tracks**

The study sites were selected for detailed study along three tracks in west and north districts of Sikkim during 2005 (Plate 3.3). These sites were located across an altitudinal gradient representing subtropical, temperate and sub-alpine regions of the BR (Table 3.2). The sites selected covered both buffer zone and core zone of KBR. The study sites were selected along three tracks: (i) Topung-Gomathang track (West Sikkim district) on which Site-I (Ngom-Phedi), Site-II (Topung), Site-IV (Dungdang-Yambung) and Site-V (Gomathang) were located, (ii) Yuksum-Kibek track (West Sikkim district) on which Site-III (Tsokha-Kibek) was located. The different component of the study that was conducted in these sites is given in Table 3.3. A brief description of these sites is given below:



Plate 3.3: The map of Sikkim showing the location of Kanchendzonga Biosphere Reserve in India and the location of study sites

# 1. Topung-Gomathang track (West Sikkim district)

*Site-I (Ngom-Phedi)*: It is located about 20 km from Nambu, the nearest village that is approachable by road. It is situated between 27°22' and 27°23'N latitude and 88°05' and 88°07'E longitude having an altitudinal range of 2,200-3,600 m representing temperate forest type.

*Site-II (Topung):* It is located about 2 km from Nambu village (27°19'N latitude and 88°09'E longitude), which has an altitudinal range of 1,560-2,500 m representing sub-tropical forests and temperate forests.

*Site-IV (Dungdang-Yambung):* The site is about 12 km form Ngom. It is located in the subalpine region between 27°22' and 27°24'N latitude and 88°4'-88°6'E longitude with altitude ranging between 3,400 and 4,000 m.

*Site-V (Gomathang):* Gomathang is located between 27°26' and 27°27'N latitude and 88°05' and 88°06'E longitude. It is situated in the sub-alpine region in the core zone of KBR with an altitudinal range of 3,700-3,950 m. The area consists of forest remnants fragmented by wind-throws that occur every winter.

## 2. Yuksum-Gomathang track (West Sikkim district)

*Site-III (Tsokha-Kibek):* The site is located between 27°25' and 27°6'N latitude, and 88°10' and 88°11'E longitude with an altitude ranging from 2,500 to 3,500 m.

# 3. Lachen-Kalep track (North Sikkim district)

*Site-VI (Kalep):* It is situated between 27°45' and 27°46'N latitude and on 88°32'E longitude in the northern part of KBR in North Sikkim district. The area consists of highly fragmented landscape with steep terrain. Frequent landslide causes forest fragments at this site.

Table 3.2: Topographical features of six study sites in three	forest types of Kanchene	Izonga Biosphere Reserve
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Site characteristics	Lower mont	áne	Montane		Upper mont	ane
Sites	Topung	Yuksum	Ngom	Bakhim	Dungdang	Kibek
Coordinates	27° 19' N, 88° 09' E	27° 23' N, 88° 12' E	27° 26' N, 88° 10' E	27° 25' N, 88° 11' E	27° 23' N, 88° 04' E	27° 26'N 88°10' E
Aspects	North	North	South	South	South	South
Slope (degree)	20–45	10–25	10 - 30	15-30	10 - 40	15-35
Elevation range (m)	1200-1910	1350-1900	1930-2560	1900–2500	2680-3000	2900-3100

Table 3.3: Studies conducted in six different sites

Site	Habitat types and occupancy	Plant population study		
	pattern	Continuous	Forest	
1999 - 1999 -		forest	fragment	
Site-I (Ngom-Phedi)	$\checkmark$	✓	✓	
Site-II (Topung)	$\checkmark$	✓	✓	
Site-III (Tsokha-Kibek)	$\checkmark$	✓	✓	
Site-IV Site IV (Dungdang-Yambung)	$\checkmark$	✓	✓	
Site-V (Gomathang)	✓	-	-	
Site-VI (Kalep)	$\checkmark$	-	-	

The three forest types are, lower montane, montane, and upper montane. As per the classification of Champion and Seth (1968), the forest at an elevation of 1,500 m a.s.l. is classified as East-Himalayan subtropical wet-hill forest ( $8BC_1$ ) under the group subtropical broad leaved hill forests. The forest at an elevation of 2000 m a.s.l. is classified as East-Himalayan moist temperate mixed coniferous forest (12C3a) under the group Himalayan moist temperate forest. The forest at an elevational range of 3,000-3,050 m a.s.l is classified as East-Himalayan subalpine-birch/fir forest ( $14C_2$ ) under the group subalpine forest. These three montane forests are referred to as subtropical, temperate and subalpine forests, respectively based on their group name.

The following three forest types representing six sites were selected for detailed plant diversity study: (i) Topung-Yuksum was the site at mid-elevation within the buffer zone representing lower montane forest. The dominant tree species were *Alnus nepalensis* D. Don, *Castanopsis tribuloides* DC., *Engelhardtia spicata* Bl., *Ficus semicordata* Sm., and *Lyonia ovalifolia* Drude. (ii) Ngom-Bakhim was the site at higher elevation within the buffer zone representing montane forest. The dominant tree species in the broadleaved temperate forest were *Acer campbellii* Hiern, *A. nepalensis*, *Betula alnoides* D. Don, *Lithocarpus pachyphylla* Rehder, *Magnolia campbellii* Hk. f. & Thom., and *Rhododendron arboreum* Sm., and (iii) Kibek-Dungdang was the site at highest elevation representing upper montane forest and falls in the core zone. The dominant tree species were *Abies densa* Griffith ex Parker, *Buddleia colvilei* Thom., *Rhododendron* spp., and *Tsuga dumosa* Eichler.

### Soil Characteristics of the Study Sites

The soil types in three forest types of KBR were of mainly Leptosols and Glaciers. The textural class of soil was sandy to loamy sandy in the lower montane forest; sandy soil in montane and sandy loam to loamy sandy in the upper montane forest in BR. Soil pH ranged from 5 to 5.2 in the lower montane forest, 4.2-4.9 in montane and 4.2-4.3 in the upper montane forest. Average soil organic carbon and total nitrogen were higher in the montane forest, while in the upper montane forest, soil available phosphorus, exchangeable potassium, and moisture content were highest. Overall soil temperature was maximum in the lower montane forest in comparison to montane and upper montane forests (Table 3.4).

 Table 3.4: Soil characteristics of the three forest types in Kanchendzonga Biosphere Reserve (after Chettri et al., 2010)

Parameters	Lower montane	Montane	Upper montane
Soil temperature (°C)	18.56	13.10	7.43
Soil organic carbon (%)	4.29	4.76	4.21
Soil exchangeable potassium( $\mu g g^{-1}$ )	16.79	19.41	22.30
Soil moisture content (%)	35.90	44.61	59.27
Soil TKN (%)	0.33	0.67	0.60
Soil pH	5.18	4.58	4.34
Soil available phosphorus (µg g <sup>-1</sup> )	15.56	26.77	42.71

## **Decadal Land Use Changes during 1999-2008**

Land use/land cover maps for 1999, 2002 and 2008 of KBR differentiated various land use/cover types. Across the years, major portion of land use was dominated by dense forests (Figure 3.2, Table 3.6 and Plate 3.4). Areas under dense forests, lakes (water bodies), and snow cover did not show any remarkable changes during the study period. Barren lands, devoid of any vegetation cover and characterized by stony rocky-lands also did not differ much in 2002 but decrease by 132.6 km<sup>2</sup> in 2008. The open forest areas also decreased by 92.6 km<sup>2</sup> in 2008. However, other land-use such as snow area, glacier bed and water bodies and meadows had increased cover in 2008 (Table 3.5).



Figure 3.2: Temporal changes in land use and land cover in KBR during 1999-2008

Table 3.5: Land	use changes	in Kanchendzonga	<b>Biosphere</b> Rese	rve during 1999–2008
I HOIV DIOT DUITO	use enunges	minuteneueneu	Diosphere reese	100 during 1999 2000

		Area (km²)		Net chang	e (km²)
Land use classes	1999	2002	2008	1999-2002	2002-2008
Barren land	600.6	608.4	475.8	7.8	-132.6
Snow	254.8	254.8	228.8	0.0	26.0
Meadow	309.4	299	345.8	-10.4	46.8
Open forests	241.8	234	137.8	7.8	96.2
Dense forests	873.6	894.4	886.6	-20.8	7.8
Glacier beds	312.0	304.2	486.2	-7.8	182.0
Water bodies	2.6	2.6	36.4	-0.0	33.8



Plate 3.4. FCC depicting the land use/landcover status of the Kanchendzonga Biosphere Reserve during 1999 (1), 2002 (2) and 2008 (3)



Plate 3.4: FCC depicting the land use/landcover status of the Kanchendzonga Biosphere Reserve during 1999 (1), 2002 (2) and 2008 (3)

### **Forest Fragmentation Dynamics during 1999-2008**

Forest fragmentation pattern during the ten years of study revealed that there was an increase in number of smaller fragments (Table 3.6, Plate 3.5 and 3.6). The number of fragments in 1999 was 875, which reduced to 533 in 2002. However, in 2008, it increased to 615. The number of forest fragments <1 ha during 1999 was 515 (112 ha), which decreased to 295 (86.3 ha) in 2002 but again increased in 2008 to 341 (108.5 ha) fragments. Forest fragments in 1-50 ha size class were also more in 1999 with 350 fragments covering an area of 2,420.9 ha; while in 2002, it was 231 fragments, covering an area of 1,668.7 ha. It increased to 267 fragments with 1,764 ha of area by 2008. Forest fragments larger than 50 ha size class were only 10 (1,368.6 ha) in 1999, which decreased to seven fragments each in 2002 and 2008, having an area of 688.9 ha and 517.5 ha, respectively (Table 3.6). During 1999, 51.3% of the forest area was in smaller fragments between 0-1 ha; 47.5% in medium size fragments and 1.1% in large fragments. By 2008, the area under smaller patches decreased to 40.8%, while, the area under medium size fragments increased to 58% and larger fragments remained the same. During the whole study period, the average annual fragmentation rate was 0.7 ha year<sup>-1</sup>, equivalent to 0.007%. The mean fragment size decreased from 4.4 ha in 1999 to 3.9 ha in 2008 (Table 3.6). This decline in mean patch size was associated with decrease in patch density and a substantial reduction in the size of the large forest fragments during the study period.

Fragment size (ha)	at No. of fragments(Area in ha)			Mean (ha)	fragme	nt size	Fragm of frag	ent dens ments/1	sity (No. 00 ha)
	1999	2002	2008	1999	2002	2008	1999	2002	2008
<1	515 (112)	295 (86.3)	341 (108.5)	0.2	0.4	0.4	0.173	0.088	0.097
1-50	350 (2420.9)	231 (1668.7)	267 (1764)	5.8	5.5	5.0	0.160	0.114	0.137
>50	10 (1368.6)	7 (688.9)	7 (517.5)	136.9	98.4	73.9	0.004	0.003	0.003
Total	875 (3901.6)	533 (2443.9)	615 (2390)	4.4	4.5	3.9	0.337	0.205	0.237

Table 3.6: Fragment dynamics in Kanchendzonga Biosphere Reserve during 1999-2008



Plate 3.5: Pattern of forest fragmentation in Kanchendzonga Biosphere Reserve



Plate 3.6: Distribution of forest fragments in three size classes in Kanchendzonga Biosphere Reserve during 1999, 2002 and 2008

#### **Intensity and Causes of Forest Fragmentation**

Forest fragments were categorized into three fragment size classes *i.e.*, <1, 1-50 and >50 ha. These fragments were created due to anthropogenic (agriculture, grazing, NTFPs cultivation/extraction, timber/poles, trekking routes, settlement, tourism, road) and natural (wind-throw, landslide, snow avalanche, wild fire) disturbances. The mean number of causative factors per fragment reduced from 3 during 1999, to 2 during 2008 (Figure 3.3). In causes general, anthropogenic of disturbances such as agriculture, NTFPs cultivation/extraction, and agriculture decreased by 87% in 2008. But disturbances from the natural causes remained same in KBR (Figure 3.4).



Figure 3.3: Mean number of causative factors per fragment during 1999, 2002 and 2008 in KBR

The percentage of fragments affected due to of trekking route, grazing, and tourism were high (Figure 3.4). Occurrences of medium and high intensity of disturbances were also common during the study period (Figure 3.5). Fragments created due to high intensity disturbance like wind-throw, landslide, snow avalanche and wildfire though were less in number, the relative frequency of occurrence of such disturbances were greater and often devastating.



Figure 3.4: Percentage of fragments affected by various causative factors



Figure 3.5: Relative frequency of occurrence of various causative factors of disturbance and their intensity in 25 fragments of KBR during 1999-2008
# CHAPTER 4 ETHNO-MEDICINAL PLANT SPECIES OF KBR AND CHARACTERISTICS OF STUDY SPECIES

#### INTRODUCTION

About 70–80% of people worldwide rely on medicinal plants to meet their primary healthcare needs (Farnsworth and Soejarto, 1991; Pei, 2001). The global demand for herbal medicine is not only large, but is also growing rapidly (Srivastava, 2000). The market for Ayurvedic medicine is estimated to be expanding at 20% per annum in India (Subrat, 2002). Factors contributing to the growing in demand of traditional medicines include the increasing human population and the inability of allopathic medicine system to reach a large portion of population in developing countries.

There are approximately 17,500 flowering plant species in India, of which at least 6,500 are used in traditional medicinal system (Ved *et al.*, 2003). Revival of the traditional medicine system in India and abroad has put additional pressure on the medicinal plant resources, particularly those growing in the wild. Habitat degradation, unscientific harvesting and over-exploitation of medicinal plants have led to the extinction of more than 150 plant species in the wild (Anon, 1997; Katariya, 1998). At least 90% of the plant species used in the herbal industry of India today is extracted from the wild, and majority of which come from the sub-alpine and alpine ecoregions of the Himalayas. Some of the species, which are in great demand from various pharmaceutical companies include, *Picrorhiza kurrooa, Podophyllum hexandrum, Nardostachys grandiflora, Dactylorhiza hatagirea, Aconitum heterophyllum,* and *Saussurea costus*. Most of these species have been listed in Appendix I and II of CITES (Uniyal *et al.,* 2002). Excessive anthropogenic pressure is the main cause of decline in the population and availability of these medicinal plants in the Himalayan region (Kala, 2000; Dhar, 2000).

A total number of 1,748 medicinal plant species have been listed. This includes 339 trees, 338 shrubs, 1,020 herbs, and 51 pteridophytes (Samant *et al.*, 1998). Among these, several

species were categorized as endangered as per current IUCN criteria under the Biodiversity Conservation Prioritization Project (BCPP) through Conservation Assessment and Management Plan (CAMP) exercises (Samant *et al.*, 1998). Some of the endangered Himalayan medicinal plant species are: *Aconitum balfourii*, *A. deinorrhizum*, *Acorus calamus*, *Angelica glauca*, *Atropa belladonna*, *Berberis kashmiriana*, *Coptis teeta*, *Dioscorea deltoidea*, *Gentiana kurrooa*, *Nardostachys grandiflora*, *Picrorhiza kurrooa*, *Podophyllum hexandrum*, *Saussurea costus*, *Swertia chirayita* and *Taxus baccata subsp. wallichiana*, and the tropical/sub-tropical species *Aquilaria malaccensis* (Batugal *et al.*, 2004).

The flora of Kanchendzonga Biosphere Reserve (KBR) in Sikkim is very diverse in terms of family, genera and species. So far, 1,225 species of angiosperms under 490 genera and 120 families, and 10 species of gymnosperms members under 9 genera and 5 families, and about 57 species of pteridophytes under 37 genera and 22 families have been described (Maity and Chauhan, 2002). Among these plants, approximately 6% are of ethno-medicinal importance (Maity, 2004). These plant species are being used as a source of medicine by the local people inhabiting KBR, often as an alternative to conventional allopathic medicine to treat different ailments. Due to its long history, traditional medicine has in fact become an integral part of the culture. It is not unusual for people living around the forest to treat common ailments using plants around them. Today, continued destruction of forest and reluctant habitat degradation due to various natural and anthropogenic causes has brought about the depletion in population rise of several medicinal plants. With the depletion of the species, its use in the traditional medicine system is also reducing. This might lead to vanishing of the associated traditional knowledge as well. Plants of global medicinal importance such as Aconitum heterophyllum, Nardostachys grandiflora, Panax bipinnatifidus, Swertia chirayita and Paris polyphylla have also importance in the local traditional medicine system in Sikkim. These species are also among the threatened species in the state.

The loss of associated traditional knowledge with different medicinal plants has been aggravated further by modernization. In general, the present generation underestimates the traditional values of the species, and does not give much attention to their conservation. Listing of currently known ethno-medicinal plant species is therefore a requirement from health, cultural and scientific perspectives. This documentation in fact, provided the basic database, from where the study species were selected for detailed habitat and population studies. A few earlier works did record a number of medicinal plants in Sikkim (Rai and Sharma, 1994), and also a compilation of ethno-medicinal plants traditionally used by the local people of Sikkim is also available (Maity, 2004). The present study undertook primary inventory of ethno-medicinal uses of several medicinal plants available in North and West Sikkim districts of KBR and added to our existing knowledge of ethno-botany in Sikkim.

## **METHODS**

### **Documentation of Ethno-Medicinal Plant Species**

The ethno-medicinal plants were inventoried through a questionnaire survey among the traditional healers of 36 villages located within and around KBR. Forty traditional healers were selected for filling the questionnaire through an intensive interaction. Prior Informed Consent (PIC) was taken before interviewing the traditional healers. The plants so documented were classified into three categories *viz.*, sub-tropical, temperate and alpine, depending on the elevation range in which they occur. The available literature was also consulted on medicinal uses of the species reported by the traditional healers. A list of threatened ethno-medicinal plant species was compiled from three main sources *viz.*, (i) Red Data Book of Indian Plants (Nayar and Sastry, 1987, 1988 and 1990), (ii) Conservation Assessment and Management Prioritization (CAMP) report, 2003 and (iii) Medicinal Plants of Indian Himalaya (Samant *et al.*, 1998).

### **Characteristics of Study Species**

#### Taxonomic Description

Three herbaceous species were selected from the documented ethno-medicinal plants for detailed population studies in relation to forest fragmentation. These were: *Swertia chirayita* 

Roxb. ex Flem. (Gentianaceae), *Paris polyphylla* Smith (Melanthiaceae) and *Panax bipinnatifidus* Seem (Araliaceae). These species were identified for the present study because they are forest inhabited species, and are widely used as ethno-medicinal plants. *Swertia chirayita* is mostly found in the sub-tropical forest. *Paris polyphylla* is found in temperate forest and *Panax bipinnatifidus* is found in sub-alpine forest. The specimens were collected and herbarium sheets were prepared following Jain and Rao (1977). The identification of the species was confirmed using the local and regional flora (Polunin and Stainton, 1984, Stainton, 1988; Kanjilal and Bor, 1997).

### Life History and Phenophasic Characteristics

Twenty five matured individuals of each of the three species were randomly selected at three sites. Different morphological parameters were measured during the peak flowering and fruiting season. The parameters are: individuals per clump, plant height, leaf number of leaves and flowers per plant, flower length and diameter, and number of fruits and seeds produced per plant. Phenological cycles from emergence period to death of the species were closely observed over a period of one annual cycle from January 2005 to December 2005. The uses of these medicinal plants were documented based on the above mentioned questionnaire survey. The chemical composition and active principles were recorded from the available literature and data bases (Devkota, 2005; Vuksan and Sievenpiper, 2005; UDWDP, 2007; Alessandra de Sousa Braz, 2009), since all these three species are well-studied from pharmaceutical aspects.

## RESULTS

#### **Ethno-Medicinal Plant Diversity of KBR**

A total of 105 ethno-medicinal plant species were recorded from KBR. A complete list of species along with their uses is given in Appendix 4.1. A maximum number of 62 species were recorded from the sub-tropical forests which are located at lower altitude of 1,500-1,700 m elevation, and 16 species were recorded from the forests of temperate region at 2,700-3,200 m elevation. Six specie *viz.*, *Betula utilis*, *Cardiocrinum giganteum*,

Dactylorhiza hatagirea, Gaultheria fragrabtissima, Hippophae salicifolia and Arisaema tortuosum were common in the sub-tropical and temperate forests, and six species viz., Ephedra gerardiana, Fritillaria cirrhosa, Juniperus recurva, Nardostachys grandiflora, Picrorhiza kurrooa and Rhododendron anthopogon were found in the alpine meadows. Five species viz., Ephedra gerardiana, Fritillaria cirrhosa, Juniperus recurva, Nardostachys grandiflora, Saussurea gossypiphora and Rhododendron anthopogon were common in alpine meadow and alpine scrub. Four species viz., Podophyllum hexandrum, Smilacina oleracea, Paris polyphylla and Valeriana hardwickii were found to occur both in temperate and sub-alpine forests. Only two species viz., Aconitum ferox and A. heterophyllum were common in the sub-alpine scrub and sub-alpine forest. Two species viz., Panax bipinnatifidus and Megacodon stylophorus occurred only in sub-alpine forest and another two species viz., Valeriana hardwickii and Holboellia latifolia had a broad range of occurrence from 1,500-3,500 m elevation i.e., sub-tropical to sub-alpine forests (Figure 4.1).



Figure 4.1: Distribution of 105 ethno-medicinal plants in different altitudinal range of KBR

A total of 55 (52%) ethno-medicinal plant species had herbaceous life-form. 21 species (20%) were trees, 16 lianas (15%), 11 shrubs (11%) and only 2 were epiphytes (2%) (Figure 4.2).



Figure 4.2: Percentage distribution of life-forms of 105 ethno-medicinal plant species of KBR

The recorded 105 ethno-medicinal plant species were spread over 64 families. About 67% of the families were represented by only one species (Figure 4.3). Araliaceae, Scrophulariaceae, Convovulaceae and Polygonaceae were represented by four species each and contributed 6% to the total species. On the other hand, 18% species belonged to Valerianaceae, Solanaceae, Saxifragaceae, Rubiaceae, Rosaceae, Ranunculaceae, Poaceae, Orchidaceae, Liliaceae, Gentianaceae, Asteraceae and Apiaceae families which were represented by three species each. Araceae and Fabaceae had each two species, and Ericaceae and Zingeberaceae had maximum representation with five species each.



Figure 4.3: Family distribution of 105 ethno-medicinal plant species of KBR

### **Medicinal Uses**

A variety of ailments were reported to be treated by different ethno-medicinal plant species. There were 52 different ailments being treated by 105 ethno-medicinal plant species. However, a maximum of 46 plant species were used for a single treatment only. 29 species were used for the treatment of two different ailments each. 18 species were used for three different ailments each, while 8 species for four different ailments each. On the other hand, there were only 3 species that were used for a more than four ailments which indicated their broad range of medicinal value. These are *Valeriana jatamansii*, *Costus speciosus* and *Centella asiatica* each was five, six and eight different ailments, respectively (Figure 4.4).



Figure 4.4: Number of species and their medicinal value

Some of the common ailments such as cough, fever, headache and other gastrointestinal disorders were being treated by a number of species (Figure 4.5). 27% of ailments were being treated by only one species, 23% by two species, 15% by three species, 12% by four species, 6% by five species, and 4% by six and seven species each. Another 10% of the common ailments were being treated by more than eight species.



Figure 4.5: Number of ethno-medicinal plant species and percentage of ailments treated

### **Threat Status**

Out of the 105 ethno-medicinal plant species recorded from KBR, 30 species (28.3%) were under various threat categories of the IUCN (CAMP Workshop, 2003) (Table 4.1). 75 species (71%) were not yet evaluated under any of the threat categories. Of the assessed ethno-medicinal plant species, three (3%) were critically endangered (CR), seven (7%) were endangered (EN), 19 were vulnerable (18%) and one species (1%) *i.e.*, *Abies densa* was near threatened (NT) (Figure 4.6).

Table 4.1: Ethno-medicinal plant species recorded from KBR under different threaten categories of the IUCN

	Hamily 27	Calegoria	
Abies densa Griffith ex R. Parker	Pinaceae	NT	CAMP, 2003
Achyranthes aspera L.	Rosaceae	NE	
Aconitum ferox Wall.	Ranunculaceae	EN	Walter and Gillet, 1998
Aconitum heterophyllum Wall. ex Royle	Ranunculaceae	EN	Walter and Gillet, 1998
Acorus calamus L.	Zingiberaceae	VU	CAMP, 1998
Aloe barbadensis Mill	Aloeaceae	NE	
Angelica archangelica L.	Apiaceae	NE	
Arisaema erubescens (Wall.) Schott	Araceae	NE	
Arisaema nepenthoides (Wall.) Mart. ex Schott	Araceae	NE	
Arisaema tortuosum (Wall.) Schott	Araceae	NE	
Aristolochia griffithii Duch.	Aristolochiaceae	VU	Walter and Gillet, 1998
Artemisia vulgaris L.	Asteraceae	NE	
Asparagus recemosus Willd.	Asparagaceae	NE	
Astilbe rivularis BuchHam ex D. Don	Saxifragaceae	NE	
Bauhinia purpurea L.	Fabaceae	NE	
Bauhinia vahlii (Wt. & Arn.) Benth.	Fabaceae	NE	
Bauhinia variegate L.	Fabaceae	NE	
Benthamidia capitata H. Hara	Cornaceae	NE	
Bergenia ciliata (Haw.) Stenb.	Saxifragaceae	VU	CAMP, 2003
Bergenia purpurascens (Hook. f. & Thoms.) Engl.	Saxifragaceae	NE	
Betula utilis D. Don	Betulaceae	NE	
Boenninghausenia albiflora (Hook.) Reichb.	Rutaceae	NE	
Campylandra aurantiaca Baker	Liliaceae	NE	
Cardiocrinum giganteum (Wall.) Makino	Liliaceae	NE	
Centella asiaticaL. (Urban)	Apiaceae	NE	
Cinnamomum impressinervium Meissn.	Lauraceae	NE	
Cissampelos pareira L.	Menispermaceae	VU	Chhetri et. al., 2005
Clematis buchananiana DC.	Ranunculaceae	VU	Chhetri et. al., 2005
Costus speciosus (Koen.) Sm.	Zingiberaceae	NE	
Curcuma zedoaria (Chirstm.) Rosc.	Zingiberaceae	CR	Chhetri et. al., 2005
Cuscuta reflexa Roxb.	Convolvulaceae	NE	
Cynodon dactylon (L.) Pers.	Poaceae	NE	
Dactylorhiza hatagirea Soo	Orchidaceae	CR	Chhetri et. al., 2005
Daphne papyracea Wall. ex Steud.	Thymeleaceae	NE	
Datura suaveolens Humb. & Bonpl. ex Willd.	Solanaceae	NE	
Dendrobrium nobile Lindl.	Orchidaceae	VU	CAMP, 2003
Dichroa febrifuga Lour.	Hydrangaceae	NE	
Digitalis purpurea L.	Scrophulariaceae	NE	
Docynia indica Decne.	Rosaceae	NE	
Drymaria cordata (L.) Willd. ex Roem. & Schult	Caryophyllaceae	NE	
Entada phaseoloides (L.) Merr.,	Fabaceae	NE	
Ephedra gerardiana Wall.	Ephedraceae	ÊN	Walter and Gillet, 1998
Euodia fraxinifolia Hk.	Rutaceae	NE	
Eupatorium cannabinum L	Asteraceae	NE	

Fraxinus florubunda Wall.	Oleaceae	NE	CAN (D. 2002
Calina cirrnosa D. Don	Lillaceae	EN NE	CAMP, 2003
Galium spp.	Rublaceae	NE	
Gautheria fragrantissima wall.	Goraniagana	NE	
Geranium nepaiense Sw. Constanthus numilus (D. Don) Eng. & Krou	Araceae	NE	
Conaianinus pumitus (D. Don) Eng. & Krau.	Floourtingene	EN	Chhatri at al 2005
Hedera venalensis Koch	Araliaceae	NF	Childen et. al., 2005
Hedrehum spicatum Rosc	Zingiberaceae	VU	Chhetri et al 2005
Heracleum wallichii DC	Aniaceae	NE	Childen et. al., 2005
Hinnonhae salicifolia D. Don	Elaeagnaceae	NE	
Holboellia latifolia Wallich	Lardizabalaceae	NE	
Imperata cylindrica (L.) Beauv.	Poaceae	NE	
Juniperus recurva BuchHam.	Cupressaceae	NE	
Kaempfera rotunda L.	Zingiberaceae	NE	
Lobelia zeylanica L.	Campanulaceae	NE	
Lycopodium clavatum L.	Lycopodiaceae	NE	
Lygodium flexuosum (L.) Sw.	Lygodiaceae	NE	
Lyonia ovalifolia Drude	Ericaceae	NE	
Mahonia napaulensis DC.	Berberidaceae	VU	CAMP, 2003
Mallotus philippinensis Muell.	Euphorbiaceae	NE	-
Megacodon stylophorus (C. B. Clarke) Sm.	Gentianaceae	NE	
Myrica esculenta Ham. ex Don	Myricaceae	NE	
Nardostachys grandiflora (D. Don) DC.	Valerianaceae	CR	Walter and Gillet, 1998
Oroxylum indicum (L.) Vent.	Bignoniaceae	VU	CAMP, 2003
Osbeckia nepalensis Hk.	Melastomaceae	NE	
Oxalis corniculata L.	Oxalidaceae	NE	
Paederia foetida L.	Rubiaceae	NE	
Panax bipinnatifidus Seem.	Araliaceae	NE	
Paris polyphylla Sm.	Trillidaceae	NE	
Phytolacca acinosa Roxb.	Phytolacaceae	NE	
Picrorhiza kurroa Royle ex Benth.	Scrophulariaceae	VU	CAMP, 2003
Pieris formosa D. Don	Ericaceae	NE	
Piper longum L.	Piperaceae	VU	Chhetri et. al., 2005
Plantago erosa Wall.	Plantaginaceae	NE	
Podophyllum hexandrum Royle.	Podophyllaceae	EN	Walter and Gillet, 1998
Polygala arillata BuchHam ex D.Don	Polygalaceae	NE	
Prinsepia utilis Royle	Rosaceae	NE	
Pteris biaurita L.	Pteridaceae	NE	
Rheum emodi Meisn.	Polygonaceae	NE	
Rheum nobile Hk. & Thom.	Polygonaceae	EN	Walter and Gillet, 1998
Rhododendron anthopogon D.Don	Ericaceae	VU	Walter and Gillet, 1998
Rhododendron arboreum Sm.	Ericaceae	VU	Chhetri et. al., 2005
Rhus semialata Murr.	Anacardiaceae	VU	Chhetri et. al., 2005
Rubia cordifolia L.	Rubiaceae	NE	
Saussurea gossypiphora D.Don	Asteraceae	NE	
Schima wallichii Korth.	Theaceae	NE	
Smilacina oleracea (Baker) Hk. f.	Convallariaceae	NE	
Smilax lanceaefolia Roxb.	Smilacaceae	NE	
Solanum nigrum L.	Solanaceae	NE	
Solanum viarum Dunal	Solanaceae	NE	
Spiranthes sinensis (Pers.) Ames	Orchidaceae	NE	
Swertia chirayita (Roxb. ex Fleming) Karsten	Gentianaceae	VU	CAMP, 2003
Swertia nervosa (G. Don) C.B. Clarke	Gentianaceae	NE	••••
Taxus wallichiana Zucc.	Taxaceae	VU	Walter and Gillet, 1998
Ihysanolaena maxima (Roxb.) Kuntze	Poaceae	NE	
Urtica dioica L.	Urticaceae	NE	<b>0.1 0 1 1 1</b>
Valeriana hardwickii Wall.	Valerianaceae	VU	CAMP 2003
Valeriana iatamansi DC	Valerianaceae	VU	CAMP 2003
	• •	× · · ·	
Viscum articulatum Burn.f.	Loranthaceae	VU	Chhetri et. al., 2005



Figure 4.6: Threat status of the 30 ethno-medicinal plant species in KBR

### **Description of Three Study Species**

### 1. Swertia chirayita Roxb. ex Flem.

*Taxonomy:* The genus *Swertia* belongs to family Gentianaceae and comprises of 100 species (Airy Shaw, 1977). *Swertia chirayita* is distributed in sub-tropical to temperate Himalayas in Nepal and India. In India, it is distributed from Kashmir to Bhutan and in Khasi hills of Meghalaya (Chanda, 1976). The plant is 30-150 cm tall. Stem is robust, branched, and cylindrical below 4, angled upwards and contains large pith. Leaves are broadly lanceolate, 5-nerved, and sub-sessile, varying in length from 8-9 cm. Flowers are small, stalked, numerous and axillary; lurid greenish yellow colour, and tinted with purple colour in large panicles. The stems and leaves are reddish in colour when mature. Fruits are small, capsulate with a yellowish colour, and capsules are egg-shaped; seeds are numerous and minute, smooth, and many angled. Dehiscence usually occurs as a narrow opening at the tip of the capsule, and seed dispersal takes place once the plant gets matured in late November or December.

Life history and phenophasic events: The life history of Swertia chirayita is similar to that of most biennial species in the genus Swertia e.g., S. punicea (Kondo et al, 1990). It is a herbaceous plant with a rosette form during the first year and an erect form with pleiochasium inflorescence during the second year. The species was distinguished into four demographic stages viz., small rosette, large rosette, vegetative aerials and flowering.

- (i) Small rosettes: Seedling rosettes were individuals that arose singularly from the soil surface during the spring season.
- (ii) Large rosettes: These are matured individuals in rosette state that survived through monsoon season. These usually consisted of 6 to 12 large rosette leaves.
- (iii) Vegetative aerials: Plants that develop into an erect vegetative stem with a few or no rosette leaves but with varied number of aboveground branching. This stage was obtained during the second year from perennating rhizomes of the first year rosettes.
- (iv) Flowering individuals: Reproductive individuals represented the ultimate stage of the species prior to senescence and were formed during the monsoon and post monsoon period.

The species was essentially in its rosette form during the first year (small and large rosettes). The large rosette survived through the winter as dormant root stock which reappeared during spring in the second year. Root stock is tap root system bearing a single erect stem which in rare cases under stress and disturbance can bear one or two small lateral shoots (Plate 4.1). Seedlings emerged by formation of two cotyledonary leaves, and its subsequent growth into four rosette leaves was the primary sign of establishment in a habitat. The emergence of seedlings (small rosette) occurred during spring with the onset of rain from early May to late June. Establishment of large rosettes began with the development of large 6-8 rosette leaves and this stage prolonged and continued till the end of November at the end of which the individuals were usually 10-14 leaved. Dormancy was initiated prior to the onset of winter season and continued till the break of spring in the second year of the plant life cycle. The growth of perennating rhizomes in the second year was the beginning of an adult stage and occurred much earlier than the emergence of small rosettes. This occurred during April to late May and rapidly developed into aerial individuals following profuse growth till July. An adult plant thus formed may be unbranched, or with two or more pairs of opposite branches. Mid-August sets off the development of flowering individuals, and peak flowering was accomplished during September to mid-October, and during this time fruiting began and

continued till late November. Fruits were formed both on branches and on stems attached by a short stalk. Seed setting typically occurred during mid-November to early-December (Table 4.2).

*Chemical constituents and active principles:* The main active principle of *Swertia chirayita* is *'Chiretin'*. Other active molecules include iridoids, xanthones, mangiferin and C-glucoflavones (Jensen *et al.*, 2002). The bitter principles are the main constituents of the plant. They are included in the Secoirodoid glucoside group. They are Amarogentin and Amaroswerin. The species has been recently used by the beverage industry as an alternative bitter product that is used in the liquor industries to impart bitter flavour to taste. *Swertia* extract contains Oleanolic acid and Swertiamarin which are used as hair growth tonic (Suzuki *et al.*, 1989). Chiraito is also used as one of the ingredients in "*Chandra Prabati*" which is an Ayurvedic drug for cancer.

*Ethno-medicinal use: Swertia chirayita* occupies one of the major positions in the trade of medicinal and aromatic plants. Around nine species of *Swertia* were reported to be in trade in different countries. Among them *Swertia chirayita* was considered superior in quality among Ayurvedic medicinal plants and occupied one of the major positions in the trade of medicinal and aromatic plants in Nepal and India. The herb is one of the most widely distributed medicinal plants with its characteristic bitter taste. The dried plant material is the source of raw drug and is commonly used for the purpose of stomach ache. The species is valued for its bitter principles which can be used as hepatic stimulant, blood purifier, tonic (NRCMAP, 2008).

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Plate 4.1: Swertia chirayita: An emerging large rosette (A); tap root system with an occasional lateral shoot (B); whole plant (C); a 2-year old plant with profuse branching (D)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
A	e n l	e n l	e n l	e n l	e n l	e n l	e m l	e m l	e n l	e n l	e m l	e n l
N	A											
	R											
A												
L												
	L											
Y	Ð											
C	F											
L	G											
E												

**Table 4.2:** Phenology of *Swertia chirayita* (e = Early; m = Mid; l = Late)

A= Emergence and establishment of small rosettes; B= Establishment of large rosettes; C= Dormancy period; D= Emergence of vegetative aerials; E= Development of reproductive individuals (flowering); F= Fruiting; G= Seed set

#### 2. Paris polyphylla Smith

*Taxonomy: Paris polyphylla* belonged to family Trilliaceae and with recent updated, it was placed in Melantiaceae. *Paris polyphylla* is distributed along the eastern Himalaya range including Nepal, Bhutan some parts of China and in most north-eastern states of India. It is found along the dense temperate forest ranging from 2,500-3,600 m elevation.

*Paris polyphylla* is a perennial plant with a thickened to cylindrical rhizome. Stem is green or purple in colour, glabrous and solitary, unbranched, 12–33 cm long. Leaves are 3-12 lanceaolate petiolate sub-sessile; leaf blades obovate, glabrous. Outer tepals typically 3, sometimes 4 or 2, green, ovate, glabrous, shortly ciliate at margin; inner tepals usually absent or if present is yellow-green in colour, filiform, erect, equal to outer ones; stamens usually 6 or 8; filaments purple, 2–3 mm; anthers 3–5 mm; ovary green, sub-globose; style ca. 1 mm; stigmatic lobes 2, 3, or 4, purple, 21–34 mm; Capsule green at maturity, globose, 0.8–1 cm diameter; seeds enveloped by red, succulent aril. Rhizome is very short about 3-6 cm, slender or thickened. Flower is solitary and borne terminally. Fruit is a berry or a berry-like capsule, bearing few to many seeds. Seeds consist of a mesophyll outer layer coat, an inner hardy coat, a large endosperm, and a small undeveloped embryo located at the tip. Seeds are released from mature capsules during autumn in the month of September.

Life history and phenophasic events: Paris polyphylla is a non-clonal polycarpic perennial plant with a short, thick non-clonal rhizome bearing several irregular constrictions which is formed annually (Plate 4.2). Seedling emergence took place during spring about a month after the emergence of stem from perennating rhizome. Germinated seeds occurred in the form of relatively massive growth of radicle which formed the rhizome system and followed by plumule and stem elongation. Growth of seedling was slow and therefore, they did not reproduce in the first year. Upper shoots withered early before the onset of winter and the plant survived as dormant rhizome during winter. Older plants flowered early during June-July and set fruits during August to September or early October. The plant produced a single berry which contained 20-50 seeds.

The life history of *Paris polyphylla* was distinguished into three demographic stages *viz.*, seedling, adult and reproductive individuals.

- (i) Seedlings: These were individuals that developed from germinated seeds. Seedlings consisted of 2-4 leaves with a short stem of 4 to 10 cm length.
- (ii) *Adult*: These were formed after at least one year from seedling stage. The stage was characterised by the size of their leaves and height of stem.
- (iii) *Reproductive*: Reproductive individuals were similar in size with that of adult stage but differed in the development of reproductive structure at the apex of the leaf whorl.

The growth of seedlings occurred during April-May followed by growth and establishment period which continued till the end of rainy season (September). However, growth of seedlings was very slow and in the first year, seedlings grew up to a maximum of 10 cm with 2-4 leaves, much of the growth therefore, took place in the rhizome component. The period of dormancy for young seedling recruits and also for plants at higher altitude started early during mid-September, while that of adult plants was much later during late October. However, dormancy in both stages continued till the start of spring in the next year. Sprouting of vegetative shoots from perennating rhizome occurred about the same time as in case of fresh seedling recruits. These exhibited pronounced growth with the onset of rainy season and development of adult plant took place during this period. Shoot growth of adult plant was more than that of seedlings, and individuals grew in height ranging from 20-50 cm with a terminal whorl of 4 to 12 leaves. Adult plant produced flower bud during July but peak flowering was observed in August. Fruiting occurred from late August to September and seed setting also started in mid-September to October (Table 4.3). The seeds had no persistent seed bank like most forest herbs (Thompson *et al.*, 1997).



**Plate 4.2:** *Paris polyphylla*: Whole plant (A); a rhizome during 2005 (B); the same rhizome during 2007 having additional constrictions during the two years of annual growth (C)

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
A	1	e n l	e m l	e m l	e m l	e n l	e m l	e n l	e n l	e n l	e n l	e n l	e n l
N	A												
U	В												
A L	С												
	D												
Y	E												
C	F												
E	G												

**Table 4.3:** Phenology of *Paris polyphylla* (e = Early; m = Mid; l = Late)

A= Emergence of seedlings; B= Establishment of seedlings; C= Dormancy period; D= Reemergence of vegetative shoots; E= Flowering; F= Fruiting; G= Seed set *Chemical constituents and active principles*: Bioprospection study on *Paris polyphylla* has resulted in the isolation of the following six known compounds (Devkota, 2005).

- (i) Przewalskinone B (1,5-Dihydroxy-7-methoxy-3-methylanthraquinone) (23) or
   Przewalskinone B (23)
- (ii) Polyphllin-C (Diosgenin-3-O-[ $\alpha$ -L-rhamnopyranosyl(1 $\rightarrow$ 3)- $\beta$ -D-glucopyranoside]) (24) or Polyphllin-C (24)
- (iii) Polyphllin-D (Diosgenin-3-O-[ $\alpha$ -L-rhamnopyranosyl( $1_{Rha} \rightarrow 2_{Glu}$ )- $\alpha$ -L-arabinofuranosyl ( $1_{Ara} \rightarrow 4_{Glu}$ )]- $\beta$ -D-glucopyranoside (25) or Polyphllin-D (25)
- (iv) Saponin-1 (Diosgenin-3-O-[ $\alpha$ -L-rhamnopyranosyl( $1_{Rha} \rightarrow 2_{Glu}$ )- $\alpha$ -L-rhamnopyranosyl ( $1_{Ara} \rightarrow 4_{Glu}$ )]- $\beta$ -D-glucopyranoside (26) or Saponin-1 (26)
- (v) Stigmasterol (27)
- (vi) Stigmasterol-3-O- $\beta$ -D-glucoside (28)

*Ethno-medicinal use: Paris polyphylla* has been used for the treatment of fever and headache. The paste of whole plant was also used for burns and wounds. The species was also used for the treatment of dysentery.

### 3. Panax bipinnatifidus Seem.

*Taxonomy: Panax bipinnatifidus* is a perennial herb belonging to family Araliaceae. The species is distributed from temperate to alpine forest in Nepal, Tibet, China and some parts of Northern India (Punja, 2001). It is also found in north-eastern states of Arunachal Pradesh and in sub-alpine forest of Sikkim. The tuberous roots of the species are long and noded. The stem is erect, 30-50 cm high, usually withers in the dry season. Leaves are palmate with 2-3 in a whorl; leaflets 5-7 long, irregularly lobed; margins toothed and ciliated. Inflorescence is solitary, simple, terminal and umbel. Pedicels articulate below small bisexual greenish white flowers, inarticulate below male flowers. Calyx shortly 5-toothed; petals 5, imbricate; stamens 5; ovary 2- or 3 (-5), carpellate; styles distinct or basally united as many as carpels. Fruit is drupe, globose, sometimes slightly compressed or triangular. Seeds laterally compressed, as many as carpels; endosperm smooth. Berry scarlet when ripe, 1-2 seeded.

Life history and phenophasic events: Panax bipinnatifidus is a long-lived, non-clonal perennial forest herb. The stem is erect and about 0.5-1.0 m long (Plate 4.3). The root system

consists of a primary storage root joined to a rhizome. The rhizome bears adventitious roots formed along several nodes which are added annually. The species was distinguished into five stages *viz.*, seedling, and 1-4 leaved stages. Recruitment of seedlings occurred with the onset of spring season. Seedlings were not found to develop into higher stages within the first year. Recruitment was very low and seeds germinated after a long period of dormancy. Growth and emergence of seedlings were similar to that of *Paris polyphylla*. Seedlings were identified by the growth of one leaf and remain one-leaved during the first few years. Shoot withered prior to the onset of winter and persisted only as rhizome which reappeared in the following year as annual shoots. This pattern of death of annual shoots was also characteristics of 2 to 4-leaved stages.

Reproductive individuals were all 3-leaved and 4-leaved stage. Some 3-leaved individuals produced flowers but failed to fruit, while all 4-leaved stage plants did bear fruits every year during the study period. Berries were borne terminally on short or long peduncle. Fruits were not observed to mature synchronously, rather maturity and dehiscence occurred at different times, and usually the central fruits matured earlier. Each individual produced about 10 to 30 seeds. The species recruited seeds during early spring (Late March to April) while the reemergence of annual shoots of seedling and matured individuals took place during midmarch. This was followed by pre-monsoon and monsoon season where seedling establishment occurred and also marked the period of shoot growth, indicating the input of resources for propagation of the underground rhizome. Flowering occurred during early June and fruiting occurred during August. Fruit setting occurred during late August to September. The plants withered during mid-September to October, and the onset of annual dormancy started very early (Table 4.4).



Plate 4.3: Panax bipinnatifidus: Whole plant (A); withering shoot during pre-winter (B); emergent shoot of 3-leaved plant along with subsequent development of inflorescence before full leaf opening (C); rhizomes of 1-leaved (D), 2-leaved (E) and 3-leaved (F)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
						1000						
AN	e n l	e n l	e m l	e m l	e m l	e m l	e n l	e n l	e m l	e n l	e n l	e n l
N	A											
U	B											
A L	С											
	D											
	Ð											
Ċ	F											
	G											

**Table 4.4:** Phenology of Panax bipinnatifidus (e = Early; m = Mid; l = Late)

A= Emergence of seedlings; B= Establishment and of seedlings; C= Dormancy period; D= Re-emergence of vegetative shoots; E= Flowering; F= Fruiting; G= Seed set

Chemical constituents and active principles: The chemical composition of different Panax species is relatively similar. However, in general, each species exerts a specific effect on the body (Vuksan et al., 2000; Radad et al., 2006). The principal active components of ginseng are the "ginsenosides" or "ginseng triterpenoid saponins". Approximately 38 types of ginsenosides have been identified (Rhim et al., 2002; Leung et al., 2007; Choi et al., 2008), which account for the pharmacological effects of these plants in the modulation of angiogenesis, for their popular adaptogenic properties and their effects on the central nervous system.

*Ethno-medicinal use:* Rhizome of *Panax bipinnatifidus* has tonic and anti-anaemic properties as well as medicinal potential to increase sexual impotency and cure stomach disorders. Locally, the rhizomes are used for treating anaemia.

#### DISCUSSION

Traditional knowledge on using plants for medicinal purposes is particularly common among the ethnic group living in and around KBR and the medicinal plants are sourced mostly from within the boundaries of KBR. Very small amount is replenished from home gardens. This study showed that the local people in the buffer zone of KBR still practice traditional health care systems and rely heavily on traditional medicine. Although 105 plant species were recorded as ethno-medicinal plants, it was however revealed from survey that in the past, there were many more plant species that had medicinal value but did not withstand the test of time, probably because of species extinction at local or regional level, or because of the loss of associated knowledge. It is noteworthy that the use of some ethno-medicinal plants however, is still common such as *Swertia chirayita*, *Picrorhiza kuroaa* and *Valeriana jatamansii*. The safety and efficacy of these medicinal plants have also been established by modern scientific research. The ethno-medicinal plant species varied widely in terms of usage. Most species were specifically used for one to a few types of ailments, while very few were used for a variety of diseases and ailments, and only three species *viz.*, *Valeriana*  *jatamansii*, Costus speciosus and Centella asiatica were used for more than five different types of ailments.

The KBR being located in mountainous terrain, the ethno-medicinal plant species were distributed across an altitudinal gradient. The maximum number of ethno-medicinal plants was recorded from the sub-tropical zone between 1,500-2,000 m a.s.l. The temperate zone harboured moderate number of species, while the higher altitude forests in the sub-alpine and alpine zone had comparatively few ethno-medicinally important plant species. From threat perspective however, the lower altitude (<1,500 m) had the least percentage of 23% of threatened plants, while the mid-altitude zone (1,500-2,500 m) and the high altitude zone (>2,500 m) contributed 40% and 37%, respectively to the overall threatened ethno-medicinal plants of KBR (Figure 4.7). Although lower altitude species located at the buffer zone of KBR are most vulnerable to human disturbances and over-exploitation, higher altitude species evidently were the most threatened component of ethno-medicinal plants, mostly because of their specialized habitat and low population sizes.



Figure 4.7: Percentage of threatened ethno-medicinal plant species in different altitudinal zone

In this study, three species viz., Swertia chirayita, Paris polyphylla and Panax bipinnatifidus were selected for detailed characterization and for population studies. Besides being important ethno-medicinal plants of Sikkim, these three species are also of global importance as raw medicine as well as important components of several pharmaceutical products. They are characteristically forest herbaceous plants having wide altitudinal distribution and are exposed to varying types of disturbance and anthropogenic activities. *Swertia chirayita* located at lower altitude from 1,700 to 2,200 m is basically a forest herb, and there is no consistency in the literature regarding the habit of *Swertia chirayita* (Joshi and Dhawan, 2005). In the present study however, the species clearly exhibited the characteristics of a semelparous biennial species *i.e.*, bi-annual plant and died after seed dispersal in the second year, which was similar to other *Swertia* species such as *Swertia franchetiana*, *S. shintenensis* and *S. punicea* (Kondo *et al.*, 1990).

*Paris polyphylla* is a mid-altitude species located between 2,500 and 3,500 m elevation in the temperate forest of KBR. The species is a long lived perennial and non-clonal which is typically different from other *Paris* species *e.g.*, *Paris quadrifolia* that exhibits pronounced clonal growth (Jacquemyn *et al.*, 2006). The production of constrictions in the rhizome body is caused annually and resulted in the formation of growth rings as in most herbaceous perennial species (Dietz and Ullmann, 1997).

*Panax bipinnatifidus* is a high altitude species located in KBR between 2,700 and 3,700 m elevation in the sub-alpine forests, characteristically dominated by trees of *Abies densa*. The species is non-clonal. *Panax bipinnatifidus* is also a long-lived perennial and ginseng species are known to live more than 100 years. All the three study species were among the widely used ethno-medicinal plant species of Sikkim. These were also most important of all the 105 ethno-medicinal plant species of KBR from economic benefit point of view. These three species are also important from conservation point of view as they have been categorised under threatened species along with the other 27 threatened ethno-medicinal plant species of KBR.

Appendix 4.1: Ethno-medicinal plant species with family and their uses

Company dicting plants	Family	Uses (allments)
Abies densa Griffith ex R. Parker	Pinaceae	Toothache, Stomach disorder
Achyranthes aspera L.	Rosaceae	cuts and wounds, blood purification
Aconitum ferox Wall. Aconitum heterophyllum Wall. ex Royle	Ranunculaceae Ranunculaceae	Food poisoning, rhizome are used to cure cough, fever, skin diseases and to relieve gout Rhizome is used to relieve body-ache, fever and nose discharge
Acorus calamus L.	Zingiberaceae	Paste prepared from rhizome is used for skin diseases; powder taken orally
Aloe barbadensis Mill	Aloeaceae	for cough, malaria and asthma Whole plant is effective in piles, muscular pain, inflammation, skin
Angelica archangelica L.	Apiaceae	ailments, arthritis and burn Whole plant is used in curing itching, skin diseases, ulcer, infection and in toxic condition
Arisaema erubescens (Wall.) Schott	Araceae	Leaf, stem and tuber as raw or liquid extract are used as antihelmenthic.
Arisaema nepenthoides (Wall.) Mart. ex Schott	Araceae	Leaf and stem extract is used as
Arisaema tortuosum (Wall.) Schott	Araceae	Leaf and stem used as raw or liquid extract is used as antihelmenthic in cattle
Aristolochia griffithii Duch.	Aristolochiaceae	Stem is used for digestive system disorder
Artemisia vulgaris L.	Asteraceae	Dried or fresh root is used against diarrhoea and dysentery. The powdered mixture of <i>Astilve rivuilaris</i> and <i>Bergenia ciliata</i> is administered to women after childbirth to ease body pain, swelling and to control excessive blaeding.
Asparagus recemosus Willd.	Asparagaceae	Abdominal discomfort, dysentery
Bauhinia purpurea L.	Fabaceae	Bark is used to control diarrhoea; root and flowers are also useful as maturant for boils and abscesses and also used against animal bite
Bauhinia vahlii (Wt. & Arn.) Benth.	Fabaceae	Fruits used in dysentery and stomach ache
Bauhinia variegate L.	Fabaceae	Flower juice is taken to cure dysentery, diarrhoea and stomach pain;
Cornus kousa	Cornaceae	Fruits, twig and leaves are used as veterinary medicine by the local people/occasionally ripe fruits are used for preparing local wine or eaten
Bergenia ciliata (Haw.) Stenb.	Saxifragaceae	Plant extract is taken to treat indigestion, fever, diarrhoea and dwentery
Bergenia purpurascens (Hook. f. & Thoms.) Engl.	Saxifragaceae	Dried roots are used as tea and gives relief from body ache.
Betula utilis D. Don	Betulaceae	Leaf decoction is diuretics; Bark paper is sacred
Boenninghausenia albiflora (Hook.) Reichb.	Rutaceae	Repellent for repel lice, fleas and other insects.

Campylandra aurantiaca Baker	Liliaceae	Bitter inflorescence purifies blood and is eaten as curry.
Cardiocrinum giganteum (Wall.) Makino	Liliaceae	Corm and leaves are used in fracture to relive pain.
Centella asiatica L. (Urban)	Apiaceae	Leaves are used for liver disorder and acts as adaptogen, anti-inflammatory, diuretic, febrifuge, hypotensive, nervine, sedative, skin Tonic
Cinnamomum impressinervium Meissn.	Lauraceae	Bark is effective against gonorrhoea; leaves extract is used in rheumatism and diarrhoea
Cissampelos pareira L.	Menispermaceae	Root extract is used in stomach and liver problem.
Clematis buchananiana DC.	Ranunculaceae	Crush roots effluvium cures sinusitis and nose-blocks
Costus speciosus (Koen.) Sm.	Zingiberaceae	Whole plant is used in cough, bronchitis, fever, rheumatism, urinary disorders, loss of appetite, loose motion and skin diseases
Curcuma zedoaria (Chirstm.) Rosc.	Zingiberaceae	Antiseptic, aids in digestion and relieve flatulence and colic
Cuscuta reflexa Roxb.	Convolvulaceae	Rhizome is used in jaundice, antiviral
Cynodon dactylon (L.) Pers.	Poaceae	Decoction of roots is used in dropsy and in secondary syphilis, piles; crushed roots are also used in chronic gonorrhoea/plant is considered sacred and has importance in various rituals
Dactylorhiza hatagirea Soo	Orchidaceae	Increase sexual potency, suppress body ache, jaundice.
Daphne papyracea Wall. ex Steud.	Thymeleaceae	Root extract acts as antidote to poison and also cures fever.
Datura suaveolens Humb. & Bonpl. ex Willd.	Solanaceae	Leaf extract is taken during swelling, body pain, asthma and skin complaints.
Dendrobrium nobile Lindl.	Orchidaceae	Eye redness
<i>Dichroa febrifuga</i> Lour.	Hydrangaceae	Leaf extract have been used during fever
Digitalis purpurea L.	Scrophulariaceae	Leaves are useful against chest pain
Docynia indica Decne.	Rosaceae	Fruits are eaten during extreme diarrhoea and dysentery.
Drymaria cordata (L.) Willd. ex Roem. & Schult	Caryophyllaceae	Leaf juices are applied for cut, wounds and eye ailments, cold and cough.
Entada phaseoloides (L.) Merr.,	Fabaceae	Juice of the bark is used for skin diseases; the kernel is used for washing hair having dandruff; seed powder is used for subsiding swollen necks and mumps
Ephedra gerardiana Wall.	Ephedraceae	Whole plant is used for asthma and high fever treatment
Euodia fraxinifolia Hk.	Rutaceae	Fruit are used in the treatment of fever and also helps indigestion

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Eupatorium cannabinum L	Asteraceae	Leaf extract checks bleeding, anti microbial
Fraxinus florubunda Wall.	Oleaceae	Poultice of bark and bark exudates is applied in the gout affected area.
Fritillaria cirrhosa D. Don	Liliaceae	Bulb is used during stomach disorder, cough, and helps in eliminating phlegm
Galium spp.	Rubiaceae	Whole plant extract is applied over wound cuts
Gaultheria fragrantissima Wall.	Ericaceae	Leaves and roots are used in rheumatism, menstrual disorder, and body ache.
Geranium nepalense Sw.	Geraniaceae	Root extract is used for stomach disorder
Gonatanthus pumilus (D. Don) Eng. & Krau.	Araceae	The paste from the extract of rhizome is effective against boil
Gynocardia odorata R. Br.	Flacourtiaceae	Edible seed oil is used to cure leprosy and skin diseases; bark is used as fish poison
Hedera nepalensis Koch	Araliaceae	Decoction of leaves is used for skin diseases
Hedychium spicatum Rosc.	Zingiberaceae	Rhizome is useful in the treatment of liver complaints, and is also used in treating fevers, vomiting, diarrhoea, inflammation, pains and snake bite
Heracleum wallichii DC	Apiaceae	Leaves are used as anti-inflammatory
Hippophae salicifolia D. Don	Elaeagnaceae	Stem used to make bangles, which is believe to give relief from orthopaedic problems
Holboellia latifolia Wallich.	Lardizabalaceae	Stem used to make bangles, which is believe to give relief from orthopaedic problems
Imperata cylindrica (L.) Beauv.	Poaceae	Extract of root is useful as antihelmenthic
Juniperus recurva BuchHam.	Cupressaceae	The branches are used to treat swelling and pain and as insect repellent
Kaempfera rotunda L.	Zingiberaceae	Root used as poultice in fracture, healing fresh woods and removes coagulated bloods from the body.
Lobelia zeylanica L.	Campanulaceae	Powder from fruit is used as insecticide, medicine for fowl infection
Lycopodium clavatum L.	Lycopodiaceae	Root and leaves are used in rheumatism
Lygodium flexuosum (L.) Sw.	Lygodiaceae	One teaspoonful of leaf powder is mixed in milk and given orally for abildran to increase memory
Lyonia ovalifolia Drude	Ericaceae	Juice extract from tender leaves are effective against skin diseases.
Mahonia napaulensis DC.	Berberidaceae	Bark extract cures eye and skin diseases
Mallotus philippinensis Muell.	Euphorbiaceae	Fruits are eaten during constipation, infestation and abdominal diseases
Megacodon stylophorus (C.B. Clarke) Sm.	Gentianaceae	Paste of the root is applied as a poultice in wounds and subsiding swellings

14 de la constructor de la construction de la const	Musicasa	The weeks of the basis is smalled on the
Myrica esculenta Ham. ex Don	Myricaceae	The paste of the bark is applied on the chest to get relief from cough and bronchitis, worms
Naraosiacnys granaytora (D. Don) DC.	valemanaceae	stomach disorder
Oroxylum indicum (L.) Vent.	Bignoniaceae	Effective against joint pain, flower is considered sacred in various rituals
Osbeckia nepalensis Hk.	Melastomaceae	Flowers and leaves paste used for cut and wound
Oxalis corniculata L.	Oxalidaceae	Whole herb is used in epilepsy, gastric troubles, and skin complaints.
Paederia foetida L.	Rubiaceae	Fruits are used in bowel complaints, asthma, diarrhoea, diabetes,
Panax bipinnatifidus Seem.	Araliaceae	Rhizome has medicinal potential to increase sexual impotency and cure
Paris polyphylla Sm.	Trillidaceae	stomach disorders Rhizome is used in skin disease, cut and wounds, fever, dysentery
Phytolacca acinosa Roxb.	Phytolacaceae	Extract of root helps in sinusitis, bleeding and infection
Picrorhiza kurroa Royle ex Benth.	Scrophulariaceae	Rhizome and leaves are used in cough and cold, anaemia, body ache, malaria
Pieris formosa D. Don	Ericaceae	Crushed leaves extract is applied in skin diseases and as insecticides
Piper longum L.	Piperaceae	Roots is used as antihelmenthic, improves appetite, abdominal pain; fruits use for anti-diarrhoeatic, anti- dysenteric piles leprosyletc.
Plantago erosa Wall.	Plantaginaceae	Plant is used for rheumatism. and dysentery
Podophyllum hexandrum Royle.	Podophyllaceae	Leaf, rhizome and root are used against gastric, ulcer, liver problem, gastric troubles
Polygala arillata BuchHam ex D. Don	Polygalaceae	Roots use for preparation of yeasts. Root juice is taken during castrointestinal disorder
Prinsepia utilis Royle	Rosaceae	Roots are used as antidote to poison
Pteris biaurita L.	Pteridaceae	Leaves are used as insect repellent.
Rheum emodi Meisn.	Polygonaceae	Dried roots are use as substitute for tea and believe to give relief from body ache. Local villagers used the attractive inflorescence to make pickle
Rheum nobile Hk. & Thom.	Polygonaceae	Decoction or powder from root is used in rheumatism, and as stimulant
Rhododendron anthopogon D. Don	Ericaceae	Ethno-botanical use of leaves and stems as incenses and insect repellent, flowers used in bronchisic
Rhododendron arboreum Sm.	Ericaceae	Dried flowers are used during
Rhus semialata Murr.	Anacardiaceae	Fruits are used as medicine for dysentery/ethno-botanically important

Rubia cordifolia L.	Rubiaceae	Root extract is useful in rheumatism and paste as antiseptic
Saussurea gossypiphora D. Don	Asteraceae	Leaves and flowers are used as antidote, urinary complaint and impotence/locally cottony woolly hairs mixed with butter are useful in asthma. Cottony wools are also used in place of cotton as butter lamp
Schima wallichii Korth.	Theaceae	Decoction of fruits as antidote against scorpion bite; bark as vermicide for curing gonorrhoea
Smilacina oleracea (Baker) Hk. f.	Convallariaceae	Headache
Smilax lanceaefolia Roxb.	Smilacaceae	Rhizome is used during epilepsy and purification of urine and stool
Solanum nigrum L.	Solanaceae	Fruits are useful for diabetic patients
Solanum viarum Dunal	Solanaceae	Useful in diarrhoea
Spiranthes sinensis (Pers.) Ames	Orchidaceae	Powder from rhizome is used as aphrodisiac
Swertia chirayita (Roxb. ex Fleming) Karsten	Gentianaceae	Whole plant is considered as tonic, astringent, stomachic, improves eye sight, pain in the joints, cures scabies
Swertia nervosa (G. Don) C.B. Clarke	Gentianaceae	Decoction of aerial shoots is used as liquid for fever
Taxus wallichiana Zucc.	Taxaceae	Barks used for treatment of headache, giddiness, feeble and falling pulse, distributes and savere billoursees
Thysanolaena maxima (Roxb.) Kuntze	Poaceae	Root decoction is used as
Urtica dioica L.	Urticaceae	Inflorescence is useful for diabetic patients and taken as vegetables
Valeriana hardwickii Wall.	Valerianaceae	Decoction of shoots effective against epilepsy and other mental diseases.
Valeriana jatamansii DC.	Valerianaceae	Rhizome is used as a stimulant, antiseptic, insect repellent and for the treatment of epilepsy, hysteria, convulsive affections, stomach ache, constipation and cholera
Viscum articulatum Burn.f.	Loranthaceae	Plant paste is used in bone fracture
Vitex negundo L.	Vitaceae	Leaves used as insecticides and for cough and cold. Erect stem are used in rituals to drive away evil spirits

#### **INTRODUCTION**

An understanding of spatial distribution of the species is fundamental to study landscape level dynamics of any species. Characterizing the habitat types and spatial occupancy pattern is a pre-requisite for habitat modelling. Biologists have conducted field surveys to map the potential distributional area of plants and animals. In spite of this, our understanding of the distribution of most species, especially those found in inaccessible areas, is still incomplete. This has been obviously due to time-intensive, costly and hazardous nature of field work. Therefore, modelling of species distributions has become necessary. Habitat Suitability (HS) models, also known as ecological niche modelling, is a good tool for decision-making within the framework of applied biology and they have mainly been used for developing strategies for conservation, planning and forest management. In recent years, our capacity to learn more about species distribution has remarkably enhanced by combining reliable point data with strong technological and analytical tools. High-speed computing, HS modelling along with interface using GIS techniques have allowed us to model the distribution of a particular species by analyzing the environmental characteristics of its known localities. These mathematically defined models can then be combined with known constraints based on the species life history to predict where else on the landscape the species might occur. A variety of environmental data are used as the basis for these models, some of which have only recently become widely available. These data include digital elevation model and its derivatives such as aspect, slope, compound topographic index and also satellite data on vegetation cover and landuse/land cover. GIS data layers providing estimates of monthly and annual mean precipitation and temperature are also available on the web (www.worldclim.org/).

Habitat mapping along with detailed population study of the species is key to develop effective measures for species conservation (Brooks et al., 2004; Samways, 2005; Giriraj et

al., 2008). Through this approach, it is possible to identify areas that are suitable for the conservation of a species (Irfan-Ullah *et al.*, 2006; Papes, 2006), and for possible reintroduction sites of threatened species (Martinez-Meyer *et al.*, 2006). For modelling, the knowledge on the ecological niche of a species is important. The niche of a species has been defined as the set of environmental and ecological conditions within which the species is able to maintain its population. Several approaches have been used to approximate species ecological niches (Nix, 1986; Austin *et al.*, 1990; Walker and Cocks, 1991; Scott *et al.*, 1993, 1996, 2002). Of these, MAXENT (Maximum Entropy) modelling for species distribution (Phillips *et al.*, 2006) has undergone rigorous testing under diverse ecological conditions and has been proved to be a robust modelling approach.

Most interpretations of ecological niche modelling are restricted to distribution mapping, although new interpretations relating to distribution are being increasingly added to enhance the utility of the models. These interpretations ought to vary depending on the approaches taken for the modelling. For example, in mechanistic approach, physiological variables are directly measured ignoring the biotic interactions. On the other hand, the correlative approach is based on observations that include effects of biotic interactions on distribution of species. The two approaches thus estimate quite different phenomena and therefore, the models need to be interpreted carefully in applications (Soberon and Peterson, 2005). Although the prime application of the modelling continues to be potential distribution area mapping, efforts to expand the application scope of the modelling to distribution related biological attributes can provide important outputs that could be utilized for species conservation.

Predictive modelling for non-endemic species at broad range is labour intensive since maximum presence data points spread from different localities is required to avoid extrapolation. All the three study species are relatively broad range in distribution but rare in their area of occurrence, a characteristic of threatened plants. *Swertia chirayita* is distributed in the sub-tropical mountains of Nepal, Bhutan and India (Bhatt *et al.*, 2007). In India, the

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species grows in sub-tropical and temperate Himalayas from Kashmir to the Khasi hills of Meghalaya (Chanda, 1976), besides it also occurs in Arunachal Pradesh and Sikkim. *Paris polyphylla* is distributed in the Himalayas, Western China and south-east Asia. In India, it is distributed in the Eastern Himalayas upto Sikkim and Arunachal Pradesh and Meghalaya. On the other hand, *Panax bipinnatifidus* is distributed along the Himalayan range in Bhutan, Nepal and India. In India, it is found in Sikkim and Arunachal Pradesh. However, in the present study, the distribution modelling of the three species is focused in the native range of Sikkim. As such, a few presence data points were sufficient for applying the model. Predictive modelling of the potential habitats of these species can help in in-situ conservation as well as relocation of these species to the suitable habitats. The objective of this chapter is to: (i) model the potential habitat distribution of three medicinally important plant species, (ii) identify the macro- and micro-scale factors shaping the potential habitats of the species.

### **METHODS**

#### **Occurrence Records**

In the present study, detailed field inventories were done through field surveys in order to record the habitat characteristics and spatial distribution of the three study species *i.e.*, *Panax bipinnatifidus*, *Swertia chirayita* and *Paris polyphylla*. Initial field surveys which were carried out in various parts of KBR revealed that the study species were known to occur predominantly in west Sikkim district part of KBR. Hence, all possible tracks in west Sikkim district were surveyed within 50 to 100 m width along both sides of the track, depending on the accessibility. Elevation, slope angle and aspect, geographic location, soil characteristics and vegetation status of the habitat of occurrence were recorded. A total of 18 records for *Panax bipinnatifidus*, 30 records for *Swertia chirayita* and 12 records for *Paris polyphylla* were collected (Table 5.1). The geographic coordinates *i.e.*, latitude and longitude of all the occurrence localities were recorded using a Global Positioning System (GPS) at an accuracy

range of 10 to 30 m. These GPS records were later converted to decimal degrees for use in modelling the habitat distribution for the species.

Provide Species	l'otat records	. Training	
Swertia chirayita	12	9	3
Paris polyphylla	22	17	5
Panax bipinnatifidus	18	13	5

Table 5.1: Number of localities used for habitat distribution modelling of three study species

### **Habitat Characteristics**

Habitat characteristics of the species were studied prior to initiation of population studies during 2004. These include vegetation type, species association, spatial occurrence, and other physiographic information such as slope, aspect and altitudinal range. Vegetation sampling was conducted through belt transects. The detailed information on species association was obtained from 40 1 m x 1 m randomly-placed quadrats in each site. Species association was estimated by Chi-square pair-wise association of attributes. A Chi-square test can indicate the probability that the two species are distributed independently, or are associated with one another (Whittaker, 1975) and Cole's coefficient of association was used to quantify the strength of association to ascertain the species those are closely associated with the study species (Cole, 1949).

### **Measurement of Microclimate and soil characteristics**

The microclimatic factors studied were light intensity, air temperature and relative humidity, which were measured 1 m above the forest floor on a particular day. The measurements were taken at four hourly intervals at ten random points in each site on the day of microclimate measurement. Light intensity was measured by a Digital Luxmeter (TES 1332A), air temperature and relative humidity by a Thermo hygrometer TH–103 (Mex–therm). Geographical parameters were also recorded. Elevation was measure using a Global Positioning System (GPS) Garmin 76. Slope and angle slope orientation was measured using a clinometer-compass instrument.

#### Soil Sampling and Processing

Soil samples were collected on a seasonal basis for three years during the study period from 2006 to 2008. Soil samples were collected randomly from each site using a steel corer (6.5 cm diameter) from the surface layer *i.e.*, 0-20 cm surface soil. One composite sample was prepared by mixing soil samples collected from the five replicates in each patch where the study species. These were then air dried and sieved through 2.0 mm sieve, and stored in polythene bags for physico-chemical analysis. The soil physical parameters studied were soil texture, water holding capacity and soil moisture content. Soil chemical properties include soil pH, soil organic carbon (SOC), total Kjeldahl nitrogen (TKN), available phosphorus (P) and exchangeable potassium (K).

#### Soil Analyses

*Soil physical properties*: Soil temperature was measured by a soil thermometer (Multi– Thermometer). Soil texture was determined by Bouyoucos hydrometer method (Allen *et al.*, 1974). Soil moisture content was determined by gravimetric method (Allen *et al.*, 1974). Water holding capacity was determined by Keen's box method, using copper cups of 5.6 cm internal diameter and 1.6 cm height (Piper, 1942).

*Soil chemical properties*: A digital pH meter (Professional Meter, PP–20, Sartorius) was used to determine the pH of soil (Anderson and Ingram, 1993). Soil organic carbon was determined by colorimetric method (Anderson and Ingram, 1993). TKN was determined by Kjeldahl digestion method followed by colorimetric analysis. Ammonium gas–diffusion technique was applied (application notes, AN–5222) for estimation of TKN, using the automated Spectrophotometric Flow Injection Analyser (FIAstar, Model 5000–Analyser, 5027–Sampler, AB, FOSS, Hoganas, Sweden). Available phosphorus was determined after extracting soil phosphorus in 0.5 M sodium bicarbonate solution by ammonium–molybdate blue method (Allen *et al.*, 1974). Exchangeable potassium was determined by extracting soil in ammonium acetate extractant at pH 7, by adding few drops of acetic acid or ammonium solution (Jackson, 1973).

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#### **Habitat Distribution Modelling**

In the present study, the variables used as predictors were remotely sensed data on Digital Elevation Model (DEM) and Enhanced Vegetation Index (EVI) were used to summarize the habitat for the species in the native range of the state of Sikkim in northeast India. 90 m digital elevation data was obtained from CGIAR-CSI (http://srtm.csi.cgiar.org, Jarvis et al., 2008). Layers on slope and aspect were derived from digital elevation model using module on ArcGIS 9.3 platform. Twenty three layers of MODIS images (MOD13Q1) with a spatial resolution of 250 m were obtained from Oak Ridge National Laboratory Distributed Active Archive Center (http://daac.ornl.gov/MODIS/modis.html). These layers correspond to the year 2006 during which the field surveys were made. These layers characterize the spatial aggregates of EVI at 16 day intervals in the native range of the species during the year of sampling. EVI has been preferred over Normalized Difference Vegetation Index (NDVI) for vegetation changes because of its improved sensitivity to saturation in the degree of greenness in the humid forested areas and higher capability to discriminate changes in vegetation across spatial and temporal scale (Huete et al., 1999). The images were downloaded in GEOTIF format and converted to raster grids in ArcGIS 9.3. In order to detect colinearity and identify the highly correlated pair of layers (r > 0.8), the 23 EVI layers were subjected to correlation test using Ecological Niche Modelling (ENM) tools version 1.3 (Warren et al., 2008). One of the EVI layer from each of the highly correlated pair of layers were excluded from the analysis. A formulation of 12 environmental data was used for habitat distributional modelling of the three species which included 3 physiographic variables viz., elevation, slope and aspect, and 9 EVI layers. All analyses were conducted at a spatial resolution of 250 m of the environmental data sets.

Habitat distributional modelling was done using MAXENT version 3.3.3e (Phillips *et al.*, 2006). MAXENT is one amongst the 'presence-only' group of species distributional modelling methods which has gained wide popularity due to a number of reasons. It estimates the maximum entropy probability distribution function to predict the geographic

location of a species based on environmental variables and reconstructs the boundaries of the ecological niche by placing constraints on the probability distribution based on the environmental parameters of the grid-cell presence record (Phillips *et al.*, 2006). Seventy five percent of the total records were used for model training and twenty five percent records were used for testing. Preliminary trial runs using different sets of training and testing records yielded different Area Under Curve (AUC) values in each run. Hence, to account for such variation and select the optimal model, we executed 20 replicated model runs for the species with a threshold rule of 10 percentile training presence. In the replicated runs, we employed cross validation technique where samples were set to default as the program is already tuned on a wide range of species datasets (Phillips and Dudik, 2008). From the replicated runs, average, maximum, minimum, median and standard deviation were generated. Model quality was evaluated based on AUC value and the model was graded following Thuiller *et al.* (2006) as: poor (AUC< 0.8), fair (0.8 <AUC< 0.9), good (0.9 <AUC< 0.95) and very good (0.95 <AUC< 1.0).

### RESULTS

#### **Habitat Characteristics**

The geomorphological habitat characteristics of the three species have been summarized in Table 5.2. The microenvironment and soil characteristics of the habitats are presented in Table 5.3. The results of analysis of associated species are presented in Table 5.4. Habitat characteristics exhibited a distinctive behaviour for each of the three species.

Table 5.2: Habitat chara	acteristics
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			n olone de la seconda de la Seconda de la seconda de la		
Swertia chirayita	1700-3000	15-40	South-west	Open forest	Sparse
Paris polyphylla	2400-3280	10-30	South	Understorey	Thick
Panax bipinnatifidus	3300-3700	5-25	South-west	Understorey	Thick

Table 5.3: Micro-environment and soil characteristics

Bisqueters	Swertig	chirit la	Partopo	ionio lla	
Micro-environment			1999 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 - 1994 -		
Air temperature (°C)	22.77	<u>+</u> 0.61	19.81	<u>+</u> 2.37	11.84 <u>+</u> 1.00
Light intensity (lux)	17,840	<u>+</u> 3897	3,620	<u>+</u> 2690	4,005 <u>+</u> 2.57
Relative humidity (%)	73.70	<u>+</u> 3.30	83.54	<u>+</u> 6.62	87.50 <u>+</u> 2.95
Soil				7. S.	
Soil texture	Loam	y sand	Sandy	/ loam	Sandy loam
Soil temperature (°C)	20.80	<u>+</u> 1.19	14.15	<u>+</u> 1.21	9.04 <u>+</u> 0.58
Water holding capacity (%)	41.40	<u>+</u> 3.98	54.32	<u>+</u> 4.69	43.70 <u>+</u> 6.37
Soil moisture content (%)	37.70	<u>+</u> 14.70	65.90	±1.41	53.13 <u>+</u> 2.90
pH	4.97	<u>+</u> 0.35	3.04	<u>+</u> 0.16	3.32 <u>+</u> 0.45
TKN (%)	0.34	<u>+</u> 0.14	0.64	<u>+</u> 0.03	0.41 <u>±0.07</u>
P (µg/g)	8.87	<u>+</u> 1.79	7.7 0	<u>+</u> 1.38	13.30 <u>+</u> 6.10
K (μg/g)	14.89	<u>+</u> 11.23	28.68	<u>+</u> 12.58	18.25 <u>+</u> 11.23
SOC (mg/g)	3.15	<u>+</u> 0.16	5.01	<u>+</u> 0.8	2.77 <u>+</u> 0.09

Table 5.4: Associated species of Swertia chiravita, Paris polyphylla and Panax bipinnatifidus

Sheries	Associated apericen	Chisquancie	
Swertia chirayita	Swertia bimiculata	7.43	0.47
Paris polyphylla	Elatostemma platyphylla	6.96	0.52
Panax bipinnatifidus	Panax pseudo-ginseng	19.93	0.80
* Significant at n< 0.01			

Significant at p< 0.01

Swertia chirayita occurred along the buffer zone of KBR from 1,700 m to 3,000 m altitude in forests, shrublands and patchily on the margins of cultivated land adjacent to buffer of KBR. It thrived on sandy to sandy-loam soil texture along the south-western slope at 20°-40°. Due to its tolerance to drier environment, it also flourished in shrublands, open ground and along the forest edges in the buffer zone of KBR (Plate 5.1).

Paris polyphylla occurred in cool temperate (2,400-3,280 m elevation) forests in moderate to deep shade condition and was commonly found in soft humid and humus rich soil (southern slope at 10°-30°). This may be attributed to its generally low colonization capacity as reported by Ehrlén and Eriksson (2000). The species are therefore, clumped to specialized habitats (Plate 5.2).

Panax bipinnatifidus occurred from 3300-3700 m elevation zone. It is a typically shadepreferring plant whose normal growth and development was ensured exclusively under forest shade without durable impact of direct sunshine, although heavy shade may also impede the growth of this plant, which periodically entered into the state of dormancy
(south-west slope at 5°-25°). Forest areas with tree cover density of 0.6-0.8 were most favourable to *Panax bipinnatifidus* development. *Panax bipinnatifidus* favours well drained although sufficiently moist weak-acid soils rich in mould. In Sikkim KBR, *Panax bipinnatifidus* thrived in the *Abies densa* dominated coniferous forests (Plate 5.3) and was found in cool microclimate condition, and on litter- rich/moss-matted well drained soils.



Plate 5.1: Overview of habitat of Swertia chirayita



Plate 5.2: Overview of habitat of Paris polyphylla



Plate 5.3: Overview of habitat of Panax bipinnatifidus

#### **Habitat Distribution Modelling**

One of the parameters used for evaluating the predictive ability of the models generated by MAXENT is the Area Under Curve (AUC) of the Receiver Operating Characteristic (ROC) curve. This is the measurement for model accuracy which ranges from 0.0-0.1 and a value of 0.5 indicates randomness. The resulting AUC ranged from 0.0-1.0, and for all the three species, the AUC produced was >0.5 which indicated a perfect fit to the model (Menke *et al.*, 2009). The mean training AUC for the habitat distribution models of all the three species showed 'good' *i.e.*, 0.91 for *Swertia chirayita* to 'very good' results *i.e.*, 0.97 for *Panax bipinnatifidus* and 0.99 for *Paris polyphylla* (Table 5.5). The mean test AUC showed 'good' results for *Panax bipinnatifidus* and *Swertia chirayita*, while for *Paris polyphylla*, the mean test AUC showed 'very good' results.

Table 5.5: Results of the 10 replicated model runs using cross validation procedure

	Mean AUCom (#SD)	Mean ANCHINESDING
Swertia chirayita	0.91 ± 0.023	0.93 ± 0.069
Paris polyphylla	$0.99 \pm 0.0007$	$0.98\pm0.004$
Panax bipinnatifidus	$0.97 \pm 0.009$	$0.92 \pm 0.105$

# Structure of the Predicted Habitat Models

EVI had maximum contribution to the structure of the modeled habitat in all the three species. The relative contribution of the environmental variables varied to a great extent. EVI layers contributed 89.3% in *Swertia chirayita*, 68% in *Paris polyphylla* and 94.9% in *Panax bipinnatifidus*. Physiographic variables collectively contributed 10.7% in the distribution of *Swertia chirayita*, 5.1% in *Panax bipinnatifidus* and a relatively higher contribution in *Paris polyphylla* with 32% to the modeled habitat (Table 5.6).

Table 5.6: Relative contribution	on of the environmental v	variables to the habita	t models
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	Percent	ontribution to the ha	
	Swerila chiravita	Raris potyphylia	
Physiography.			
Elevation	2.8	27.3	4.6
Slope	7.4	1.1	0.2
Aspect	0.5	3.6	0.3
	A STARLE MONTRY	1432.0	

Enhanced vegetation index (EVI)	Swertia chirayita	Paris polyphylla	Panax bipinnatifidus
EVI 097 (7 Apr-22 Apr)	1.5	1.5	20.6
EVI 145 (25 May-9 Jun)	37.5	4.4	18.0
EVI 161 (10 Jun-25 Jun)	0.0	0.7	13.6
EVI 177 (26 Jun-11 Jul)	0.1	10.4	0.2
EVI 193 (12 Jul-27 Jul)	1.6	3.0	6.4
EVI 209 (28 Jul-12 Aug)	16.9	22.7	18.4
EVI 225 (13 Aug-28 Aug)	0.0	2.9	4.4
EVI 273 (30 Sep-15 Oct)	6.3	19.8	13.2
EVI 289 (16 Oct-31 Oct)	25.4	2.6	0.1
	89.3	68.0	

In *Swertia chirayita*, maximum contribution was evidently by EVI 145 representing spring season with 37.5%, followed by EVI 289 which corresponds to autumn season (Figure 5.1). Among physiographic variables, slope (7.4%) was a strong determinant for the distribution of *Swertia chirayita*. For *Panax bipinnatifidus*, EVI 097 corresponding to early spring had maximum contribution to the predictive distribution pattern with 20.6% (Figure 5.2). Premonsoon vegetation index (EVI 145) and monsoon data (EVI 209) had equal importance to the model prediction of 18% and 18.4%, respectively. These were followed by other fewer contributors such as early monsoon EVI 161 (13.6%) and post monsoon EVI 273 (13.2%). Among physiographic variables, elevation had more importance with 4.6%. Elevation particularly had high importance in the distribution of *Paris polyphylla* with 27.3% (Figure 5.3). Among EVI variables, EVI 209 (monsoon) and EVI 273 (post monsoon) had high percent contribution with 22.7% and 19.8%, respectively.

## **Geographic Distribution of the Potential Habitats**

By and large, the potential habitats of the three species were more confined towards the southern part where major vegetation types were represented due to diverse altitude and topography. In *Swertia chirayita*, the potential habitats showed a more or less continuous distribution pattern in the southern part of Sikkim and got weaken towards the northern part of the state (Figure 5.1). The distribution of potential habitats for *Paris polyphylla* and *Panax bipinnatifidus* was patchy but showed a restricted range (Figure 5.2 and 5.3). The highly suitable habitats for *Paris polyphylla* and *Panax bipinnatifidus* were perched mostly in the higher elevations.



Figure 5.1: Potential habitat distribution of *Swertia chirayita* in Sikkim. The colour ramp in the map represents different levels for habitat suitability based on probability scores



**Figure 5.2:** Potential habitat distribution of *Paris polyphylla* in Sikkim. The colour ramp in the map represents different levels for habitat suitability based on probability scores



Figure 5.3: Potential habitat distribution of *Panax bipinnatifidus* in Sikkim. The colour ramp in the map represents different levels for habitat suitability based on probability scores

#### DISCUSSION

#### **Habitat Distribution Modelling**

The fitting of the model parameters on the habitat distribution of the Swertia chirayita, Paris polyphylla and Panax bipinnatifidus was fairly high and accurate. Since the 'presence only' data was used, the maximum achievable AUC was less than 1.0 (Sánchez and Arroyo, 2008) and values 0.5 or less corresponds to randomness thereby, rendering the model useless (Phillips et al., 2006). Variables with AUC values above 0.75 were considered to be strong predictors by Swets (1988) and also by Hosmer and Lemeshow (2000) in their study of ant species occurrence in Argentina. The average AUC values for all the species revealed that the habitat distribution models generated by MAXENT are of high quality and could discriminate between suitable and unsuitable habitats. Therefore, the models for the three species had AUC very close to 1.0 which per se indicated their capacities for very strong predictability. Therefore, MAXENT successfully distinguished between the suitable and unsuitable habitats for the selected species within the native range of KBR as revealed by model calibration. The models could effectively capture the range of physiographic and vegetation characteristics defining the habitat boundary. The results are also consistent with field observations implying the usefulness of the predicted models in in-situ conservation of the species.

#### **Factors Shaping the Potential Habitats**

EVI collectively contributed most to the structure of the modeled habitat indicating its overall importance in defining the habitat within the native range of KBR habitat of the species. The relatively high importance of vegetation parameters such as herbaceous cover, NDVI and EVI in predicting distribution of species was also reported by Stohlgren *et al.* (2001) who used NDVI, EVI and other physiographic predictors, in predicting the distribution of African honey bees. Variation in individual contributions made by the nine EVI layers to the modeled potential habitat was observed within and between the species. In case of *Panax bipinnatifidus*, the major factors which defined the boundaries of its habitat

were the layers corresponding to important events for the species, such as bud initiation, vegetative growth and flowering time. For *Paris polyphylla*, the vegetation component of the potential habitat was mostly defined by the layer which corresponds to flowering and fruiting period of the species. The potential habitat of *Swertia chirayita* was defined mostly by the layers corresponding to the flowering and fruiting period of the species. Amongst all the physiographic variables, elevation was relatively more important in determining the habitat of *Panax bipinnatifidus* and *Paris polyphylla*, while for *Swertia chirayita*, slope angle was important. Therefore, vegetation cover was found to be an important factor governing the habitat suitability for the species. The decisive role of vegetation cover in shaping the potential habitats of the three species was conspicuous from the contributions made by the respective EVI layers to the overall structure of the habitat model. Overall, this indicated a minor role played by physiography on the spatial occupancy of the study species with exception of *Paris polyphylla* where elevation had relatively higher contribution to the distribution model.

Therefore, the current distribution of habitats of the three species is skewed more towards the southern part of KBR, where majority of the areas are covered under protected area networks. This is because different vegetation and forest types are represented in the southern part of KBR along West Sikkim district. Northern part is predominated by landuse classes of non-forest origin such as, snow and glaciers, alpine scrub, alpine pastures, alpine barren lands, landslides and rock outcrop areas. Since bounding is implemented in the model, therefore, the habitat area predicted represents the habitat suitability within a local area bounded by Sikkim political boundary. This in itself renders the model more accurate as greater extrapolation often resulted in greater uncertainty (Stohlgren *et al.*, 2001). The predicted potential habitats of the three species were seen in areas beyond KBR and some portion were well covered by the Protected Area Networks outside the Biosphere Reserve (BR) such as, Barsey Rhododendron Sanctuary (104 km<sup>2</sup>) in West Sikkim, Fambonglho

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Wildlife Sanctuary (51.76 km<sup>2</sup>) and Pangolakha Wildlife Sanctuary (128 km<sup>2</sup>) in East Sikkim, and Maenam Wildlife Sanctuary (35.34 km<sup>2</sup>) in South Sikkim.

In addition to habitat distribution modelling, the associated species identified through association analysis, soil and micro environmental features of the habitats where the species occurred did provide adequate empirical data on habitat characteristics of the species, which worked as strong indicators of distribution of the three species in KBR.

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# CHAPTER 6 POPULATION DYNAMICS IN RELATION TO FOREST FRAGMENTATION

#### INTRODUCTION

Traditionally, population ecology has been dealing with the studies in demography and temporal dynamics of species population at a local scale. Considering the rapid and large scale changes in land-use, environmental stochasticity and external influence, on natural populations during the past decade, the study of populations at a higher spatial scale today has become inevitable. In order to understand the population behaviour of the species, which is vital for developing its management strategy, population dynamics at landscape scale need to be understood. Several studies in recent times have explored population dynamics particularly with reference to habitat fragmentation (Bruna and Oli, 2005).

Fragmentation of habitats and forests has brought about drastic changes in the dynamics and configuration of natural populations which are being divided into remnant patches in a landscape. These effects have imposed extreme pressure on the persistence of several species. Plant populations in such habitats have decreased population fitness which may consequently lead to decline in population sizes due to their vulnerability to environmental and demographic stochasticity. This has been the case for ethno-medicinal plants inhabiting the Eastern-Himalayan region due to forest fragmentation. The problem is further aggravated with over-exploitation due to their medicinal values. By repeated census of the population on a regular basis, changes in a population over time can be determined (Simberloff, 1988; Primack and Hall, 1992; Sckemske et al., 1994). Census record can help to distinguish population trends of increase and decrease. Inclusion of spatial characteristics in the population study is important because the size configuration of the population patches can indicate the vitality of separate populations. The number of populations, their size and characteristics are all important considerations in a population study. This was argued by Primack (1995) to be of particular importance for species that is linked by migration *i.e.*, having metapopulation characteristics.

Seedling stage of the plant life cycle is often characterized by high mortality. Therefore, this stage is an important determinant of the overall population dynamics of the species. Survival of seedlings under natural conditions depends on the actions of a large number of climatic, edaphic and biotic variables of the environment. Studies carried out by Harper (1977), Silvertown and Dickie (1981) and Fenner (1985) show that most of the seedlings survive within a narrowly defined limits of moisture, temperature and light. Burton and Muller-Dombois (1984) studied the effect of light on survival and growth of seedlings of different species. Van der Toorn and Pons (1988) studied the influence of photon flux density on survival and growth of *Plantago lanceolata* and *P. major*. All these studies explicitly reveal that light favourably affects the growth and survival of plant populations. Sorensen and Ferrell (1973) investigated the role of temperature on growth of juveniles, while the effect of soil moisture was studied by McLeod and Murphy (1977), Mueller-Dombois *et al.* (1980) and Schulte and Marshall (1983).

Distribution and abundance of a species in the community is significantly influenced by its associates (Putwain and Harper, 1970; Rai and Tripathi, 1984). The neighbouring plants, whether of same or of different species, generally affects the growth of seedlings in various ways (Fenner, 1978; Gross and Werner, 1982; Gross, 1984). Studies of Newell *et al.* (1981) on Viola species reveals that competition may be a cause of seedling death. A number of workers have studied the effect of interspecific competition on growth and survival of various plant species (Sagar, 1959; Sagar and Harper, 1961; Harper and McNaughton, 1962; Harper and Clatworthy, 1963; Cavers and Harper, 1967; Bergh, 1968; Palmblad, 1968; Marshall and Jain, 1969; Tripathi and Harper, 1973; Pradhan and Tripathi, 1980). These studies reveal that seedlings of different species may die due to a number of causes and only a few generalizations can be made in this regard, since even in the same species, causes of mortality may vary from place to place and from one season to another season. High seedling mortality in populations of different plant species have been observed by many workers (Williams, 1970; Sarukhan and Harper, 1973; Sharitz and McCormick, 1973; Hett 93

and Loucks, 1976; Bazzaz and Harper, 1976; Yadav and Tripathi, 1981; Silvertown and Dickie, 1981; Law, 1981; Rai and Tripathi, 1984; Pandey and Dubey, 1989). And a number of authors (Hett, 1971; Sharitz and McCormick, 1973; Mack, 1976; King, 1977; Watkinson and Harper, 1978; Regehr and Bazzaz, 1979; Solbrig *et al.*, 1980; Gross, 1980; Augspurger, 1983; Mack and Pyke, 1984) have attempted to specify the causes of seedling mortality in the field and they have even tried to quantify them. There have been many studies on the responses of plant populations to variation in a single factor, but only a few of them evaluate the relative importance of multiple factors (Newman, 1964, 1965; Sharitz and McCormick, 1973; Greig-Smith and Sagar, 1981; Mack and Pyke, 1983; During *et al.*, 1985; de Jong and Klinkhamer, 1988).

The natural population is usually a mixture of individuals of different ages or cohorts. Early cohorts are often large in size and they show high fecundity and exhibit greater survival rate (Cook, 1980; Zimmerman and Weiss, 1984; Kalisz, 1986; Miller, 1987). Some studies have however, reported lower survival rate of early emerging cohorts (Baskin and Baskin, 1972; Van der Toorn and Ten Hove, 1982). Findings of a large number of studies on different species show that established plants affect the survival and growth of newly recruited individuals in the population (Friedman, 1971; Andel Van and Rozema, 1974; Gupta and Tripathi, 1979; Singh, 1980; Yadav and Tripathi, 1981; Rai and Tripathi, 1984). It has also been established that the species arriving late in the established population show very low density and survival in comparison to those which arrive early (Tamm, 1956; Sagar and Harper, 1960; Cavers and Harper, 1967; Putwain et al., 1968; Hawthorn and Cavers, 1976; Weaver and Cavers, 1979; Weiss, 1981; Rai and Tripathi, 1984; Kataoka et al., 1989; Pandey and Dubey, 1989). Lee and Hamrick (1983) and Mack and Pyke (1983) have recorded the difference in survival and reproductive behaviour of different cohorts recruited to the same natural population. Newell et al. (1981) studied the demography of Viola blanda and V. pallens in relation to habitat types. Seedling recruitment, age specific survival and reproduction in the population of Avena sterilis were studied by Fernandez-Quintanilla et al.

(1986). Lovett Doust (1981) studied the population dynamics of *Ranunculus repens*. Survival, fecundity and growth of wild cucumber, *Echinocystis lobata* were studied by Silvertown (1985). Young and Evans (1990) studied the survival and growth of *Artemisia tridentata*. Germination, emergence, growth and survival of *Ambrosia trifida* were investigated by Abul-Fatih and Bazzaz (1979). Among-site differences in seedling size, growth and survivorship in *Solidago flexicaulis* were studied by Krannitz and Carey (1988). Several studies (Cook, 1980; Solbrig *et al.*, 1980; Fowler and Antonovics, 1981) have reported lower rate of mortality in larger seedlings than the smaller ones. Sharitz and McCormick (1973) demonstrated that colonizing species having high fecundity suffer mortality during seedling establishment.

Survivorship curves of many annual plant species show a uniform mortality risk throughout the life of the population. However, certain species like *Danthonia caespilosa* (Williams, 1970), *Galinsoga ciliata* and *G. parviflora* (Rai and Tripathi, 1984) and *Parthenium hysterophorus* (Pandey and Dubey, 1989) differ in this respect by showing high mortality during young stage. Deevey's type I (Deevey, 1947) survivorship curve characterised by less risk of death during young and middle ages, and high mortality risk at old age is rarely found in plant populations. Canfield (1957) reported such a survivorship curve in case of *Trichache californica, Bouteleva hirsuta* and *B. chondronoides* populations. According to Mack (1976) and Watkinson and Harper (1978), the species that reproduce large number of seeds show Deevey's type II and type III survivorship curves in contrast to those which produce lesser number of seeds and exhibit type I survivorship curve.

The interaction among environment factors and variation in the time of seedling emergence give rise to different successive cohorts of seedlings in most plant populations (Benjamin, 1990). Much work has been done to understand the behaviour of these cohorts during the seedling stage (Misra *et al.*, 1992; Bender *et al.*, 2000). The cohort dynamics of several weedy species of North-Eastern Hill University, India have been studied by Yadav and Tripathi (1981, 1982) and Misra (1992). The present research therefore, did not give much emphasis on the survivorship pattern of such seedling cohorts. Instead, the present work focused on the survival of plants from one stage in the life cycle to the next and the survivorship pattern here was presented more discretely than the earlier survivorship studies of plant populations (Eriksson and Eriksson, 2000; Dinnetz and Nilsson, 2002).

The main objective of this chapter is to analyse and compare the population dynamics of the three selected species *viz.*, *Swertia chirayita*, *Paris polyphylla* and *Panax bipinnatifidus* in continuous forest (CF) and forest fragments (FF) in Khanchendzonga Biosphere Reserve (KBR). The study was expected to provide an insight into the impact of forest fragmentation on populations of the three study species. The following questions were attempted to answer:

(i) How do dynamics of the populations are affected due to forest fragmentation?

(ii) Which of the environmental factors most affected the population dynamics?

(iii) Why all the three study species had a very low population size?

#### **METHODS**

#### 1. Delineation of Sub-populations

The sub-populations are the population units that assemblage to form a larger population or metapopulation. It is also defined as a patch which is an integrated population unit in a landscape. The regional distribution of the three species was determined by investigating all possible forest habitats within the study area. Populations were systematically surveyed by walking fifty transects of 50 m x100 m dimension along the accessible trekking path. All the populations encountered were mapped. A population was defined as an individual patch separated by a distance of at least 100 m from the nearest neighbor patch. This criteria was also followed by (Kolb (2005) and Jacquemyn *et al.* (2006). Patch isolation was measured as the mean distance between the two nearest populations. To estimate the area occupied by the population, the outermost plants of a population were marked on a map and the area of the convex polygon defined by these plants was determined using Geographical Information System (GIS) software ArcMap 9.3.

The sub-populations were categorized according to the habitats of occurrence (Table 6.1). Swertia chirayita populations occurred in four habitats or group type (henceforth referred to as group) viz., continuous forest (CF), forest fragments (FF) and shrubland populations (SH). Populations of Paris polyphylla and Panax bipinnatifidus occurred in groups *i.e.*, CF and FF.

Table 6.1: Details of population groups of Swertia chirayita, Paris polyphylla and Panax bipinnatifidus

STOLEN CONTRACTOR	Group/Habitat	Solution and the second s
Swertia chirayita	i. Continuous forest-sub-tropical	CFS
	ii. Continuous forest-temperate	CFT
	iii. Forest fragments	FF
	iv. Shrublands	SH
Paris polyphylla	i. Continuous forests	CF
	ii. Forest fragments	FF
Panax bipinnatifidus	i. Continuous forests	CF
	ii. Forest fragments	FF

#### 2. Population Size

Population size was estimated during peak flowering season for all the three species. Mature individuals were counted in the case of *Swertia chirayita*. For the two perennials *Paris polyphylla* and *Panax bipinnatifidus*, the population size was determined by counting the total number of individuals including all stages in a life cycle. This was chosen because unlike *Swertia chirayita*, all the life cycle stages of these two species grow simultaneously during the growing season. For populations having very few individuals, the total number of plants was counted, while for larger populations, estimates were based on the total number of reproductive individuals per unit area. This was determined through laying 3-10 replicated quadrats of 1 m x 1 m size depending on the population size and area of occupancy. The population size of each patch was extrapolated by multiplying the area of each patch with the number of reproductive individuals per unit area of that population.

#### **3.** Annual Growth Rate $(\lambda_A)$

Since all the three species exhibited discrete population growth pattern, the population growth rate between three transition years *i.e.*, 2005-2006, 2006-2007 and 2007-2008 was calculated following the formula:  $\lambda = N_{(t+1)}/Nt$  following Morris and Doak, 2002; where N<sub>t</sub> is

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the population size at time t and  $N_{(t+1)}$  is its size after time t. A subscript '<sub>A</sub>' was added to  $\lambda$  to differentiate it from the finite rate of increase expressed in chapter 7. Populations with  $\lambda_A$  >1 indicates an increase in population size;  $\lambda_A = 1$  shows no growth, whereas,  $\lambda_A < 1$  signifies a decline in growth.

#### 4. Population Dynamics in Continuous Forest and Forest Fragments

To initiate demographic studies, the life cycle of the three species was divided into discrete life-stages based on preliminary survey prior to conducting detailed demographic studies. Demographic studies on the three species were conducted for four years beginning 2005. For *Swertia chirayita*, the complete demographic data pertaining to three generations were studied (2005, 2006 and 2007). While for long-lived *Paris polyphylla* and *Panax bipinnatifidus*, a 4-years demographic census was completed during 2005 and 2008. The study was performed in permanent plots which were established in each population (n= 72 plots for *Swertia chirayita*, n= 44 plots for *Paris polyphylla* and n= 28 plots for *Panax bipinnatifidus*) (Table 6.2).

<b>Children</b>		Code	No. of	No. of plots	Total	
			papulation	of codines 2	noed plate	
Swertia	i. Continuous forest-sub-tropical	CFS	7	4	28	1
chirayita	ii. Continuous forest-temperate	CFT	3	4	12	1
	iii. Forest fragments	FF	3	4	12	1
	iv. Shrublands	SH	5	4	20	1
Paris	i. Continuous forests	CF	7	4	28	4
polyphylla	ii. Forest fragments	FF	4	4	16	4
Panax	i. Continuous forests	CF	4	4	16	4
bipinnatifidus	ii. Forest fragments	FF	3	4	12	4

 Table 6.2: Number of populations and total number of sampling plots of Swertia chirayita, Paris polyphylla and Panax bipinnatifidus

The size of the plot was decided considering the plant demography and area of occupancy of plants. For *Swertia chirayita*, the plot size was 1 m x 1 m and each plot was demarcated by putting boundary structures made of bamboo. For *Paris polyphylla* and *Panax bipinnatifidus*, the plot size was 2 m x 2 m and the plants were identified, tagged and counted during successive censuses. The populations of the two latter species were very sparse *i.e.*, 0.39

 $\pm 0.03$  plants/m<sup>2</sup> for *Paris polyphylla* and 0.22 plants/m<sup>2</sup> for *Panax bipinnatifidus*. Demographic census therefore, was based on the number of individuals that was recorded in each of the plots. For tagging, plants within the plots were tagged with a small engraved aluminium tag. In order to avoid the attention of the people, the tags were placed at the base of the plant but below the soil surface. The tag was then relocated during regular seasonal census from 2005 to 2008. Randomly selected 10 places in each population were tagged for studying growth and phenological aspect of the species. Although census was carried out three times a year from 2005 to 2008, the demographic data was presented bi-annually for *Swertia chirayita* because demographic stages were best represented during those time intervals. The demographic data of *Paris polyphylla* and *Panax bipinnatifidus* were presented at yearly interval because of their slow growing and long-lived nature. This was according to Jacquemyn *et al.* (2008) who studied *Paris quadrifolia* and Nantel *et al.* (1995) who studied *Panax quinquefolium*.

### 5. Measurement of Micro-environmental and Soil Factors

Micro-environment and soil factors were measured during pre-monsoon, monsoon and post monsoon period. Climatic variables such as light intensity, relative humidity and air temperature were recorded at 1m above ground level at each population of the three species at five hourly intervals during the day of measurement. Each parameter was measured at ten random points adjacent to the demographic plot of all populations. Light intensity was measured by a Digital Luxmeter (TES 1332A), while air temperature and relative humidity were measured by a Thermo hygrometer TH–103 (Mex–therm). The mean values for the microclimatic parameters were used for comparing the variables among the populations of each of the three species.

Soil samples were collected on a seasonal basis for three annual cycles during 2006 and 2008. One composite sample for each population patch was prepared by mixing soil samples collected from the five replicate points in each patch. Soil temperature was measured using a digital portable soil thermometer (Multi–Thermometer). Soil pH was estimated by a digital

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portable pH meter. Edaphic variables *viz.*, soil moisture content (SMC), water holding capacity (WHC), soil organic carbon (SOC), total Kjeldahl nitrogen (TKN), available phosphorus (P) and exchangeable potassium (K) were estimated in the laboratory following the methods described in Chapter 5.

#### **Statistical Analyses**

Comparative analysis of the survivorship curves between the CF and FF populations was analyzed using survival analysis of Mantel-Cox (log-rank test), Gehan-Breslow-Wilcoxon test and Kaplan-Meier survival function (Hutchings *et al.*, 1991; Lee, 1992; Krebs, 1999). The Gehan-Breslow-Wilcoxon test gives weights to early deaths while Mantel-Cox is more reliable if the rate of death is more or less similar through time. To identify the most important environmental variables related to the growth rate and survival of plant populations, forward stepwise multiple regression analysis was performed considering environmental parameters as explanatory variables and plant density as dependent variable using Statistica-version 6 (Statistical Software Inc. 2001). The analysis was performed by adding parameters sequentially starting from no variable in the model, and then adding the most significant explanatory variables, *i.e.*, the one with the lowest p- value, at each step until all variables were added. The variables which had significant correlation with the species parameter were tested for their difference in different groups i.e., CFS, CFT, FF and SH in *Swertia chirayita*, and CF and FF in *Paris polyphylla* and *Panax bipinnatifidus*.

#### RESULTS

#### **Demographic Stages**

*Swertia chirayita*: The species had three major stages namely (i) Small rosette:- Seedling rosette individuals that arose singularly from the soil surface during the spring season, (ii) Large rosettes:- Mature individuals in rosettes states that survived through monsoon season, (iii) Vegetative aerials:- Plants that develop into an erect vegetative stem with a few or no rosette leaves but with various aboveground branching, and (iv) Flowering:- Reproductive individuals representing the ultimate stage of the species prior to senescence (Plate 6.1).

**Paris polyphylla:** The species comprises of three stages *viz.*, (i) seedling (ii) subadult/juvenile, and (iii) reproductive adult. All stages had similar morphology except size. The plant had a stout rhizome and a single shoot which terminates in a single bud that develops into flower or that may abort (Plate 6.2).

**Panax bipinnatifidus:** The life history of *Panax bipinnatifidus* consisted of 5 stages *i.e.*, seedling stages and four other leaf-stages from 1-leaved to 4-leaved stage. 3 and 4-leaved stage are reproductive individuals (Plate 6.3).



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Small Rosette



Large Rosette



Vegetative Aerial

Flowering

a manager of





Seedling



Adult



Flowering

Plate 6.2: Life stages of Paris polyphylla



Seedlings



1-leaved

2-leaved



3-leaved

4-leaved

Plate 6.3: Life stages of Panax bipinnatifidus

## **Spatial Characteristics of Populations**

Swertia chirayita: The metapopulation of Swertia chirayita in KBR consisted of a total of 18 populations (Table 6.3). These were classified into 4 demographic groups based on their similarity of habitat of occurrence within the CF, FF and SH (designated as CFS, CFT, FF and SH). Of these, 7 populations occurred in CFS, 3 each in CFT and FF, and 5 others are located in the SH along KBR boundary. The mean patch was 2.56 ha in CFS, 1.55 ha in CFT, 0.47 ha in FF and 1.32 ha in SH. The average density of plants in CFS, CFT, FF and SH was 2.56  $\pm 0.75$ , 1.55  $\pm 0.11$ , 0.38  $\pm 0.22$  and 1.21  $\pm 0.54/m^2$ , respectively. The mean isolation of populations was 0.29  $\pm 0.13$ , 0.54  $\pm 0.05$ , 0.28  $\pm 0.83$  and 0.31  $\pm 0.16$  km in CFS, CFT, FF and SH, respectively.

이 같은 사람이 많은 것이 없다.		stan na		(Ma) × Propile ii	ne s Ruo unio	
				densus/r	it zhaisand	Manager
	CFS	TCF1	2.40	35	0.21	
		TCF2	1.55	23	0.19	
		TFE1	2.90	60	0.38	
		TFE2	6.30	65	0.55	0.29 (0.13)
		TFE3	1.20	19	0.26	
		TFE4	0.10	2	0.32	
		TFE5	3.50	58	0.17	
Swertia	CFT	YCF3	1.50	12	0.59	
chirayita		YCF4	1.76	17	0.54	0.54 (0.05)
		YFE6	1.40	11	0.49	
	FF	TFF1	0.20	2	0.37	
		TFF2	0.90	9	0.25	0.28 (0.83)
		CFF3	0.30	3	0.21	
	SH	TS1	0.70	11	0.43	
		TS2	3.40	82	0.50	
		TS3	1.20	17	0.30	0.31 (0.16)
		TS4	0.30	4	0.18	
	_	CS5	1.00	11	0.45	

Table 6.3: Sp	patial characteristics o	populations	of Swertia chirayii	ta in KBR
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<sup>a</sup> (mean distance from three nearest population). Value in parentheses is the corresponding standard deviation of the mean



Figure 6.1: Location of 18 populations of Swertia chirayita in the buffer zone of KBR

**Paris polyphylla:** Paris polyphylla metapopulation had 11 populations out of which 7 were in CF and 4 were in FF. The mean patch size of individual population patches was 2.85  $\pm 0.93$  ha and 1.06  $\pm 0.45$  ha in CF and FF, respectively. The density of plants in CF was 0.43  $\pm 0.62/m^2$  and in FF it was 0.35  $\pm 0.10/m^2$ . The mean isolation of populations was 0.75  $\pm 0.25$ km in CF and 0.44  $\pm 0.17$  km in FF (Table 6.4).

	CF	BCF1	7.37	0.7	1.09	and an
		KBCF2	3.04	0.5	0.84	
		KCF3	5.41	0.4	0.49	
		YCF4	0.42	0.2	2.10	0.75 (0.25)
Paris		NCF5	2.26	0.6	0.23	. ,
polyphylla		TBCF6	1.04	0.3	0.40	
		ANCF7	0.41	0.3	0.24	
	FF	BFF1	0.22	0.2	0.97	
		NFF2	2.56	0.7	0.30	0.44 (0.15)
		FFF3	0.45	0.3	0.27	0.44 (0.17)
		FTFF4	1.01	0.2	0.31	

Table 6.4: Spatial characteristics of populations of Paris polyphylla in KBR

<sup>a</sup> (mean distance from three nearest population). Value in parentheses is the corresponding standard deviation of the mean



Figure 6.2: Location of 11 populations of Paris polyphylla in the buffer zone of KBR

**Panax bipinnatifidus:** Panax bipinnatifidus metapopulation had only 7 populations, out of which, four were in CF and 3 in FF. The mean area of population patch in CF was  $3.7 \pm 0.98$  ha, while that in FF it was  $1.19 \pm 0.42$  ha. Total plant density was  $0.04 \pm 0.01/m^2$  in CF and  $0.02 \pm 0.00/m^2$  in FF populations. The mean patch isolation of CF populations was  $0.32 \pm 0.01$  km and that of FF populations it was  $1.26 \pm 0.74$  km (Table 6.5).

		Populatio	n Papiling	(hr) Kapolation	Populou Trankani	n iolinging Kurter
	CF	DCF1	7.22	0.06	0.30	
		DCF2	1.25	0.05	0.31	0.00 (0.01)
		KCF1	3.86	0.03	0.34	0.23 (0.01)
		KCF2	2.55	0.03	0.34	
Panax	FF	NFF1	0.83	0.01	2.95	
bipinnatifidus		DFF2	2.22	0.03	0.32	1.26 (0.74)
		PFF3	0.54	0.02	0.97	

Table 6.5: Spatial characteristics of populations of Panax bipinnatifidus in KBR

<sup>a</sup> (mean distance from three nearest population). Value in parentheses is the corresponding standard deviation of the mean



Figure 6.3: Location of 7 populations of Panax bipinnatifidus in the buffer and core zone of KBR

#### **Relationship between Plant Density and Patch Size**

The density of plants in *Swertia chirayita* populations was strongly correlated with the patch size (r= 0.87). This was also observed in *Paris polyphylla* (r= 0.70), while in *Panax bipinnatifidus*, no relationship was obtained. However, there was an increase in plant density with increasing patch size (Figure 6.4).

The relationship between plant density and patch isolation however, did not show any significant correlation in *Swertia chirayita* and *Paris polyphylla*. In *Panax bipinnatifidus* however, there was a negative linear relationship (r= 0.77) indicating the negative impact of population isolation on plant density.



Figure 6.4: Relationship between plant density of *Swertia chirayita*, *Paris polyphylla* and *Panax bipinnatifidus* and patch characteristics of the populations

# Annual Growth Rate $(\lambda_A)$

Swertia chirayita: Most populations of CFS group had constant growth rate during the first and second transition years, while there was an increase in population size during the third year transition with 5 populations having  $\lambda_A > 1$  (Figure 6.5). All populations of CFT and FF progressively declined in growth during the transition phases. Although 2 population (TFF1 and TFF2) had increased growth ( $\lambda_A > 1.0$ ) during first transition year, all the three populations had annual growth rate fell below 1.0 during the second and decreased much further in the third year (Figure 6.5). The populations of SH on the other hand, fluctuated between growth and decline among the three transition years with only TS4 showed progressive decline throughout. While in first transition year there were 2 populations *i.e.*, TS4 and TS1 with  $\lambda_A > 1$ , in the third transition year 3 populations were more than 1.0 (TS1, TS3, TS2). The FF populations were lose to the constancy in the first and second transition years but nevertheless had declined rate. Overall, CFT and SH populations had relatively more deviation from the constant growth rate (Figure 6.5).





**Paris polyphylla:** In *Paris polyphylla*, 4 populations of CF (BCF1, YCF4 and NCF5) had alternate growth and declined during the three transition years (Figure 6.6). 3 populations (TBCF6, KCF3 and ANCF7) declined gradually towards the third year. While KBCF2 declined sharply in the second year and showed little growth during third transition year. On the other hand, three populations of FF (BFF1, NFF2, FFF3) also showed alternating trend 110

where there was growth in the second year but declined in the third year. One FF population *i.e.*, FTFF4 however, declined progressively till the third year (Figure 6.6).



Figure 6.6: Annual growth rates of Paris polyphylla

**Panax bipinnatifidus:** Panax bipinnatifidus populations in CF had declining growth rate during the first phase but two populations viz., KCF1 and KCF2 showed significant growth with  $\lambda_A > 1$  (Figure 6.7). While the other two CF populations (DFC1, DCF2) were characterized by gradual increase during the three years and  $\lambda_A > 1$  was reached in the third year. Only KCF1 again declined sharply in the third year by more than 70%. One FF population *i.e.*, NFF1 had  $\lambda_A > 1$  in the first year and increased marginally during the second year; however, it declined unexpectedly to  $\lambda_A < 1$  in the third year. DFF2 population on the other hand, showed a decline in population during the first year but showed growth in the second and third year. PFF3 population had alternate growth and declined during the three transition years (Figure 6.7).

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Figure 6.7: Annual growth rates of *Panax bipinnatifidus* 

The scatter plot in Figure 6.8 revealed the relationship between annual growth rate and patch size. Out of the three species only *Swertia chirayita* had significant positive correlation with patch size of the populations.



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Figure 6.8: Relationship between annual population growth rate and patch size of Swertia chirayita, Paris polyphylla and Panax bipinnatifidus

# **Population Dynamics**

#### Swertia chirayita

*Survivorship pattern*: The population dynamics for the three cohorts recruited in 2005, 2006 and 2007 were studied. All the three cohorts of the populations exhibited concave survivorship curve (Figure 6.9). A notable decline in population was observed during the transition from seedling stage to vegetative-rosette stage, and the individuals exhibited higher survival during latter stage *i.e.*, from vegetative-aerial to reproductive stage. Overall, the populations in CF and forest edge had better survivorship than those in FF and SH (Figure 6.9).



Figure 6.9: Survivorship curves for different populations of *Swertia chirayita* along the four life history stages: small rosette (SR), large rosette (VR), vegetative aerial (VA) and flowering (Fl)

The difference in survivorship curves between the four groups was analyzed by pair-wise comparison of CFS group with CFT, FF and SH separately (Table 6.6). Significant difference in the shape of survivorship curve was observed between CFS and FF, while no significant difference was observed in other paired comparisons. However, those significant different was also revealed by only log-rank test ( $\chi^2$ = 4.24, p= 0.039), and Gehan-Breslow-Wilcoxon test did not reveal any significance difference between any pairs (Table 6.6).

 Table 6.6: Pair-wise comparison (log-rank test (Mantel-Cox) for the survivorship of plants between CFS, CFT, FF and SH of Swertia chirayita

Contract Contract	0.032010	CES BL	CES-SH	CRI-SH	CRAF	SHORE
Ing-rank (Mantel-Cox) test.		and the second se				
Chi square	1.877	4.241	0.4478	0.5421	0.4584	2.051
df	1	1	1	1	1	1
p value	0.1707	0.0394	0.5034	0.46	0.4984	0.1521
		*				Ma
p value summary	กร	+	ns	ns	ns	INS
Hazard ratio	ns	, ,	ns	ns	ns	INS
Flazard ratio Ratio	ns 0.6823	0.5533	ns 0.8337	1.236	0.8115	0.6529
P Value summary Flazard ratio Ratio 95% CI of ratio	ns 0.6823 0.3949	0.5533 0.315	ns 0.8337 0.4894	1.236 0.7029	0.8115 0.4434	0.6529 0.3643

\* -Significant, ns- Not significant

Since the curve was Deevey type II, the differences in survivorship curves of CFT and FF was significantly different as represented by the Kaplan-Meier curve (Figure 6.10). The slope of the curve was quantified in Prism 5.0 by Hazard ratio (a measure of how rapidly plants were dying) using the Mantel-Haenszel method. The slope had a hazard ratio of 0.6 (95% CI= 0.32 to 0.97) which indicated that the rate of deaths in CFS was 40% lower than that of FF populations.



Figure 6.10: Kaplan-Meier estimate of the survival function between CFS each with (A) CFT, (B) FF and (C) SH

## Paris polyphylla

Survivorship pattern: The survival of seedlings exhibited an identical pattern in all the populations where the annual mortality was more or less uniform except in two populations *i.e.*, YCF and ANCF population of CF where all seedlings died in 2007 (Figure 6.11). Similarly, seedling survivorship in FF populations also had similar pattern. Five out of seven CF populations had seedlings survival till 2008 while in FF, two out of four populations had seedlings that survived till 2008. However, the overall seedling survival till 2008 in CF was 3.9% while in FF it was 5.9%. Survival of adult individuals depicted a sharp decline during the first year, followed by low mortality in subsequent years and for CF1 population, it was
constant till the year 2008. ANCF had no adult survival and so in FFF population. The reproductive individuals had better survivorship compared to the two preceding stages both in CF and FF (Figure 6.11).



Figure 6.11: Survivorship curve for different stage individuals of Paris polyphylla

The test for survival curve did show significant difference in average survivorship both by log-rank test ( $\chi^2$ = 3.86, p= 0.049) and by Gehan-Breslow-Wilcoxon test ( $\chi^2$ = 4.23, p= 0.039) (Table 6.7). Hazard ratio of 0.63 (CI at 95% = 0.39 to 0.99) was observed for Kaplan-Meier curve suggesting that the rate of death in CF was 47% less than that of FF (Figure 6.12).

 Table 6.7: Comparison (log-rank test (Mantel-Cox) and Gehan-Breslow-Wilcoxon test) of survivorship of plants between CF and FF of Paris polyphylla

	And
Log-rank (Mantel-Cox) test	
Chi square	0.02789
df	1
p value	0.8674
p value summary	Ns
Gehan-Breslow-Wilcoxon test	
Chi square	0.01696
df	1
p value	0.8964
p value summary	Ns
Hazard ratio	
Ratio	1.036
95% CI of ratio	0.6838 to 1.570



Figure 6.12: Kaplan-Meier estimate of the survival function between CF and FF

# Panax bipinnatifidus

Survivorship pattern: The survivorship curve for seedlings was concave and consistent for all populations (Figure 6.13). The one-leaved and three-leaved stages had better survivorship in all populations and there was approximately only a linear increase in mortality during the study period. Two-leaved stage on the other hand, represented a transient stage with fewer individuals and was encountered in only one population of CF *i.e.*, DCF1 and only 4.7% survived till the year 2008. The occurrence of four-leaved stage was also very rare and found only in DCF2 and KCF1 populations of CF. 100% mortality was observed in KCF1 during the first year of census *i.e.*, 2006 while in DCF2, mortality was observed in the first year, after which, it remained constant till the third year, and declined in the fourth year with 16.7% survival.



Figure 6.13: Survivorship curve for different stage individuals of Panax bipinnatifidus

The survivorship curves between the populations of CF and FF did not show any significant difference by both log-rank test and Gehan-Breslow-Wilcoxon test (Table 6.8). The difference in the slope as seen in Kaplan-Meier curve was almost overlapping, and the deviation was marginally visible at the third year (Figure 6.14). Hazard ratio of 0.63 (CI at 95% = 0.39 to 0.99) also inferred marginal difference in the rate of death between CF and FF populations.

 Table 6.8: Comparison of survivorship of plants (log-rank test (Mantel-Cox) and Gehan-Breslow-Wilcoxon test) between CF and FF of Panax bipinnatifidus

Log-runk (Mantel-Cox) test	
Chi square	3.859
df	1
p value	0.0495
p value summary	*
Gchan-Breslow-Wilcoxon test	
Chi square	4.277
df	1
p value	0.0386
p value summary	*
Hazard ratio	
Ratio	0.633
95% CI of ratio	0.399 to 0.999



Figure 6.14: Kaplan-Meier estimate of the survival function between CF and FF

#### **Effects of Micro-environment on Population Dynamics**

The relationship between micro-environmental variables and different attributes of population was analyzed using the stepwise forward regression model (Table 6.9, 6.10 and 6.11). Twelve micro-environmental variables were used and regressed against population attributes, and each was evaluated independently and only those variables having F value  $\geq 1$  (standard value) were entered into the model.

Swertia chirayita: In Swertia chirayita, only a few variables met the criteria of  $F \ge 1$  in multiple regressions (Table 6.9). The pre-monsoon was the time of seedling recruitment and the emergence of vegetative aerial plants. Regression of each of these with pre-monsoon micro-environmental conditions revealed that seedling recruitment was independent of the environmental conditions. Whereas, the survival of plants into vegetative aerial was correlated with three soil parameters *viz.*, soil moisture content, soil TKN and soil temperature, each with a standard coefficient of 1.08 at p< 0.0, -11.08 at p< 0.05 and 2.39 at p< 0.01, respectively (Table 6.9). The post-monsoon phase marked the growth and survival of vegetative rosettes, reproductive plants and the overall annual growth rate of the population. Multiple regressions yielded only soil chemical properties to significantly relate with population characteristics of *Swertia chirayita*. The growth and survival of vegetative rosette was significantly correlated with soil moisture content with regression coefficient of 1.19

2.21 at significant level p< 0.05. Survival of reproductive plants was dependent on pH and available phosphorus, each with a coefficient of 0.47 and 0.76 which were significant at p< 0.05. The mean annual growth rate of populations for three transition years *i.e.*, from 2005 to 2008 was correlated with soil moisture content (regression coefficient= 0.04) and TKN (coefficient= -1.43) each significant at p< 0.01.

**Table 6.9:** Regression models of population growth rate and survival rate at seedling, adult and reproductive stage with environmental variables and soil physical-chemical properties in *Swertia chirayita*. The independent variables of the equations were significant at p< 0.05 (t-Test) after running forward stepwise multiple regression analysis

of Content Variable (a)	Regression equation.	n Adjusted	piere
Swertia chirayita		18	
Population growth rate (annual)	Y = -12.19 + 0.35 x pH + 0.51 x AT	0.47	p< 0.01
Aerial vegetative (pre monsoon)	Y = -101.26 + 1.09  x SMC + 2.39  x ST -11.08 x TKN	0.61	p< 0.01
Large rosette (post monsoon)	Y = -55.58 + 2.21  x SMC	0.55	p< 0.01
Reproductive (post monsoon)	Y = -8.37 + 0.77 x P + 0.47 x pH	0.38	p< 0.02

AT= Air temperature; ST= Soil temperature; SMC= Soil moisture content; N=Total Kjeldahl nitrogen; P=Available phosphorus

**Paris polyphylla:** The species being perennial, all life stages were represented throughout the growing season of the species. Therefore, the population attributes were correlated with the mean annual micro-environmental conditions. A stepwise forward multiple regression yielded six variables that strongly influenced population at different life stages (Table 6.10). These were three soil chemical properties *viz.*, pH, TKN and available phosphorus, and three micro-environmental parameters *viz.*, soil temperature, light intensity and relative humidity. Variation in pH significantly affected seedlings and adult individuals (coefficient = -12.07 and 14.73, respectively at significant level p< 0.05). TKN and available phosphorus affected seedling survival only (each with a coefficient of -83.03 and -5.32, respectively at significant level p< 0.05). Seedling was also influenced by light intensity with a coefficient of -1.04 at significant level p< 0.01. Light intensity was also correlated with adult survival with a regression coefficient of -1.60 which was significant at p< 0.01. Reproductive individuals on the other hand, showed significant correlation with soil temperature with a coefficient of -49.81 which was significant being significant at p< 0.05. The annual growth rate trend 120 during the four years of study showed dependency on only one variable *i.e.*, relatively

humidity.

**Table 6.10:** Regression models of population growth rate and survival rate at seedling, adult and reproductive<br/>stage with environmental variables and soil physical-chemical properties in *Paris polyphylla*. The<br/>independent variables of the equations were significant at p< 0.05 (t-Test) after running forward<br/>stepwise multiple regression analysis

	Repression equation	.0	Adjusted	p (2-51
Paris polyphylla Population growth rate	$Y = -3.88 + 0.04 \times RH$	11	0.72	p< 0.00
Seedling	Y = 137.46 – 1.04 x LI – 12.07 x pH – 5.32 x P – 83.03 x TKN		0.94	p< 0.00
Adult	Y = 35.60 - 1.60  x LI - 14.73  x pH		0.84	p< 0.00

RH= Relative humidity; LI= Light intensity; P=Available phosphorus; TKN=Total Kjeldahl nitrogen

**Panax bipinnatifidus:** Regression of *Panax bipinnatifidus* populations with microenvironmental variables did not yield any relationship with most of the parameters. Seedlings were however influenced by the level of exchangeable potassium in the soil and a regression coefficient of 1.18 that was significant at p< 0.05 was observed (Table 6.11). Individuals in one-leaved through 4-leaved stages did not show any relationship with the soil and micro-environment. The overall dependency of the population was however revealed in the relationship of the mean annual growth rate which was correlated with two parameters *i.e.*, soil water holding capacity and pH, each had regression coefficient of -0.05 and 0.67, respectively both significant at p< 0.01 (Table 6.11).

Table 6.11: Regression models of population growth rate and survival rate at seedling, adult and reproductivestage with environmental variables and soil physical-chemical properties in Panax bipinnatifidus.The independent variables of the equations were significant at p< 0.05 (t-Test) after running</td>forward stepwise multiple regression analysis

		er alle stilluster	
<b>Panax bipinnatifidus</b> Population growth rate	Y = 0.67 - 0.05  x WHC + 0.67  x pH	0.83	p< 0.01
Seedling survival	Y = 91.60 + 1.18 x K	0.76	p< 0.05

WHC= Water holding capacity; K= Exchangeable potassium

Each of the micro-environmental variables that were significantly correlated with population attributes were analyzed for the significance of variation between the four demographic groups (CFS, CFT, FF and SH) of *Swertia chirayita* and between the CF and FF for *Paris* 

polyphylla and Panax bipinnatifidus (Table 6.12), and their concentration in the different groups were also compared (Figure 6.15, 6.16 and 6.17). In Swertia chirayita, the difference in environmental condition among the groups was significant in all correlated microenvironment except available phosphorus, as revealed by Kruskal-Wallis test (Table 6.12). Soil moisture content was significantly different among the four groups during pre-monsoon  $(\chi^2 = 6.41, p = 0.04)$  and post-monsoon  $(\chi^2 = 6.24, p = 0.04)$  period. The variation in pH among populations was also significant during pre-monsoon  $(\chi^2 = 9.10, p = 0.03)$  and post-monsoon  $(\chi^2 = 8.13, p = 0.04)$  period. In Paris polyphylla, out of all six micro-environmental variables that were found to influence population growth and survival, only relative humidity was significantly different between the populations of CF and FF as revealed by Kruskal-Wallis test  $(\chi^2 = 4.34, p = 0.03)$  (Table 6.12). For Panax bipinnatifidus on the other hand, only exchangeable potassium concentration was significantly different  $(\chi^2 = 4.5, p = 0.03)$  between CF and FF populations (Table 6.12).

 Table 6.12: Kruskal-Wallis test for the difference in selected micro-environment between the four groups (CFS, CFT, FF and SH) of Swertia chirayita and between the CF and FF of Paris polyphylla and Panax bipinnatifidus during pre-monsoon and pot-monsoon period

Swertia chirayit	a										
		2. X X			i estatu				3.955		
		2		2°						2	D
	0 <u></u>	<u> </u>	<u> </u>	<u> </u>	Р	<u> </u>	P	<u> </u>	<u> </u>	<u> </u>	<u> </u>
Pre-monsoon	3	6.41	0.04	9.10	0.03	10.32	0.016	6.890	0.08	13.17	0.004
Post-monsoon	3	6.24	0.04	8.13	0.04	10.90	0.012	3.92	0.27	6.33	0.09

Paris polyphylla

Panar hininnatifidus

ւ սոսո օւբսոսայա	144.3						
		STATE OF LEVEL	(%))	i je pl	1 2	K K	(URIO)
		x <sup>2</sup>	р	r i	р	x²	P
Mean annual	1	0	1	2.577	0.101	4.5	0.0339

SMC= Soil moisture content; pH= Soil pH; N=Total Kjeldahl nitrogen; P=Available phosphorus; ST= Soil temperature; LI= Light intensity; RH= Relative humidity; WHC= Water holding capacity; K= Exchangeable potassium

### DISCUSSION

The population dynamics of the three species described their status in their respective habitats of occurrence. Each being forest inhabited was influenced by the pattern of change in landscape particularly forest fragmentation which has been occurring in KBR.

#### Swertia chirayita

The populations of Swertia chirayita occurred in a wide range of habitats (CFS, CFT, FF and SH). Its occurrence in varied habitats with varied environmental conditions was probably because of its broad range of tolerance to light and moisture, and also to different soil types as reported by Joshi and Dhawan (2005). In KBR, 56% of the populations occurred in forest habitats (7 in CFS and 3 in CFT) indicating forest as the preferred habitat. This is in consistent with a few other species of Swertia such as S. angustifolia (Bhatt et al., 2007). The populations are patchily oriented in the habitat, each with small patch size separated by suitable as well as unsuitable habitats. The positive correlation of patch size with plant density was obvious. Patches with small sizes had relatively low plant density, signifying vulnerability of small populations to demographic stochasticity and genetic effects in the form of inbreeding depression. On the other hand, threats to large population come from patch isolation as the mean isolation of population patches was found to increase to some extent with increasing patch size. This indicated that there is a potential threat to large population because distantly located populations are vulnerable to numerous disturbances and also limiting seed dispersal to other populations. However, since Swertia chiravita dispersed exclusively through seed dispersal, the constraint that patch isolation can cause on populations may not be so severe. This was supported by Johansson and Ehrlén (2003) who predicted that habitat isolation should constrain the distribution of the vegetatively dispersed plants more than those which are sexually dispersed. Nonetheless, the mean patch size and plant density among the habitat groups was larger in the populations of CF (CFS and CFT) and least in the populations of FF. SH represented a transition habitat for the populations of Swertia chirayita, and it had moderate mean patch size and plant density

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compared to FF populations. The populations of CFT and FF were evidently more susceptible to the negative effects due to their reduced patch size and plant density. This was also in the case of FF populations because of their relatively small patch size and low plant density, while the low plant density coupled with high isolation of the population patches in CFT was detrimental to the populations in this habitat. The significant relationship between patch size and annual growth rate trend also provided important insights into the role of patch size in propagating the population of the species. The survivorship curve of *Swertia chirayita* was concave which indicated high mortality among the young or early stages, and in this case, the vegetative rosettes thereby following Deevey type-III survivorship curve. Consequently, high mortality at this stage resulted in significant variation of survivorship among the populations. The latter was proven by standard comparison of survivorship curves (log-rank and Gehan-Breslow-Wilcoxon tests), although only the average survivorship between CFS and FF was shown to be significantly different.

Seedling recruitment did not have significant relationship with any of the microenvironmental variables. This may be the possible explanation for the fact that *Swertia chirayita* is able to establish in a wider range of habitat than other forest plant species although the survival in subsequent stages differed in different habitats and as discussed above, the survival was significantly different in CF and FF. The effect of microenvironment in *Swertia chirayita* was however significant in latter stages with vegetative rosettes having significant relationship with soil moisture content, TKN and soil temperature. The soil moisture on the other hand, was important to the development of vegetative aerials since emergence from perennating rhizome required moisture and hence, the significant relationship. Reproductive plants were significantly correlated with pH and available phosphorus. Optimum soil pH is required such that nutrients are available to the plants and available phosphorus is known to stimulate flowering (Erel, 2008). The annual growth rate was correlated with soil moisture content and TKN. Therefore, it is indicative that the positive annual growth rate seen in CFS was partly due to TKN and soil moisture

content. Moreover, there was significant difference in the level of these parameters between the groups with CFS having more TKN and soil moisture than FF or CFT and SH (Figure 6.15).



Figure 6.15: Levels of micro-environment and soil properties (soil temperature, pH, moisture content, TKN and available phosphorus) that were significantly related to *Swertia chirayita* in the four habitat groups

# Paris polyphylla

The total area occupied by CF populations was 20 ha which was much higher than that occupied by FF populations (4.3 ha). This disparity in the size of the population patches

indicated that there was more habitat availability and better habitat quality for the species in CF. There was also a difference in density of plants between the two habitats, and a linear relationship between plant density and patch size of the population was observed. Therefore, it is evident that the populations in CF had better fitness than in FF. However, the isolation of the population patches in CF was more than those of FF populations signifying that the FF populations may be more connected by inter-dispersal. Better fitness of CF populations was also apparent in the annual growth trend where annual growth rate was greater than those in FF particularly in the first and second transition years. Comparison of survivorship curve between CF and FF populations however, did not reveal any significant difference suggesting that, for *Paris polyphylla*, some processes are affected by forest fragmentation such as the patch characteristics (size, plant density) and overall growth rate as seen above. However, other population aspects such as survivorship were not noticeably affected.

Effect of micro-environment on *Paris polyphylla* as analyzed by multiple regression, revealed varied dependency of plants at different stages. Seedlings survival was affected by maximum numbers of parameters *viz.*, soil pH, TKN, available phosphorus and light intensity. Adult plants were related with pH and light intensity. Reproductive plant survival was correlated with soil temperature. The correlation of *Paris polyphylla* to many environmental parameters partly elucidated the habitat specificity of the species within the forest. The mean annual growth rate was correlated only with relative humidity. Although humidity did not have any relationship with the survival and growth, it did however have some significance in the growth of the populations; this may be the primary reason why *Paris* thrives better in moist and deep shade environment. This finding is consistent with a study on *Paris quadrifolia* by Jacquemyn *et al.* (2005). This species is identical in habitat and morphology with *Paris polyphylla*. These workers established that the growth of shoot showed strong differences along an environmental gradient of soil and light conditions. Moreover, out of the six parameters (Figure 6.16) those at some point of time were important to *Paris polyphylla*, only relative humidity was significantly different in CF and FF.



Figure 6.16: Levels of micro-environment and soil properties (light intensity, relative humidity, soil temperature, pH, TKN and available phosphorus) in CF and FF that were significantly related to *Paris polyphylla* 

# Panax bipinnatifidus

The total area occupied by *Panax* was 14.88 ha in CF and 3.59 ha in FF. Plant density increased with patch size and so did isolation as in the case of *Swertia chirayita*. Mean annual growth rate of both CF and FF populations was below 1.0, and both increased to more than 1.0 in second transition year. However, in the third year, the FF populations fell below 1.0, which also explained the fact that significant variation in annual growth rate of *Panax* was observed only during the third transition year. The mean annual growth rate of *Panax* was

comparatively lower than that reported by Farrington *et al.* (2004) on *Panax pseudo-ginseng* from 1999-2004, in which the populations maintained the growth rate around the constant value of 1.0. Survivorship trend showed a concave curve during the first year, but it was almost constant in most populations during second and third year. Although this trend is universal, the overall survivorship pattern in this study differed significantly between populations of CF and FF as revealed by log-rank test and Gehan-Breslow-Wilcoxon test, suggesting a negative impact of forest fragmentation on the populations of FF. *Panax bipinnatifidus* did not show significant relationship with micro-environment except at seedling stage which had some relationship with exchangeable potassium level of the soil and also water holding capacity and pH. The range of these parameters did not vary much as depicted in Figure 6.17. However, Kruskal-Wallis test showed that the level of exchangeable potassium in the soil differed significantly between CF and FF habitats, which could be another reason for low survival of seedlings in FF populations. Hence, this signifies the possible role of exchangeable potassium in seedling growth and survival of the species. The average annual growth rate was correlated with water holding capacity and soil pH.



Figure 6.17: Levels of soil properties (water holding capacity, soil pH and exchangeable potassium) in CF and FF that were significantly related to *Panax bipinnatifidus* 

# CHAPTER 7 METAPOPULATION DYNAMICS AND EXTINCTION RISK ANALYSIS

### **INTRODUCTION**

An increasing number of species are facing the risk of extinction because they occur in specific or highly fragmented areas, have a reduced number of populations or individuals, or are declining (World Conservation Union, 1994). Currently, habitat loss due to anthropogenic activity is considered to be the single most important cause of loss of plant diversity (Wilcove *et al.*, 1998). Besides, human-caused changes in the environment, natural stochasticity in populations also pose additional threat to species persistence (Nakaoka, 1996; Menges, 1997; Holsinger, 2000; Vucetich *et al.*, 2000).

Patterns of birth and death that primarily determine population size vary widely among and within species (Roff, 1992). The persistence of a population depends on a numerical equilibrium over time. The simulation models based on the schedules of fecundity, recruitment and survival, and the integration of such parameters into the models allow exploration of their relative importance (Schemske *et al.*, 1994; Horvitz and Schemske, 1995; Caswell, 2001). Thus these simulation models help us in understanding the mechanisms that govern population dynamics and in estimating extinction probabilities, particularly for threatened species (Schemske *et al.*, 1994; Menges, 2000).

Although several instances are there where management recommendations for threatened plants are based on deterministic models (Manders, 1987), these models being static, do not take into account the random and unpredictable changes in environmental conditions, or any other factors resulting in stochastic extinction. On the other hand, stochastic computer simulation models, based on the temporal variation in observed demographic parameters, are considered valuable for Population Viability Analysis (PVA) investigations (Shaffer and Samson, 1985; Menges, 1992). Variations in these parameters may be obtained from longterm demographic monitoring data and are influenced by several ecological and genetic factors. Influential factors include environmental variability, intraspecific and interspecific 129 competition, herbivory, mutualism, pathogens, pollen limitation, dispersal, heterozygosity and allelic diversity (Schemske *et al.*, 1994). The distribution and dynamics of organisms at a large spatial scale have been the focus of ecological research since early part of the twentieth century (Wright, 1931; Fisher, 1937; Skellam, 1951, 1952; MacArthur and Wilson, 1967; Levins, 1969, 1970). The theories of island biogeography and metapopulation dynamics have been particularly influential in this respect as they have offered a quantitative basis to analyse regional scale ecological dynamics (Hanski and Simberloff, 1997).

The original metapopulation theory based on source-sink relationship (Levins, 1969, 1970) has subsequently been extended to encompass a wider class of population structure, including non equilibrium and mainland-island forms (Hastings and Harrison, 1994; Harrison and Taylor, 1997; Hanski, 1999). In the metapopulation context, the key elements are the processes of inter-population migration, local population extinction and regional distribution of suitable habitats as discrete patches within a larger matrix of unsuitable habitat. Some authors have defined plant metapopulation dynamics as 'the product of local population dynamics and dispersal (Husband and Barrett, 1996). Antonovics *et al.* (1994) described it as a system of interconnected populations. Even globally, very few studies are available on plant metapopulations (Husband and Barrett, 1996).

Studies on population dynamics of any plant species in India at a spatially large scale have not been undertaken (Hanski, 1999). Metapopulation theory states that the scaling from local to regional dynamics may not be straightforward, and that the regional scale availability of habitat, migration and extinction play a role in determining whether a system of local populations of a species can persist.

The identification of populations that shows a metapopulation structure is not an easy task. For instance, the persistence and dynamics of metapopulations are critically dependent on the amount and regional configuration of suitable habitats (Hanski, 1997). In contrast, the dynamics of a population existing in an undisturbed continuous area of suitable habitat would be an extrapolation of local processes. Thus, determining the form of regional dynamics is not simply a matter of typology, and this links directly to demographic parameters and ecologically important aspects of population organization (Thomas and Kunin, 1999).

Many of the world's species are threatened with extinction from habitat loss, overexploitation, introduced species, pollution, demographic, genetic and environmental tluctuations, and natural catastrophes (World Conservation Monitoring Centre, 1992). As a consequence of this, metapopulation models have further broadened to address the conservation needs of plants and animal species and hence PVA was developed to assess extinction risk and to compare management options. PVA is a method for predicting the future fate of plant and animal populations based on demographic and environmental and genetic parameters (Shaffer, 1981; Gilpin and Soule, 1986; Boyce, 1992; Ferson and Akcakaya, 1993; Norton, 1995). PVA process, based on computer simulation models, provides an important framework for interdisciplinary discussion and synthesis, and is now an influential and widely applied management tool in conservation biology. PVA is used for ranking management strategies according to their relative impacts on the persistence of wildlife populations (Clark et al., 1991; Lindenmayer et al., 1993). Exploring management options helps to build populations up to adequate size and to reduce the risks of extinction (Given 1994). Most studies on PVA have assessed the impact of various management regimes on plant populations (Haig et al., 1993; Lindenmayer and Possingham, 1996; Drechsler, 1998). However, such applications for threatened plant populations are almost absent in India.

Many computer programs are used for evaluating PVA, the World Conservation Breeding Specialist group of the World Conservation Union (IUCN) has conducted more than 80 PVAs using VORTEX (Lacy, 1993), other generic package such as ALEX (Possingham and Davies, 1995), SPOMSIM (Moilanen, 2004). GAPPS and INMAT are also used. RAMAS Metapop is one such tool that has been used to conduct PVA for threatened taxa (Beissinger and Mccullough, 2002). It is used by ecologists, resource managers, and population

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biologists worldwide who need to predict population structure and size through time and assess population and species extinction risks. The greatest strength of RAMAS Metapop is that it can be used for stage structured populations besides its ability to model age structure. This allows the use of RAMAS Metapop for PVA of plants species, as most plant PVAs are stage or size-classified (Lefkovitch matrix models).

In this chapter, the results of the study on metapopulation dynamics of *Swertia chirayita*, *Paris polyphylla* and *Panax bipinnatifidus* have been presented. The models were developed based on RAMAS Metapop version 5.0. The software was used to explore various model scenarios by using multiple simulations. The program simulates the future of metapopulations using projection matrix models (Caswell, 2001) by using transition matrices of populations in different demographic groups each with corresponding standard deviation matrices to model environmental stochasticity. Additionally, management interventions were simulated to explore the most effective strategy for conservation of the species. The study had the following specific objectives:

- 1. To evaluate and compare deterministic growth rate ( $\lambda$ ) in continuous forest and forest fragments.
- 2. To identify the demographic processes (s) which contribute(s) most to  $\lambda$ , thereby important in maintaining a viable population.
- 3. To simulate the future metapopulation trend and to assess extinction probability

Besides these, the goal of the metapopulation model is to determine the Minimum Viable Population (MVP) size and to simulate management interventions to explore the most effective strategy for conservation of the species. These were estimated and discussed in Chapter 8.

### **METHODS**

The projection matrix model was used for metapopulation analyses of the three species. Field demographic data was used to estimate the vital rates for each stage. Vital rates considered in this study were survival, and fecundity rates. Stage abundance (column vector of the matrix,  $n_t$ ) was defined as the total population size in each population at each life cycle stage. This was enumerated by determining density per unit area in each population through sampling in 50 1 m x 1 m quadrats for each life cycle stage. The density so obtained was multiplied by the respective patch sizes of each population to obtain the total stage-specific population size. Demographic data from the previous chapter was used and vital rates for stage elements were calculated as the average changes over a period of six months for *Swertia chirayita*, and one year for *Paris polyphylla* and *Panax bipinnatifidus*.

The projection matrix model takes the form:  $A.n_t = n_{t+1}$  which is represented as:

<b>— —</b>		<b>—</b> —		
$a_{11}$ $a_{12}$ $a_{13}$		<b>n</b> 1		
$a_{21}$ $a_{22}$ $a_{23}$	Х	n <sub>2</sub>	=	<b>n</b> <sub>t+1</sub>
$a_{31}$ $a_{32}$ $a_{33}$		n <sub>3</sub>		
— — — — Matrix A	Col	umn vect	or n <sub>t</sub>	

Where, A= Projection Matrix;  $n_t$ = Total population at time t;  $n_{t+1}$ = Total population size at time t+1.  $a_{11}$ ,  $a_{21}$ ,  $a_{31}$ ...are the stage elements representing probabilities of survival and transition.

### **Estimation of the Matrix Elements**

Separate transition matrices and corresponding standard deviation matrices were constructed for each habitat/demographic groups as described in Table 5.1 of chapter 5. The matrices were grouped based on the assumption that the vital rates of populations belonging to the same group would be identical because they experience similar environmental and demographic factors that determine their dynamics. The populations within each group were sufficiently close to justify this assumption. Several studies have taken such approach of grouping based on habitat for demographic analysis such as PVA (Akcakaya, 2005). The Tield demographic data obtained from the study of population dynamics were used for constructing stage-based transition matrices. Each element of the matrix representing survival rates and fecundity of plants were summarized from field data following Caswell

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(1989). Survival rate is the proportion of individuals that survived from a previous stage to the next. Therefore survival rate for stage 2 ( $S_2$ ) is calculated as:

 $S_2 = N_3/N_2 \label{eq:S2}$  Where  $N_2$  and  $N_3$  are the number of individuals at stage 2 and 3 respectively.

Fecundity (F) was calculated using the average number of seedling per flowering or reproductive plant in each population. All reproductive plants were assumed to produce the same average number of seedlings (Akcakaya, 1991). Fecundity is the only entry in the matrix that does not represent a probability, rather, it represents the average number of seedlings a single flowering plant produce in a given year:

### Fecundity (F) = $S_{(t+1)}/A_t$

Where,  $A_t$  is the number of adults at time t and  $S_{t+1}$  is the total number of seedlings recruited at time t+1.

Although estimation of seed number would have been ideal for fecundity data, the fate of seeds could not be followed in the plots for the three species because of logistic constraints that need weekly monitoring given the species biology. Therefore, the stage specific fecundities (fx) by a less direct method as described above *i.e.*, considering seedling population data was followed (Damman and Cain, 1998) Stage-based transition matrices (Caswell, 2001) were built from field data collected over 4 years period *i.e.*, 2005, 2006, 2007 and 2008.

### **Deterministic Population Projection**

Projection is carried out by a series of multiplication of the matrix (A) as:  $n_{t+2} = A \cdot n_{t+1}$ ;  $n_{t+3} = A \cdot n_{t+2} \cdot \dots \cdot n_{t+1}$  until a stable configuration is reached. The finite rate of increase ( $\lambda$ ) was calculated as:

### Finite rate of increase $(\lambda) = n_{t+1}/n_t$

Sensitivity determines how much various life-history stage transitions affect the population dynamics by examining how changes in a particular stage affect the magnitude of the leading eigen value.

# Sensitivity $(S_{ij}) = w_i/W$

Where, wi is the population vector at the i<sup>th</sup> generation and W is the sum of all population vector.

One problem with this approach is that some of the variables, *i.e.*, survival rates, are intrinsically restricted in their range to values between 0.0 and 1.0, while others, *i.e.*, fecundities, may be very large.

Elasticity is a measure of proportional effect, *i.e.*, the effect that a change in a given matrix element has as a proportion to the change in that element:

#### Elasticity (Eij) = $(aij/\lambda)$ Sij

Where, Eij is the elasticity value and represents the proportion of  $\lambda$  due to transition  $a_{ij}$  (de Kroon *et al.*, 1986).

### **Matrix Model Formulation**

Demographic data was used to construct a stage structured projection matrix model (basemodel) (Caswell, 2001; Lefkovitch, 1978) for each group. We also made use of data from biotic interference to create an alternate model. Therefore, two different matrices designated as M1 (base-model) and M2 matrices (alternate model) were used. M1 contained the vital rate elements that was estimated from actual survivals in the demographic plots, while M2 matrix contained vital rate elements that was estimated by adding actual survivals including those plants that would have survived if there was not apparent disturbance in the sites. During estimation of M2 matrix, care was taken to include those individuals only whose mortality was evidently caused by unnatural processes such as disturbance. The method of using the two model matrices was followed to compare the population performance in two contrasting scenarios *i.e.*, with disturbance in natural conditions (M1) and without disturbance (M2). Disturbances from all sources and forms were included such as grazing, landslide, weeding activities in trekking sites, uprooted plants, wild animal disturbance etc. All demographic groups of each species had a separate M1 and M2 model. We modelled the stochastic population dynamics using Monte Carlo based software RAMAS Metapop version 5.0 (Akçakaya, 1998). Parameters that were included in the Model are vital rates, demographic and environmental stochasticity, and initial population structure/stage abundance. Constraints were imposed within RAMAS Metapop to ensure that all simulated survival rates lie within the bounds of 0.0 and 1.0 with minimal truncation of the

distribution. Environmental stochasticity was modelled through introducing random fluctuations in stage specific fecundities and survivals. For this, the program assigns during each time step to each transition rate a random value drawn from a specified log-normal distribution whose mean and standard deviation are given by the empirical matrices. A lognormal distribution for stochastic simulations was chosen because several matrix elements had small mean values, but large standard deviations (Akçakaya and Root, 2003; Regan, 2004). Since no catastrophic event disturbed the populations during the 4 years of study period, changes in population size most likely reflected true environmental and demographic stochasticity. Demographic stochasticity was modelled using binomial distribution (Akçakaya, 1991, 2002). Demographic stochasticity is the variation in the average chances of survival that occurs because a population is made up of a finite integer of individuals. For demographic stochasticity can be represented as:

### Demographic stochasticity = $S_i N_i(t)$

The number of survivors for ith stage was drawn from a binomial distribution with two parameters viz., Survival rate 'S' and Ni(t) (as sample size).

In the first set, all simulation was run with 1000 replications until time to extinction is achieved. In the second set, a threshold population size ( $N_e$ ) was set for quantification of quasi-extinction (Ginzburg *et al.*, 1982). The quasi-extinction time was quantified because the estimation of time to extinction of a species is usually achieved at longer time which all the three species studied could not afford. The species studied are critically endangered and are exposed to unstable land use dynamics. Besides, population falling below critical size may not be viable due to demographic and genetic effects. Therefore, it was pertinent to evaluate risk estimation at an early time (Bretagnolle and Inchausti, 2005). A threshold size of Ne= 1000 was set for *Swertia chirayita* since the population was modelled in terms of abundance. For *Paris polyphylla* and *Panax bipinnatifidus*, the threshold size of Ne= 100 was set, as these two species are long-lived perennials and are likely to persist at longer duration with more than 100 individuals.

### **Statistical Analyses**

X

Analysis of variance (ANOVA) (fixed effect model) was performed to test the variation of growth rates between the population groups. Significance of variation in fecundity was examined. Since samples were uneven among the groups, a non-parametric Kruskal-Wallis test was conducted at 95% confidence limit using SYSTAT version 10.0. Grime triangular plot was used to categorize the demographic events *i.e.*, survival, growth and fecundity of each species to visualize its contribution to population growth rate.

# RESULTS

### **Matrix Elements**

The matrix elements with survival and transition of the three studied species are presented in

	m - Maissiehement.	2005-2006	2006-2007	2807,2008	Mean	
Swertta c	hirayita					
CFS	$a_{14}$	114	119	115	116	2.6
	a <sub>21</sub>	0.245	0.238	0.22	0.235	0.012
	a <sub>32</sub>	0.127	0.161	0.113	0.134	0.025
	a43	0.143	0.131	0.139	0.138	0.006
CFT	a <sub>14</sub>	63	62	61	62	1
	a <sub>21</sub>	0.128	0.129	0.168	0.142	0.023
	a <sub>32</sub>	0.048	0.077	0.038	0.054	0.02
	a <sub>43</sub>	0.068	0.056	0.117	0.080	0.032
FF	a14	76	81	78	78	2.5
	$\mathbf{a}_{21}$	0.112	0.119	0.133	0.122	0.01
	a <sub>32</sub>	0.053	0.106	0.056	0.072	0.029
	a <sub>43</sub>	0.012	0.022	0.028	0.021	0.008
SH	a <sub>14</sub>	93	108	100	100.333	7.506
	a <sub>21</sub>	0.248	0.202	0.208	0.219	0.025
	a <sub>32</sub>	0.093	0.122	0.081	0.099	0.021
	a <sub>43</sub>	0.065	0.101	0.146	0.104	0.040
Paris poly	phylla					25.00
	a <sub>11</sub>	0.292	0.161	0.185	0.212	0.069
CF	a <sub>13</sub>	0	0	0	0	0
	a <sub>21</sub>	0.051	0.223	0.083	0.119	0.091
	a <sub>22</sub>	0.369	0.168	0.113	0.216	0.134
	a <sub>31</sub>	0	0	0.013	0.004	0.007
	a <sub>32</sub>	0.058	0.165	0.154	0.125	0.058
	a33	0.336	0.479	0.528	0.447	0.099
гF	<i>a</i> <sub>11</sub>	0.204	0.199	0.25	0.217	0.028
	a <sub>13</sub>	0	0	0	0	0

Table 7.1: Estimated projection matrix elements for three species \*\*

Ren Vietnen	- Alexander and	2005-2006	2006-2007	2007-2008	Medit	
	a <sub>21</sub>	0.028	0.129	0	0.052	0.068
	a <sub>22</sub>	0.208	0.262	0.125	0.198	0.068
	a <sub>31</sub>	0	0	0	0	0
	a <sub>32</sub>	0.083	0	0.1	0.061	0.053
	a <sub>33</sub>	0.313	0.4	0.417	0.376	0.055
Panax bipinn	atifidus					
CF	a <sub>11</sub>	0.088	0	0	0.029	0.050
	a <sub>14</sub>	0	0	0	0	0
	a <sub>15</sub>	0	0	0	0	0
	<b>a</b> <sub>21</sub>	0.150	0.071	0	0.074	0.075
	a <sub>22</sub>	0.135	0.452	0.533	0.373	0.210
	a <sub>32</sub>	0.006	0	0	0.002	0.004
	a <sub>33</sub>	0.031	0	0	0.010	0.018
	a <sub>43</sub>	0.031	0.25	0	0.093	0.136
	a44	0.520	0.509	0.844	0.624	0.189
	a <sub>54</sub>	0	0	0.031	0.010	0.018
	a <sub>55</sub>	0.145	0.5	0.375	0.340	0.179
FF	a <sub>11</sub>	0.012	0	0	0.004	0.0069
	a <sub>14</sub>	0	0	0	0	0
	a <sub>15</sub>		0	0	0	0
	a <sub>21</sub>	0.124	0	0	0.041	0.072
	a <sub>22</sub>	0.118	0.5952	0.383	0.366	0.238
	a <sub>32</sub>	0.010	0	0	0.004	0.006
	a <sub>33</sub>	0.15	0	0	0.05	0.086
	a <sub>43</sub>	0	0.1667	0.333	0.167	0.167
	a44	0.279	0.7222	0.833	0.612	0.293
	a <sub>54</sub>	0.089	0.1111	0	0.067	0.059
	a <sub>55</sub>	0	0.1667	0.333	0.167	0.167

<sup>\*</sup> Matrix elements that were not listed were zero in each of the three years; <sup>\*</sup> elements shown in bold represent fecundity

# **Deterministic Analyses**

# 1. Finite Rate of Increase $(\lambda)$

The asymptotic growth rate ( $\lambda$ ) of all the three species fell below 1.0 depicting a general decline in population (Table 7.2). For *Swertia chirayita*, the population groups in the descending order of  $\lambda$  was sub-tropical continuous forest (CFS)> shrubland (SH)> temperate continuous forest (CFT)> sub-tropical forest fragments (FF). For *Paris polyphylla* it was greater in CF than FF by 32%. The CF populations of *Panax bipinnatifidus* on the other hand had marginally greater  $\lambda$  (1% higher) than that of FF. The trend of  $\lambda$  in yearly matrix differed among the three species. For *Swertia chirayita*, the overall growth pattern in the base model

(M1) was better in the transition matrix of second year for all populations except for CFT group which although had lower growth rate than other groups, had a relatively steady increase in  $\lambda$  over the three years period. This steady rise may be attributed to the fact that the CFT populations were located in the core zone of the Kanchendzonga Biosphere Reserve (KBR) which has over the years been given more protection than the sites harbouring other (CFS, FF and SH) populations. *Paris polyphylla* likewise, the  $\lambda$  value in CF increased in the second year matrix and declined in the third year matrix. Nevertheless, both the second and third year matrices yielded a growth in population with  $\lambda > 1$ . The FF matrices had gradual decline in  $\lambda$  in the second year matrix and increased by 1.7% in the third year matrix. *Panax bipinnatifidus* had constant  $\lambda$  in CF populations but differed marginally among the yearly matrices of FF and showed a small increase with the years.

**Table 7.2:** Yearly projection of  $\lambda$  from three transition matrices (2005-2006, 2006-2007, 2007-2008) of *Swertia* chirayita, Paris polyphylla and Panax bipinnatifidus

Wood of the state of the	these are	0.48.6cm	rio (Mb)		Atterns		Nonabero I	
	- 2005 - F.			illean de la companya de la company La companya de la comp				
	Imatric							
Swertia chirayita		- 1995 - 1995	1 de 1.	100 C 100		10	1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-	
CFS	0.844	0.880	0.795	0.839	*0.830	0.937	0.868	0.878
CFT	0.405	0.434	0.461	0.433	0.439	0.472	0.590	0.500
FF	0.274	0.384	0.360	0.339	0.396	0.484	0.428	0.436
SH	0.645	0.746	0.704	0.698	0.728	0.882	0.784	0.798
Paris polyphylla								
CF	0.698	1.065	1.027	0.960	0.807	*0.952	1.082	0.932
FF	0.578	0.400	0.417	0.641	0.674	0.749	0.751	0.724
Panax bipinnatifidus		12				6		1310
CF	0.625	0.626	0.626	0.626	0.720	0.622	0.652	0.678
<u>FF</u>	0.614	0.623	0.625	0.612	0.620	0.671	0.633	0.641

\*  $\lambda$  less than that of the corresponding M1 model

The M2 yearly matrices had the similar trend with M2 matrices for all the three species and with an observed increase in  $\lambda$  as expected for all groups except for *Swertia chirayita* in first year matrix for CFS and in the second year matrix of *Paris polyphylla* which depicts a decline in  $\lambda$  compared to that of the M1 matrix. This is caused by the reduced vital rates in one of the matrix elements due to increased in the number of survivors in the preceding stages. There is significant variation in  $\lambda$  between the groups of *Swertia chirayita* in both M1 and M2 matrix (F = 75.26, p = 0.00 in M1 and F = 32.78, p = 0.00 in M2) (Table 7.3).

	S. S. A.	df.	MIS STA	A STATE	
Swertia chiraylta					
M1 model	0.482	3	0.161	75.263	0.000
M2 model	0.426	3	0.142	32.783	0.000
Paris polyphylla				÷	
M1 model	0.324	1	0.324	12.91	0.022
M2 model	0.074	1	0.074	7.11	0.055
Panax bipinnatifidu	8	-			
M1 model	0.00	1	0.00	1	0.374
M2 model	0.00	1	0.00	1.96	0.230

Table 7.3: Analysis of variance (ANOVA) of  $\lambda$  between groups in M1 and M2 models

The confidence interval (*CI*) of mean  $\lambda$  provides accurate measure for estimating the variation and also to identify the source of variation (Bruna and Oli, 2005; Caswell, 2001). As depicted in Figure 7.1, there is no overlapping of *CI* of CFS and SH with that of CFT and FF and partial overlapping is detected in CFS with SH, and CFT with FF. This implied that the significant difference in  $\lambda$  between the groups comes mostly in their disparity of CFS and SH growth rate from CFT and FF. While for *Paris polyphylla*, significant variation in  $\lambda$  between CF and FF was observed only in M1 matrix model (F= 12.91, p= 0.02) although partial overlapping in confidence interval was seen in M1 and total overlapping in M2 was observed. *Panax bipinnatifidus* showed total overlapping of confidence intervals between  $\lambda$  of CF and FF populations, hence the insignificant variation.





Figure 7.1: 95% confidence interval of  $\lambda$  in different demographic groups of Swertia chirayita, Paris polyphylla and Panax bipinnatifidus

### 2. Elasticities

Three demographic processes contribute to the finite rate of increase of *Paris polyphylla* and *Panax bipinnatifidus i.e.*, fecundity (F) growth (G) and survival (L). However, for *Swertia chirayita*, there was no vital rate element under survival (L) category since the plant is a viennial and individuals get transformed from one stage to another during the short census interval. Because  $\lambda$  is the rate at which individuals on average, multiply, it can also be interpreted as the fitness of the populations of the three species. Demographic contribution to overall growth of the population by different phase of the life cycle of *Swertia chirayita*, *Paris polyphylla* and *Panax bipinnatifidus* was represented by the relative sensitivity of survival, growth and fecundity to  $\lambda$ . Apparently for *Swertia chirayita*, the contribution of growth is more in all the habitat groups both in M1 and M2 matrices. Figure 7.2.A shows that growth elasticities is higher in all groups compared to fecundity. However, the CFS 141

populations in M2 matrix scenario had relatively higher elasticities than other groups in both M1 and M2. This is consistent with the finding of Dinnetz and Nilsson (2002) on a semelparous perennial *Saxifraga cotyledon*, that growth and survival contributes more to the variation in  $\lambda$  than do reproduction and seed bank. *Paris polyphylla* exhibited a slight variation in the elasticities values (Figure 7.2.B), survival evidently had high elasticity values in all populations, but highest values were noted in FF populations in both M1 and M2. While in CF populations growth had more elasticities in both the demographic scenarios. Fecundity had the least elasticities as in the case of *Swertia chirayita. Panax bipinnatifidus* demonstrated the actual behaviour of an iteroparous forest herb as survival had the highest elasticities signifying it had relatively less contribution to  $\lambda$  (Figure 7.2.C).





Figure 7.2: Elasticities of Swertia chirayita (A), Paris polyphylla (B) and Panax bipinnatifidus (C) in M1 and M2 scenarios

Grime plot is the 3-D representation of the relative importance of demographic stages in the overall growth of population (Silvertown *et al.*, 1996; Gibson, 2001). There was some variation in the elasticities of M1 and M2 matrices especially for *Swertia chirayita* and *Paris polyphylla*. This variation was seen in the relative importance of growth, fecundity and survival on  $\lambda$ . For *Swertia chirayita*, growth forms were the most important stage which contributed significantly to  $\lambda$ . In M1 model, all populations were influenced explicitly by growth, in M2 model however, the relative importance of fecundity became more pronounced as the population groups increased with increase in fecundity (Figure 7.3). The importance of growth was also seen in *Paris polyphylla* where  $\lambda$  showed increase with growth. With the increase in survival, there was a small increment in  $\lambda$  as seen in M2 model. While for *Panax bipinnatifidus*, survival played a major role in the contribution to  $\lambda$  and was of greatest magnitude compared to that in other two species. Maximum elasticities were occupied by survival. Grime plot did not give any indication of the relative importance of other demographic processes.



C. Panax bipinnatifidus

Figure 7.3: Grime plot representing triangular ordination of survical, growth and fecundity elasticities for M1 and M2 matrix models for *Swertia chirayita* (A), *Paris polyphylla* (B) and *Panax bipinnatifidus* (C).

#### **Stochastic Risk Analyses**

### **Trajectory Summary**

A statistical summary of the abundance of the metapopulation (and each of its populations) as it changes through time is depicted in Figure 7.4. The average,  $\pm 1$  standard deviation, minimum and maximum abundances are all model outputs. The  $\pm 1$  standard deviations are displayed symmetrically around the mean, regardless of the actual distribution. The model predicted a continuous decline in the course of time, both in terms of metapopulation size and number of occupied habitat patches (Figure 7.4) for Swertia chirayita and Panax bipinnatifidus. This reaffirmed the threat status of these two species. On the other hand, Paris polyphylla metapopulation exhibited a stochastic trend with some growth at least during a few time steps in the future, usually a characteristic feature of a stable population. A very large decline was expected in Swertia chirayita and Panax bipinnatifidus during the next few years both in M1 and M2 scenarios. This would lead the metapopulation to extremely low abundance. This trend for rare species is reported by many workers (Schtickzelle et al., 2005) in their PVA models. The trajectories for Swertia chiravita and Panax bipinnatifidus stabilized after the sharp decline, and although in a stochastic simulation the projection tends to lead to an almost deterministic decline in the future in both M1 and M2 scenario. For Paris polyphylla however, the M1 predicted the similar trend in the initial time steps of the simulation but became more undulating as metapopulation size stabilized. M2 model of Paris polyphylla typically had an unpredictable trajectory with growth and decline in most time steps.



Figure 7.4: Trajectories summaries over 100 years of (A) Swertia chirayita, (B) Paris polyphylla and (C) Panax bipinnatifidus. The mean and 95% confidence intervals about the means after 1000 replications are presented. Minimum and maximum abundance (in case of Swertia chirayita) and population size (in Paris polyphylla and Panax bipinnatifidus) are represented by red arrows. Some of the maximum confidence intervals and range are not shown to confine the scale of the curve for better representation

# Metapopulation Viability

The change in the number of occupied populations of *Swertia chirayita* through time is depicted in Figure 7.5. The persistence of all 18 populations was projected to maintain for the next 4 years. However, the curve depicted a sharp decline in the number of patches and only 50% of which would remain extant within 12 years and most of which would remain are populations of CFS habitat. This was evident from the local occupancy of populations shown in Figure 7.5, where maximum time steps during the simulations were occupied by the populations of CFS. The metapopulation occupancy of *Paris polyphylla* also showed similar trend and approximately 50% of the population would be extant by 25<sup>th</sup> year, while 146

in M2 model, the reduction by half the number of sub-populations would not occur before 40 years and some patches may remain till 100 years. In the case of *Panax bipinnatifidus*, the decline in number of patches occurred at regular pace in both M1 and M2 unlike *Swertia chirayita* and *Paris polyphylla*. Model prediction showed that approximately 50% of the population would remain extant till 12<sup>th</sup> and 13<sup>th</sup> year in M1 and M2 scenarios.



M1



C. Panax bipinnatifidus

Figure 7.5: Metapopulation occupancy of Swertia chirayita (A), Paris polyphylla (B) and Panax bipinnatifidus
 (C) (line represents mean value of the 1000 replications; bars show standard deviation, 95% confidence interval), (one time step= 6 months for Swertia chirayita and one year for Paris polyphylla and Panax bipinnatifidus)

The statistical summary of the occupancy rate (the proportion of time patches remained occupied) is shown in Figure 7.6. Each population was represented by a histogram with the average,  $\pm 1$  standard deviation, minimum and maximum number of time steps that each population would remain extant.





A. Panax bipinnatifidus

**Figure 7.6:** local occupancy of *Swertia chirayita* (A), *Paris polyphylla* (B) *and Panax bipinnatifidus* (C) In *Swertia chirayita*, maximum time that a sub-population would remain extant was 56 time step or 27 years in M1, and 81 time steps or 40 years in M2 (T-FE2 population of CFS habitat) while lowest time a population persisted was 7 years (T-FF of FF habitat).

In *Paris polyphylla*, the average time the sub-population would persist through time was predicted at 30 ( $\pm$ SD 15.6) years in M1 and 45 ( $\pm$ SD 25.5) years in M2 scenario. The CF populations had 41 (( $\pm$ SD 8.9) years in M1 and 62 ( $\pm$ SD 12) years in M2 models while FF populations had persistence time of 13 ( $\pm$ SD 2.4) years in M1 and 15 year ( $\pm$ SD 2.9) year in M2. The sub-population BCF1 of continuous forest had the most persistence time with 52 and 77 years in M1 and M2 model respectively. While BFF of forest fragments had the least persistence time with 11 and 13 years in M1 and M2, respectively.

The average persistence of populations of *Panax bipinnatifidus* on the other hand, was 12 ( $\pm$ SD 25.5) years in M1 and 13 ( $\pm$ SD 3.4) years in M2. Habitat-wise, the persistence of CF populations was 14 ( $\pm$ SD 2.4) years in M1 and 15 ( $\pm$ SD 2.5) years in M2, and that of FF

populations was 9 ( $\pm$ SD 1.6) years in M1 and 10 ( $\pm$ SD 1.8) years in M2. Among all populations, DCF had highest persistence time of 18 years in M1 and 19 years in M2 scenarios. Whereas, PFF of forest fragments was projected to have 7 and 8 years persistency in M1 and M2, respectively.

A linear relationship between local occupancy of populations with patch size was obtained which implied that the persistence of species was dependent on patch size. The predictive capacity of patch size on local occupancy as revealed by adjusted  $r^2$  did not vary much in M1 and M2 of *Swertia chirayita* (0.38 in M1 and 0.37 in M2). In *Paris polyphylla*, the linear predictability was more with  $r^2$ = 0.52 and 0.45 in M1 and M2, respectively. While in *Panax bipinnatifidus*, there was a strong correlation of the size of population patch with the persistency of the sub-populations (in M1  $r^2$ = 0.78 and in M2 it was 0.81) (Figure 7.7).



Figure 7.7: Relationship between patch size and local occupancy of patches of Swertia chirayita (A), Paris polyphylla (B) and Panax bipinnatifidus (C)
### **Probability of Extinction**

*Time to extinction:* Time to extinction refers to the complete extinction of the populations while extinction risk is the probability of extinction in 100 years. The extinction curves for all the three species approached extinction probability 1.0 much before 100 years (Figure 7.8, 7.10 and 7.12). Therefore, estimating quasi-extinction was a requirement for all the three study species. The probability of extinction was represented in a range of risk from lower range of quasi-extinction ( $Q_E$ ) to the higher end of complete extinction (E). Quasi-extinction time is determined by providing a threshold population size to the model. The threshold population size is decided based on the actual current population size. For example, species with higher number of existing individuals such as Swertia chiravita, the threshold population could be taken as 1000. While species with fewer numbers of individuals as in case of Paris polyphylla and Panax bipinnatifidus the threshold population size was fixed at 100. The model output using the above mentioned threshold population size was the quasiextinction time. In other words, the quasi-extinction time as estimated by the model, represents the time required to reach the respective threshold population size after which, the population is presumed to proceed towards extinction due to genetic drift, inbreeding effect and external forces operating. On the other hand, the extinction time was estimated through the model by not setting any population threshold size. Therefore, the extinction time as estimated by the model refers to the time when the population actually reaches zero.

The quasi-extinction and extinction times have been represented in Figure 7.9, 7.11 and 7.13, where the beginning of the horizontal bars for each population groups represented the quasiextinction time, and the end of the bar represented the extinction time. These ranges provide additional information for characterizing the risk of extinction from a critical threshold population size to complete extinction.

The probability of extinction of *Swertia chirayita* was different for different groups (Figure 7.8). The extinction probability curves revealed a more or less identical shape for FF, CFT and SH population groups in both M1 model (Figure 7.8a) and M2 models (Figure 7.8b).

However, the CFS and metapopulation extinction curves were relatively smoother in M2 model than in M1 model.



Figure 7.8: Extinction probability of Swertia chiravita in M1 (a) and M2 (b) model

In Figure 7.9, CFT and FF were predicted to have the shortest quasi-extinction and extinction times (CFT:  $Q_E - 5$  years both in M1 and M2 model; E- 10 years in M1- 4 years in M2; FF:  $Q_E - 4$  years in M1 and 6 years in M2 model; E - 12 years in M1 and 16 years in M2). CFS had significantly longer persistence and a relatively extended range of predicted extinction. In M1 model, the quasi-extinction time was 25 years, in M2 model it was 54 years while times to extinction were 48 and 88 years, respectively. The overall 153

metapopulation had a quasi-extinction time of 25 years in M1 model while in M2 projection it was 46 years. The trend revealed an approximate doubling of persistence time in M2 scenarios in comparison to M1 scenarios in CFS and SH groups and metapopulation. Thus, groups with lower risk *i.e.*, SH and CFS had a greater persistence time range *i.e.*, up to 90 years, whereas, high risk groups *i.e.*, CFT and FF were predicted to be extinct within 20 years. Therefore, across the scenarios and population groups, the species is facing a projected extinction within 100 years.



Figure 7.9: Range of risks of Swertia chirayita from quasi-extinction (left end of bar) to extinction time (right end of bar)

The risk curves for *Paris polyphylla* exhibited a wide disparity in isolated scenario between FF populations and that of CF where the latter is highly skewed while the former is relatively smooth. However, the risk of CF populations and metapopulation was almost uniform throughout, this is seen both in M1 (Figure 7.10b) and M2 models (Figure 7.10b). Additionally, the prediction between M1 and M2 model showed wide differences although the pattern is similar. The risk in M1 of CF was 0.86 while in the metapopulation scenario it was 0.97. In M2 the CF was predicted to have 0.43 risk probability and 0.42 for the entire metapopulation.



Figure 7.10: Extinction probability of Paris polyphylla in M1 (a) and M2 (b) model

In Figure 7.11, the range of extinction was only shown for FF populations as it had probabilities of quasi-extinction and time to extinction well within 100 years. In the isolated scenario it had a time to extinction range from  $Q_E=20$  to E=36 years and in the M2 from  $Q_E=33$  to E=46 years (Figure 7.11). CF and metapopulation did not have any risk of neither quasi-extinction nor time to extinction within 100 years and is not revealed in the figure. Plotting risk results beyond 100 years is impractical as pointed out by Menges (1990). The close range probability of extinction in CF and metapopulation suggested that the role the forest fragment populations play in the persistence of metapopulation is very insignificant.



Figure 7.11: Range of risks of *Paris polyphylla* from quasi-extinction (left end of bar) to extinction time (right end of bar). Only FF populations appeared in the plot while CF and metapopulation plots went beyond the scale.

*Panax bipinnatifidus* had similar pattern of prediction with skewed curves in the isolated scenarios as well as metapopulation scenarios (Figure 7.12). Contrastingly, the risk curves in both M1 and M2 model did not show much variation as that of the other two species. Moreover, the range of probability from quasi-extinction to time to extinction did not show apparent variation between the M1 and M2 scenarios (Figure 7.13). In the isolated scenario the FF populations had an extinction risk ranging from Ne= 10 to E= 25 in M1 model, and Ne= 11 to E= 28 in M2 model. CF had  $Q_E= 16$  to E= 33 in M1, and  $Q_E= 22$  to E= 39 years in M2. Metapopulation in M1 had extinction risk that ranged from  $Q_E= 17$  to 38 years and  $Q_E= 23$  to E= 42 years in M1 and M2, respectively.



Figure 7.12: Extinction probability of Panax bipinnatifidus in M1 (a) and M2 (b) model



Figure 7.13: Range of risks of *Panax bipinnatifidus* from quasi-extinction (left end of bar) to extinction time (right end of bar)

### **Interval Extinction Risk**

Analysis of interval extinction risk provide important information on the species persistence at interval range, this has got more implications on the management of species. The species time was systematically reduced, and the response of the model was measured by examining Expected Minimum Abundance (EMA)/Expected Minimum Population size (EMP). The two high risk species *Swertia chirayita* and *Panax bipinnatifidus* did not produce interval extinction risk at 50 to 100 years duration during the simulation because complete extinction is realised before that time. Hence, for the purpose of characterising the interval risk the duration for model prediction was explored at 25 years duration. *Swertia chirayita* metapopulation had a 50% probability of falling below 30 individuals within 25 years in M1 scenario, and 7,194 individuals in M2 scenario. The projected EMA of *Swertia chirayita* was 249 and 8,981 in M1 and M2 scenarios respectively. While for *Panax bipinnatifidus*, there was >90% probability in M1 that all plants would be extinct and >80% in M2. However, the minimum Expected Metapopulation Size (EMS) was yielded at 3 and 8 in M1 and M2, respectively signifying that there is approximately 10-20% chance that few individuals would persist after 25 years.

*Paris polyphylla* is a comparatively lower risk species. As indicated by the extinction risk, there is approximately 10% probability that the metapopulation would persist in 100 within 100 years. Being lower risk, exploration of interval risk is valuable information for conservation (McCurthy, 1996). Hence interval risk was explored at 50 and 100 years. The interval extinction risk curve at 100 years duration indicate that there was a 50% risk that the metapopulation size would fall below 229 at least once during the next 100 years, while in M2 model, the metapopulation was likely to fall below 5,346 at the same risk probability. At 50 years, there was 50% chance that metapopulation would fall below 236 in M1 and 4,224 individuals in M2 model. The minimum EMS (McCurthy, 1996; McCurthy and Thompson, 2001) in 50 years was 12,405 in M2, and 1,524 in M1 model. The EMS drop to 1,023 in M2 at 100 years, while in M1 it fell to 129 individuals. The fact that the EMS was able to be

estimated at 100 years both in M1 and M2, strongly indicate that *Paris polyphylla* metapopulation in KBR was still viable under the current demographic scenario (M1) and also under the expected demographic scenario of M2 model.

#### DISCUSSION

The use of M1 and M2 matrices in population modelling in this chapter took care of the disturbance factor that has often been reported to impact  $\lambda$ . For instance, Nantel *et al.* (1996) reported that  $\lambda$  declined with increasing harvest of the plants. Inclusion of alternate matrix model is important since change in  $\lambda$  has important implication to the persistency of species (Morris and Doak, 2005). Deterministic analysis yielded the finite rate of increase ( $\lambda$ ) which is the determining factor in the future growth of the metapopulation. In each of the species, it was seen that the average  $\lambda$  was greater in continuous forest populations than those in the forest fragments. Lower  $\lambda$  in populations of forest fragment may be attributed to various reasons, the most important being reduced quality of habitat patches owing to disturbance and change in micro-climatic conditions. In addition, some factors those that are intrinsic and population specific could have also contributed to this pattern. In Swertia chirayita, CFS which was a continuous forest, populations had highest  $\lambda$  and was 50% higher than that of forest fragment populations in M1 and 44% higher in M2 mean matrix. On the other hand, CFT which was also continuous forest populations had lower  $\lambda$  than shrub-land populations and it was only 13% and 6% higher than the forest fragment populations in M1 and M2 respectively. Shrub-land populations had higher  $\lambda$  than both CFT and FF by 26% and 35% in M1, and 29% and 36% in M2 matrix. The impact forest fragmentation had on  $\lambda$  of Swertia chiravita populations was evident from the fact that the CFS which is represented by seven populations had higher  $\lambda$  than the other populations particularly that of forest fragments. In Paris polyphylla, the effect of forest fragmentation was most pronounced among all the three species. Its metapopulation had also better persistence than the other two species. Populations of continuous forest had 32% higher  $\lambda$  in M1, and 21% in M2 than that of forest fragments. Panax bipinnatifidus however, showed little variation (statistically insignificant)

in  $\lambda$  in response to forest fragmentation. CF populations had only 2% in M1 and 4% in M2, higher  $\lambda$  values than FF. This is in contrast to *Swertia chirayita* and *Paris polyphylla* where  $\lambda$ was significantly higher in continuous forest than forest fragment populations. Bruna and Oli (2005) also reported significantly higher  $\lambda$  in continuous forest populations than those in forest fragments. Further,  $\lambda$  in *Panax bipinnatifidus* was much lower compared to the other two species. This  $\lambda$  value was also lower when compared with that of populations of other *Panax* sp. In the case of American ginseng, Nantel *et al.* (1996) reported  $\lambda$  value close to 1.0. The low growth rate of *Panax bipinnatifidus* may be due to its low population density, small size of the population, greater demographic and environmental stochasticity.

Different demographic processes that contributed to  $\lambda$  differed among habitats and between species. In *Swertia chirayita*, growth influenced most in  $\lambda$  than fecundity, as revealed by the elasticities values. This is the characteristic feature of semelparous species which reproduce only once in a lifetime. The growth of *Swertia chirayita* which included transition of small rosettes to large rosettes, large rosette to aerial vegetative and vegetative aerials to reproductive stages, covered major portion of the species life history. However, since the species is non-clonal and propagates only through seeds, the role of fecundity cannot be undermined. In FF and CFT populations, the relative contribution to  $\lambda$  by fecundity was higher than the other groups. *Paris polyphylla* on the other hand, showed survival as the main contributor to  $\lambda$  in CF populations, although the distribution of elasticities did not vary much among growth, survival and fecundity as depicted in the grime plot. In FF populations however, survival was important by a huge proportion as revealed by elasticities and this was also observed in *Panax bipinnatifidus* both in CF and FF of M1 and M2 models.

The relatively less importance of fecundity in all the three species may be explained based on the argument of Silvertown *et al.* (1992), who stated that there is often a trade-off between the different processes. Trade-offs between growth and seed reproduction (fecundity) and survival and fecundity have been frequently observed in plant demographic studies (Silvertown, 1987). However, the relationship between survival and growth and their trade-offs against each other is not very common in nature. It was reported that vital rates with a major impact on population growth often exhibited the lowest levels of variability and vice-versa (Ehrle'n and van Gronendael, 1998; Gaillard *et al.*, 1998, Lande, 1988; Pfister, 1998; de Kroon *et al.*, 2000; Pico, 2000; Sæther and Bakke, 2000; Zuidema and Franco, 2001). Pico *et al.* (2003) also reported significant negative correlation between CV of matrix elements with elasticities. In this study, elasticities varied among populations and also between M1 and M2 matrices in all the species. However, Spearman rank correlation test revealed no significant relationship between demographic traits (survival, growth and fecundity) and vital rates. This indicated that the variation in vital rates did not influence the relative importance of life stage to  $\lambda$  in all the three species studied. It can however be inferred that the vital rates and the input of life-stage processes to  $\lambda$  in M2 differed from that of M1 owing to the natural perturbation caused by disturbance to populations. However, the relative change imposed on the elasticities of survival, growth and fecundity did not show significant variation especially in the survival process that had more than 90% elasticities as in case of *Panax bipinnatifidus*.

The trajectory figures showed marked improvement from the actual M1 to the expected M2 scenario. This primarily resulted from the increase in  $\lambda$  in all three species. Although maximum rise in  $\lambda$  was observed in *Swertia chirayita*, the relative change in projected abundance did not yield substantial difference between the two models (M1 and M2). Trajectories of population size in M1 and M2 of *Panax bipinnatifidus* also had similar characteristics. These observations implied that for populations whose  $\lambda$  values were drastically lower than 1.0, their proportionate increase may come from management. Protection may not be sufficient to check the decline of abundance/population size. In fact, a testament to this comes from the comparison of the trajectory trend observed in M1 and M2 scenarios of *Paris polyphylla*. *Paris polyphylla* had  $\lambda$  closer to a constant value of 1.0 as discussed above. The trajectory of M1 showed a declining but relatively stable population size with some increase is expected at some point of time in the future. Although the

difference in  $\lambda$  between M1 and M2 was much lower compared to that of *Swertia chirayita* and *Panax bipinnatifidus*, the projected trajectory in M2 varied significantly from M1. The trajectory trend was typical to a stable metapopulation.

The time to extinction curves had a skewed distribution in all the three species. This confirms to the conclusion of Levington and Ginzburg (1984), who concluded that skewed distribution and high variance are typical of time to extinction curves especially for threatened and rare plants. Simulation results imply a relatively high risk of extinction within the next specified 100 years. The level of risk and skewed behaviour is similar in M1 and M2 models for all the three species but with an extended predicted persistency in M2 model as expected. Apart from the increased susceptibility of sub-populations (particularly that of Swertia chirayita and Panax bipinnatifidus in the isolated scenario) to chance events that might result in the extinction of the population within the predicted time, it is possible that the metapopulation of Swertia chirayita and Panax bipinnatifidus in KBR has reached a critical minimum size below which it is impossible to maintain a stable metapopulation. For this reason, analysis of interval extinction risk provided valuable insights so far as species management is concerned. Swertia chirayita and Panax bipinnatifidus did not yield EMA/EMS at a specified time of 100 years or even at 50 years, while Paris polyphylla did and indicated that the two former species are facing imminent extinction unless timely intervention is undertaken.

# CHAPTER 8 GENERAL DISCUSSION

Kanchendzonga Biosphere Reserve (KBR) is one of the richest areas of biodiversity in the Himalayan range. In addition to the high species diversity and endemism, the region also plays an important role in maintaining altitudinal connectivity between the habitat types that make up the larger Himalayan ecosystem. Topographic diversity and associated variation in altitudinal, climatic and edaphic conditions in the Biosphere Reserve (BR) have resulted in the formation of diverse ecosystems in KBR that harbour rich variety of flora. Such diverse and spatially segregated ecosystems have ecological implications for species richness and their distribution.

Six terrestrial ecosystems were identified and characterized in the core and buffer zones of KBR (Chettri et al., 2006). The species richness and diversity varied in different ecosystem types, indicating the role of altitude and other associated physical and biological factors in determining the species diversity of various ecosystems in the BR. In this study, forest fragmentation was identified as the most important factor reducing the plant diversity of KBR. In addition to fragmentation, several other anthropogenic and natural factors have brought about extreme pressure on the persistence of threatened species in KBR. Several ethno-medicinally important plant species inhabiting the forests of KBR are also under threat due to these factors. In addition, over-exploitation of their populations for their medicinal value has put additional pressure on these species, many of which have reached on the brink of extinction. Empirical assessment of their populations is a pre-requisite for developing any effective in-situ conservation strategy. Therefore, the present research work was undertaken to prepare a list of ethno-medicinal plants, and their conservation status, and uses under uraditional medicine system. The habitat distribution modelling of the three study species viz., Swertia chirayita, Paris polyphylla and Panax bipinnatifidus was undertaken to characterize the habitats of the species and map their distribution within KBR. Their metapopulation status was ascertained, characterized and Population Viability Analysis

(PVA) was performed. The extinction probability analysis was also undertaken to evaluate their extinction risk. Generation of these empirical data and analyses are expected to help in preparing an efficient management plan for the threatened ethno-medicinally important plant species of KBR.

### **Ethno-medicinal Plants of KBR**

A total of 105 ethno-medicinal plants were recorded. These plant species were used for the treatment of at least 52 types of human ailment and also as veterinary medicine. The human ailments include asthma, diabetes, epilepsy, jaundice, rheumatism, gout, cancer and arthritis. Many of these treatments are still a challenge even in allopathic system of medicine. Among the ethno-medicinal plant species, *Centella asiatica* was used for eight different ailments for its diuretic, hepato-protective, sedative, nervine, anti-inflammatory and hypotensive properties. The second species that was used to treat maximum number of ailments was *Costus speciosus* and was used for treatment of bronchitis, fever, rheumatism, urinary disorder, loss of appetite, and skin disorder. The third most important ethno-medicinal plant in terms of number of ailments treated was *Valeriana jatamansii* which was used as stimulant, antiseptic and for treatment of epilepsy, hysteria, gastrointestinal disorder and cholera.

The distribution of the ethno-medicinal plants within KBR also varied with altitude. Maximum number of species (62%) were found at lower elevation range of 500-2,000 m *i.e.*, in tropical and sub-tropical zone. The temperate region (elevation range of 2,000-3,000 m) had moderate number of ethno-medicinal plant species (28%). Sub-alpine and alpine zone (elevation range of 3,000-4,000 m) had the least (10%) number of species because of limited vegetation and habitat availability owing to harsh climatic conditions. However, maximum number of threatened ethno-medicinal plants were located at higher altitude *i.e.*, in temperate and sub-alpine zone. This has important research and conservation implications because most of the higher altitude species had specific micro-environmental requirements and are rare even in their area of occurrence. The three study species were among the most important medicinal plant species and their medicinal properties were of local as well as global importance. They also occupy an important position in the trade of medicinal and aromatic plants worldwide. All the three species grow at higher elevational of KBR. Although *Swertia chirayita* was also recorded at lower elevation of 1800 m, the species flourished at higher altitude in KBR *i.e.*, 2,300 m in the temperate climate. *Paris polyphylla* thrived at an altitude range of 2,500-3,500 m while *Panax bipinnatifidus* inhabited the narrow range along the sub-alpine forest at 2,700-3,700 m elevation.

### Habitat Types and Occupancy Pattern of the Study Species

The habitats of all the three study species were essentially forest vegetation. For *Swertia chirayita* however, it can also be open grassland vegetation. It is also found in degraded lands as transient populations. *Paris polyphylla* and *Panax bipinnatifidus* however, are exclusively forest species occurring in moist environment, under deep shade and on thick forest floor litter. The habitat specificity and occurrence of each species could be traced through identifying their associated species. *Swertia chirayita* was commonly found with *Swertia bimiculata* as determined by Cole's coefficient of association. *Paris polyphylla* was significantly associated with *Elatostemma platyphylla* ( $\chi^2$ = 6.96, p= 0.01) with 0.52 association strength. *Panax bipinnatifidus* on the other hand, was mostly found with *Panax pseudoginseng* which were significantly associated ( $\chi^2$ =19.93, p= 0.01) with a very high strength of association of 0.80. These associations are important indicators for locating the species and are also useful for conservation and monitoring of vegetation changes (Munishi *et al.*, 2011).

Identification of their habitat of occurrence within KBR was carried out by habitat distribution modelling and hence a spatial occupancy pattern of the three species was mapped in the entire KBR using MAXENT (Maximum Entropy) software package. The potential habitats of occurrence of the three species as predicted by the models were restricted to the southern part of KBR. Their actual and existing metapopulations were also

identified in this region. As expected, the habitat of Swertia chirayita was very sparse towards the high altitude areas of northern region. Nevertheless, the habitat availability for Swertia chirayita was well-spread out although the existing metapopulation was confined to the southern part of KBR. For Paris polyphylla and Panax bipinnatifidus, the potential habitats were predicted to be at higher elevation and were sparsely distributed in KBR. The most important parameter that contributed to the habitat model was Enhanced Vegetation Index (EVI) and the poorest contributor was physiographic predictors or Digital Elevation Model (DEM) parameters. This was true for all the three species. In where in Swertia chiravita, the contribution of EVI was 89.3% and DEM was 10.7%. In Paris polyphylla, it was 68% by EVI and 32% by DEM, and in Panax bipinnatifidus, EVI made up for a substantial 94.9% compared to just a meagre 5.1% by DEM predictors. The important contribution of vegetation to the distribution of species was in conformity with the most habitat models (Stohlgren et al., 2001). This may be attributed to the species specialized niche that was found under specific vegetation cover where the species occur. Swertia chirayita had a broad habitat space consistent with the range of vegetation where it occurred. In other words, it thrived in sub-tropical forest, temperate forests, as well as shrubland and open meadows. Paris polyphylla and Panax bipinnatifidus had noticeably narrow habitat occupancy pattern in KBR and was consistent with their habitat characteristics *i.e.*, specific shaded and moist environment under the forest canopy.

Model accuracy was high as reflected in the high AUC values (AUC>0.9) for all the species. The values were 0.91, 0.99 and 0.97 for *Swertia chirayita*, *Paris polyphylla* and *Panax bipinnatifidus*, respectively. The models with AUC= 0.75 are considered to be strong predictors (Swets, 1988). More recently, Baldwin (2009) considered AUC= 0.81 as highly informative and accurate. Therefore, it may be concluded that MAXENT successfully modelled and generated map of potential habitat occupancy of the three species with very high accuracy. Further, the generated maps would provide data for monitoring temporal dynamics of the species (Ndirima, 2007).

#### **Spatial Structure of the Populations**

The spatial patch occupancy pattern of *Swertia chirayita*, of *Paris polyphylla* and *Panax bipinnatifidus* revealed that their habitats were not uniformly distributed in KBR. This is a usual phenomenon for most organisms (Husband and Barrett, 1998), which are patchily distributed across the landscape (Hanski and Gilpin 1997; Hanski, 1999). The patchiness of the habitat by and large influences the configuration of the actual population patches.

The landscape at which Swertia chirayita occurred was typically a mosaic structure of temperate forests at higher altitude and subtropical forests at lower altitude often interspersed by agricultural land, grassland, shrublands and human settlements. Forest fragments were numerous and small, and much of these were located outside the core zone of KBR. This physiognomy of landscape provided a good habitat for Swertia chirayita. Its metapopulation in KBR consisted of 18 populations, which were spread out in four demographic or habitat groups viz., continuous forest sub-tropical (CFS) which consisted of a maximum of seven populations, continuous forest temperate (CFT) with three populations, shrubland (SH) with five populations, and sub-tropical forest fragments (FF) with three remnant populations. Such diversity in habitat for a species is not common as most of the literature on inetapopulation studies focused on discrete populations with equally discrete but single distinct type of habitat. Such studies typically follow the Levins' concept of metapopulation (Levins, 1970). The metapopulation of Swertia chiravita however, did conform to the important rule of metapopulation in which populations must essentially be isolated from one another by inhabitable space, or it should be sufficiently spaced from one another to limit natural dispersal (Hanski, 1999; Frekleton and Watkinson, 2002).

The populations are therefore patchily oriented with small patch size separated by suitable as well as unsuitable habitats. There was an expected positive correlation of patch size with plant density, and patches with small sizes had relatively low plant density suggesting vulnerability of small populations to external perturbations. Large population of CFT and CFS were similarly exposed to threat because of relatively wide isolation of the population

patches. This was evident from the fact that the mean isolation of population patches increased with increasing patch size. The mean patch size and plant density among the habitat groups were larger in the populations of continuous forests (CFS and CFT) and least in the populations of FF. SH had moderate mean patch size and plant density compared to FF populations.

The patch characteristics of *Swertia chirayita* were clearly impacted by the forest fragmentation especially that of CFT and FF populations. This was detrimental to the species distribution. Hanski (1999) concluded both local habitat availability (often measured as patch size) and habitat isolation are important determinants of species distributions. There are also empirical evidences from several studies that both patch size and isolation influence patch occupancy patterns (Hanski, 1994a and b; Hokit *et al.*, 1999; Hames *et al.*, 2001). In addition, patch quality may affect species occupancy and turnover rates (Summerville and Crist, 2001; Fleishman *et al.*, 2002). In this backdrop, the populations in CFT and FF are particularly vulnerable as they had smaller patch size and low plant density.

The typical habitat of *Paris polyphylla* was dense temperate forests where there were very few forest fragments. The existing forest fragments were generally connected to the continuous forest (CF) by forest corridors of varying sizes and distances. Altogether, a total of 11 identified populations constituted the metapopulation of *Paris polyphylla*. Seven populations were located in the CF spread across the south-western part of KBR, and four were located in FF distinctively along the tourist trekking areas where forest fragments were prominent. The populations were more or less discrete and isolated.

The patch size of the populations was significantly correlated with plant density. Although there was no significant relationship between patch size and patch isolation, the density of plants in a population however decreased with increasing patch isolation. While the patch size-plant density correlation was as expected, the depressing effect that patch isolation had on plant density of individual populations was however unexpected. Nevertheless, in relation to forest fragmentation, the metapopulation was apparently affected as the total area 168 occupied by CF population was 20 ha which was much higher than that occupied by FF population which was only 4.3 ha. This high disparity in the population patch size of CF and FF populations *per se*, was in conformity with the literature which suggests that forest fragmentation had direct impact on population size (Adriaens et al., 2009).

*Panax bipinnatifidus* represented the smallest metapopulation among the three study species. Although the habitat micro-environment was suitable and spatial habitat was available for the species to colonize and grow, the sub-alpine forest was characteristically and highly fragmented. Numerous small FF were completely isolated and many others had partially and poorly connected corridors. There were a total of 7 populations that constituted the metapopulation of the species in KBR all of which were identified in the southern and northwestern part of KBR. Four populations were in CF and 3 were in FF.

The plant density-patch size relationship followed similar trend with the other two species, although it was not significant as in the case of *Paris polyphylla*. There was however a significant negative relationship between patch isolation and plant density. While the rationale behind this trend was difficult to explain and required more intricate investigation, there was possibility that distantly isolated populations were subjected to poor population fitness in terms of population dynamics and overall population health thereby, causing the decline in plant density with increase in patch isolation. The total area occupied by the *Panax bipinnatifidus* populations in CF was 14.88 ha and 3.59 ha in FF. This confirmed that forest fragmentation did play a negative role.

### Annual Growth Rate $(\lambda_A)$

In *Swertia chirayita*, the annual growth rate ( $\lambda_A$ ) between transition years was greater in CFT and SH than the other populations. All the populations of CFS group exhibited more or less constant growth rate during the first and second transition years (2005-2006 and 2006-2007), and 5 populations had increased growth rate in the third transition year (2007-2008). All CFT and FF populations had a decreasing trend in the growing rate from the beginning transition year till the end. This may be attributed to the habitat characteristics of the species, which is prone to human disturbances and cattle grazing. A significant positive correlation was obtained between the annual growth rate of *Swertia chirayita* and its patch size. This was in turn due to the positive patch size-plant density relationship (r= 0.8, p= 0.00) which had obvious impact on the growth of the populations.

In *Paris polyphylla*, the trend in annual growth rate did not reveal any definite picture. Nevertheless, four populations of CF had alternate growth and decline rates during the three transition years. Overall, there was a decrease in annual growth rate during the third transition year. For three FF populations, the growth rate increased during the second transition year but declined in the final transition year. This shows that the annual growth rate of perennial *Paris polyphylla* does not give a definite trend in population growth. There was also no significant relationship between annual growth rates and patch size.

Panax bipinnatifidus exhibited similar indefinite trend in annual growth rate as Paris polyphylla. There was a decline in two populations of CF in the second year while two others had considerable increase in annual growth rates. The third transition year was marked by the growth of three populations while the fourth one had decline 70% from the second year's annual rate. In FF populations, two populations *i.e.*, PFF3 and NFF1 fluctuated in annual growth rate during the three transition years On the other hand, one FF (DFF2) population had progressively increased in growth rate in the second and third transition years. The overall trend was reduction in FF population growth rate during the entire study period, indicating the negative effects of forest fragmentation on the annual growth rate of *Panax bipinnatifidus*. As in the case of *Paris polyphylla*, the annual growth rate of *Panax bipinnatifidus* populations did not have significant relationship with patch size.

### **Population Dynamics**

The dynamics of *Swertia chirayita* populations yielded concave survivorship curves for all of the 18 populations. In other words, there was high mortality in the early phase of life cycle *i.e.*, at vegetative rosette stages representing Deevey type-III survivorship curves. High mortality during early phase of the life cycle is typical to many plant populations (Harper,

1977; Rockwood, 2006). There was no visible difference in survivorship curves among the individual populations. However, a critical analysis using pair-wise standard tests for survivorship curves revealed a significant difference between CFS and FF population groups. CFS exhibited better survival than FF populations. This was concluded based on: (i) the Kaplan-Meier curve where CFS slope was more gentle than that of FF and (ii) hazard ratio where the death rate of CFS was less *i.e.*, 0.55 than FF (1.0). This implied that forest fragmentation had a detrimental effect on the survivorship of *Swertia chirayita*.

The survivorship curves of *Paris polyphylla* were more or less similar in all population groups except in two CF populations *i.e.*, YCF and ANCF which exhibited a steep decline and followed type III survivorship curve. In all other populations, all the individuals survived throughout the study period. A comparison of the survivorship curves did not reveal any significant difference between CF and FF populations, and the Kaplan-Meier curves of CF and FF were almost overlapping throughout. This suggests that demographic processes of *Paris polyphylla* were not influenced by fragmentation.

The survivorship of *Panax bipinnatifidus* exhibited a concave curve signifying a high mortality during seedling stage compared to matured stages *i.e.*, one to four leaved stages. This is consistent with the findings of Portela *et al.* (2010) who reported that survivorship of three perennial species *viz.*, *Astrocaryum aculeatissimum, Euterpe edulis* and *Geonoma schottiana* exceeded 85% for adult stages. The overall survivorship pattern of *Panax bipinnatifidus* differed significantly between populations of CF and FF. This was revealed by log-rank test and Gehan-Breslow-Wilcoxon test. Moreover, the Kaplan-Meier curve displayed wide variation in the survival curves between the two groups. This strongly implied a negative impact of forest fragmentation on populations of *Panax bipinnatifidus*. This was also evident from the CF to FF hazard ratio of 0.63.

## **Effects of Micro-environment on Population Dynamics**

Multiple regression analyses revealed the correlation of different population parameters with various micro-environmental factors, which did differ from species to species. In Swertia

chiravita, the pre-monsoon period was the time of seedling recruitment and the emergence of vegetative aerial parts. Regression analysis between seedling population parameters and premonsoon micro-environmental conditions did not yield significant indicating that Swertia chirayita seedlings can establish in a wide range of soil and habitat conditions. Similar findings on two generalist species Polygonella robusta and Lechea deckertii were also made by Maliakal-Witt et al. (2005) where seedlings growth were not affected by the microenvironment. Therefore, the occurrence of the species in diverse habitats was due to the wide tolerance of seedlings to a range of micro-environmental conditions. The vegetative aerial plants were significantly correlated with three soil parameters viz., soil moisture content, soil TKN and soil temperature which indicate the importance of these parameter to the growth of aerial plants from vegetative rosettes. Reproductive plants were significantly correlated with soil pH and available phosphorus. Phosphorus has been reported to play a major role in the development of flower buds (Erel, 2008). The annual growth rate was correlated with soil moisture content and TKN. This strongly suggests that the positive annual growth rate as obtained in CFS populations was partly due to TKN and soil moisture content. There was a significant difference in the levels of these parameters among the groups, and CFS populations had significantly higher levels of soil TKN and soil moisture than FF, CFT and SH populations.

In *Paris polyphylla*, seedlings survival was significantly correlated with soil pH, TKN, available phosphorus and light intensity. Adult plant density was related to pH and light intensity, whereas, reproductive plant survival was correlated with soil temperature. The correlation of *Paris polyphylla* to many environmental parameters partly elucidated the habitat specificity of the species within the forest. The mean annual population growth rate was correlated only with relative humidity. This may be the reason why *Paris polyphylla* thrives better in moist and deep shade environment. This finding is consistent with a study on *Paris quadrifolia* by Jacquemyn et al. (2005). These workers established that the growth of shoot showed strong differences along an environmental gradient of soil and light 172

conditions. Panax bipinnatifidus at seedling stage showed significant relationship with soil exchangeable potassium, water holding capacity and pH. Kruskal-Wallis test revealed that the level of exchangeable potassium in the soil differed significantly (p < 0.01) between CF and FF habitats. Availability of exchangeable potassium in fragment populations could be a limiting factor for low survival of seedlings in FF populations. Moreover, the role of potassium on seedling growth has also been demonstrated by Hanley *et al.* (2007), Paysoy *et al.* (2010) and Kadam and Wadje (2011). The average annual growth rate was correlated with water holding capacity and pH which indicated that the population growth of the species was improved with dependent on these factors.

#### **Population Viability Analysis**

The viability analysis in the present research used different model scenarios (M1 and M2 model) to simulate the future of the species based on certain ecological and demographic features of the three species populations following Akcakaya (2000). The PVA presented are demographically structured models. The individual populations of each of the study species were classified into distinct groups called population groups that are actually based on their habitat or occurrence wherein they shared similar micro-environmental conditions and hence had similar demography of Akcakaya (2005).

The PVA for the three species had four important outputs *viz.*, (i) finite rate of increase (ii) elasticity analysis (iii) metapopulation occupancy and, (iv) extinction risk analysis. Additionally, threat status of the three species was assessed using IUCN criteria and management options were explored to suggest conservation measure of the species.

In both the scenarios, the metapopulation of *Swertia chirayita* had <20% extinction probability in 20 years and therefore, surpassed the endangered category thereby, placing it in vulnerable category (Table 8.1). This is not inconsistent with the status currently assigned to the species which is endangered. However, since 20% risk is projected at 28 years, this difference of 8 years may not be a significant difference and therefore, the species is very close to the endangered category. *Paris polyphylla* on the other hand, had >10% risk in 100 years and is therefore a vulnerable species. This is in conformity with the existing categorization. *Panax bipinnatifidus* had >20% risk probability in 20 years and is therefore an endangered plant of KBR.

IUCN Criteria		
Extinction risk	Time (years)	Status
5%	10 years	Critically endangered
20%	20 years	Endangered
10%	100 years	Vulnerable
<10%	100 years	Lower risk
Status of the study species		
Species	Extinction risk	Threat status
Swertia chirayita	20% in 28 years	Vulnerable
Paris polyphylla	86% in 100 years	Vulnerable
Panax bipinnatifidus	70% in 20 years	Endangered

 Table 8.1: Threat categorization of Swertia chirayita, Paris polyphylla and Panax bipinnatifidus as per IUCN criteria

It is therefore, pertinent to seek management options for the three study species particularly for the endangered *Panax bipinnatifidus*. Management options for *Swertia chirayita* can be effectively carried out by seed sowing and constituting medicinal plant reserves around the elevational predictions. For the perennial species *Paris polyphylla* and *Panax bipinnatifidus*, seed sowing is not feasible because of long seed dormancy and low seed production. Therefore, conservation efforts for the genus *Paris* and *Panax* are carried out exclusively by seedling plantation. Consequently, for *Paris polyphylla* and *Panax bipinnatifidus*, introduction of juveniles into the metapopulation is the only management intervention suggested. In this regard, simulation experiment was conducted for these two perennial species to estimate the impact on recovery that can be achieved by introducing individuals into their metapopulations in KBR (Figure 8.1).



Figure 8.1: Impact of introduction on extinction risk of Panax bipinnatifidus (A) and Paris polyphylla (B)

The simulation results suggest that the introduction of around 10,000 *Panax bipinnatifidus* seedlings every year for 3 years in each population might improve the status of the species from endangered to vulnerable category. For *Paris polyphylla* however, introduction of approximately 75,000 plants for three years is expected to improve the conservation status of the species. If the management intervention succeeds in bringing the species at lower risk status, then estimation of the Minimum Viable Population (MVP) size would be possible. MVP is the minimum threshold that a species can persist for 100 years. In the figure, once lower risk status is achieved, an approximately 2,00,000 individuals of *Paris polyphylla* is required to be introduced to reach its MVP size.

## Conclusion

The metapopulations of all the three species may be characterized as remnant populations in KBR. This was evident from the fact that all the species had  $\lambda$ <1.0. Eriksson (1996) defined a remnant plant population as system of local populations in which some are maintained despite having a local population growth ( $\lambda$ ) below 1.0 (also see Frekleton and Watkinson, 2002).

Concrete empirical evidences were provided regarding the negative impact of forest fragmentation on the three study species. These includes patch characteristics, annual growth rate, population dynamics, environmental parameters correlation, finite rate of increase, metapopulation occupancy and extinction probability. The effects of forest fragmentation on the three species can therefore be summarized as follows:

- Most of the populations in the three species are separated from one another by nonforested land and in case of *Swertia chirayita* by agricultural land as well.
- This caused the isolation of populations and led to decreased patch size and the edge of which was vulnerable to disturbance.
- The significant positive correlation of plant density with patch size in *Swertia chirayita* and *Paris polyphylla* indicated a significant role of patch size in overall growth.
- Increased patch isolation on the other hand had a detrimental effect on plant density of *Panax bipinnatifidus* populations as revealed by its significant negative correlation.
- The importance of patch size was also reflected in its relationship with local occupancy of individual populations.
- The demographic rates of CF populations and those of forest fragments were significantly different in populations of *Swertia chirayita* and *Panax bipinnatifidus* as revealed by pairwise log-rank test of their survivorship curves.
- Swertia chirayita was most affected by fragmentation among all the three species. Besides low survivorship, the difference in  $\lambda$  of CF and FF populations was highly

significant in M1 as well as M2 model indicating two-fold impact of fragmentation on the species.

- *Paris polyphylla* survivorship on the other hand, was not affected by forest fragmentation (log-rank test of survivorship curves between CF and FF populations).
- Negative impact came in the form of disturbance because the difference in λ between CF and FF populations was significantly different in both M1 and M2 model.
- The impact of disturbance mediated by fragmentation on λ was also evident by the stochastic risk assessment, where the difference in predicted extinction risk was very high in M1 compared to M2 model in both *Swertia chirayita* and *Paris polyphylla*.
- In the case of *Panax bipinnatifidus*, forest fragmentation significantly impacted the survivorship of plants as revealed by the comparison of survivorship curves between CF and FF populations. Contrastingly, the CF populations also had very low λ as in FF populations. λ did not vary much between these two groups and consequently model prediction in M2 model also did not reveal much variation from the M1 model.

Of the three species, *Swertia chirayita* which occupied the buffer zone was the most affected species, particularly in the face of rapid land-use dynamics and human activities surrounding the zone. Patches of agricultural field prevalent in the fringe areas could hinder the dispersal capacity of populations as the land is often cleared for cultivation mostly from autumn to winter season which incidentally is the dispersal period for *Swertia chirayita*. Besides, grazing and human disturbances had a detrimental role in the persistence of populations in FF. Strengthening the boundary along the entire buffer zone where *Swertia chirayita* is occurring can go a long way in protecting the populations.

Paris polyphylla represented a mid-altitude ethno-medicinal plant which was relatively less exposed to disturbance. The major disturbance occurred in the form of grazing and seasonal make-shift habitation which was common in the past, however, such activities have been stopped during the last two years as it was banned by the State Forest Department. This has markedly improved the forest habitat conditions. Nonetheless, the populations of *Paris* polyphylla in FF are still affected by environmental edge effects and tourism trekking path. Populations of *Panax bipinnatifidus* were located in an extremely fragmented sub-alpine forests which is per se, the major threat to *Panax bipinnatifidus* as it is exposed to open and drier environment. Biotic interference by deer and wild boar was minimal. Windthrow and forest fire were the major causes of FF in the area where *Panax bipinnatifidus* was found. Although the entire area is sub-alpine forest which is the habitat of the species, the area of occurrence is limited to a certain region surrounded by a large suitable but unoccupied habitat. This poor dispersal was probably due to its seed size and seed predation by insect and deer. There is therefore, immense scope for effective management of *Panax bipinnatifidus* populations, and its closely associated species *Panax pseudoginseng*. The effective conservation of the species can be achieved through transplantation of individuals in such large empty habitats.

# SUMMARY

Forest fragmentation was identified as the most important factor reducing the plant diversity of Kanchendzonga Biosphere Reserve (KBR) located between 27°06' and 28°05' North latitudes, and 88°02' and 88°47' East longitudes in the Eastern Himalayan state of Sikkim in north-eastern India. In addition to fragmentation, several other anthropogenic and natural factors have brought about extreme pressure on the persistence of threatened species in KBR. Several ethno-medicinally important plant species inhabiting the forests of KBR are also under threat due to these factors. In addition, over-exploitation of their populations for their medicinal value has put additional pressure on these plant species, many of which have reached on the brink of extinction. Empirical assessment of their populations is a prerequisite for developing any effective in-situ conservation strategy. Therefore, the present research work was undertaken to prepare a list of ethno-medicinal plants, and their conservation status, and uses under traditional medicine system. The research aimed to assess the population ecology of threatened and ethno-medicinal plant species in relation to forest fragmentation. The habitat distribution modeling of the three study species viz., Swertia chirayita, Paris polyphylla and Panax bipinnatifidus was undertaken to characterize the habitats of the species and map their distribution within KBR. The spatial and temporal dynamics of their populations in forest fragments (FF) and continuous forest (CF) were studied to assess the impact of forest fragmentation on population ecology of these species. Their metapopulation status was ascertained, characterized and Population Viability Analysis (PVA) was performed. The extinction probability analysis was also undertaken to evaluate their extinction risk. Generation of these empirical data and analyses are expected to help in preparing an efficient management plan for the threatened ethno-medicinally important plant species of KBR.

### **Ethno-medicinal Plants Species of KBR**

A detail inventory of ethno-medicinal plants species present in KBR was prepared both through primary and secondary survey. Primary data was collected through a questionnaire survey among the forty traditional healers residing in 36 villages located around KBR. Prior Informed Consent (PIC) was taken before interviewing the traditional healers. The available literature was also consulted on medicinal uses of the plant species reported by the traditional healers. The plants so documented were classified based on their uses, ecologic distribution, taxonomic classification and threat status.

A total of 105 ethno-medicinal plant species were recorded belonging to 64 families. Dominant families were Ericaceae and Zingeberaceae with five species each. Fabaceae and Araceae had four species each, while 12 families viz., Valerianaceae, Solanaceae, Saxifragaceae, Rubiaceae, Rosaceae, Ranunculaceae, Poaceae, Orchidaceae, Liliaceae, Gentianaceae, Asteraceae and Apiaceae were represented by three species each and contributing 18% of the total plant species. On the other hand, about 67% of the families were represented by only one species. Araliaceae, Scrophulariaceae, Convovulaceae and Polygonaceae were represented by four species each and contributed 6% to the total species. On the other hand, 18% species belonged to Valerianaceae, Solanaceae, Saxifragaceae, Rubiaceae, Rosaceae, Ranunculaceae, Poaceae, Orchidaceae, Liliaceae, Gentianaceae, Asteraceae and Apiaceae families which were represented by three species each. Araceae and Fabaceae had two species each, and Ericaceae and Zingeberaceae had maximum representation with five species each. The ailments treated were grouped into 52 types. Common ailments such as cough, fever, headache and other gastrointestinal disorders were being treated by a number of species. 27% of ailments were being treated by only one species, 23% by two species, 15% by three species, 12% by four species, 6% by five species, and 4% by six and seven species each. Another 10% of the common ailments were being treated by more than eight species. In terms of medicinal value of the plant species, most species were used for a few ailments *i.e.*, one to four only, while three species were used for treatment of a variety of ailment types. Valeriana jatamansii was used for treatment of five ailments, Costus speciosus was used for six ailments and Centella asiatica was used for a maximum of eight different ailments. From threat perspective, 75 species were not evaluated

and a total of 30 species were found to be threatened. Of these 30 species, three (3%) were critically endangered, seven (7%) were endangered, 19 were vulnerable (18%) and one species (1%) *i.e.*, *Abies densa* was near threatened.

#### **The Study Species**

Three species were selected from the documented ethno-medicinal plants for detailed population studies in relation to forest fragmentation. These were: *Swertia chirayita* Roxb. ex Flem. (Gentianaceae), *Paris polyphylla* Smith (Melanthiaceae) and *Panax bipinnatifidus* Seem (Araliaceae).

To study the life history and phenology, twenty five matured individuals of each of the selected species were randomly selected at three sites. Different morphological parameters were measured during the peak flowering and fruiting season. The parameters were: individuals per clump, plant height, leaf number of leaves and flowers per plant, flower length and diameter, and number of fruits and seeds produced per plant. Phenological cycles from emergence period to death of the species were closely observed over a period of one annual cycle from January 2005 to December 2005.

Although the habit of *Swertia chirayita* had been a subject of debate, in this study it was established as a biennial species. The life cycle was distinguished into four demographic stages *viz.*, small rosette, large rosette, vegetative aerials and flowering. Seedlings emergence (small rosette) occurred during spring with the onset of rain from early May to late June. The species attained its rosette form during the first year as two distinct stages *i.e.*, small and large rosettes. The large rosette reappeared after dormancy during spring in the second year and initiation of flowering or reproductive stage developed during late monsoon period and completed during autumn. Fruiting commenced simultaneously around the same period and seed setting started before the onset of winter.

*Paris polyphylla* is a polycarpic perennial plant with a short, thick non-clonal rhizome bearing several irregular constrictions which is formed annually. Life stages of the species are represented by distinct types of annual shoots. Three stages were recognized viz.,

seedling, adult and reproductive individuals. Seedling recruitment took place during spring about a month after the emergence of stem from perennating rhizome. Germinated seeds gave rise to radicle which later formed the rhizome system, and followed by plumule which formed the stem. The growth of seedlings occurred during spring and continued till the end of rainy season (September). Seedlings did not attain maturity during the first year during which it grew up to a maximum of 10 cm with 2-4 leaves. Much of the growth however, took place in the underground rhizome component. Dormancy period continued throughout the winter season. Spring season marked the emergence of adult plants which grew upto 20-50 cm height with a terminal whorl of 4-12 leaves. Adult plant produced flower bud during July and peak flowering was observed in August. Fruiting occurred from late August to September, and seed setting started in mid-September to October.

Panax bipinnatifidus is a non-clonal perennial herb. The stem is erect that arose singularly from a primary storage rhizome root joined to a chain of nodes which are added annually. The species is distinguished into five stages viz., seedling, and four stages based on the number of leaves i.e., 1-4 leaved stages. Seedlings were recruited during spring and they did not develop into higher stages *i.e.*, 2-4 leaved stages within the first year. Seedlings are identified by the growth of one leaf and remained one-leaved during the first year. Shoot withered prior to the onset of winter and persisted only as rhizome which reappeared in the following year as annual shoots. This pattern of death of annual shoots was also characteristic of 2 to 4-leaved stages. Reproductive individuals were all 3-leaved and 4leaved stages. Some 3-leaved individuals produced flowers but failed to fruit, while all 4leaved stage plants did bear fruits every year during the study period. Berries were borne terminally on short or long peduncle. Fruits were not observed to mature synchronously, rather maturity and dehiscence occurred at different times, and usually the central fruits matured earlier. Each individual produced about 10 to 30 seeds during early spring (Late March to April) while the re-emergence of annual shoots of seedlings and mature individuals took place during mid-march. Seedling establishment and shoot growth took place during

pre-monsoon and monsoon season. Flowering occurred during early June and fruiting occurred during August. Fruit setting took during late August to September. The plants withered during mid-September to October.

## Habitat Characteristics of the Study Species

Habitat characteristics of each species such as vegetation type, species association, spatial occurrence, slope, aspect, and altitudinal range were recorded. Vegetation sampling was conducted through belt transects. The detailed information on species association was obtained from 40 1 m x 1 m randomly-placed quadrats at each site. Species association was estimated through chi-square pair-wise association of attributes. Cole's coefficient of association was used to quantify the strength of association. Microenvironment and soil characteristics studied were, light intensity, air temperature and relative humidity, which were measured 1.0 m above the forest floor on a particular day. The measurements were taken at four hourly intervals at ten random points in each site on the day of microclimate measurement.

*Swertia chirayita* occurred along the buffer zone of KBR from 1,700 m to 3,000 m altitude in forests, shrublands and patchily on the margins of cultivated land adjacent to buffer zone of KBR. It grows typically on sandy to sandy-loam soil texture with a range of pH from basic to weak acid condition. It flourished along the south-western slopes at 20°-40°. Due to its tolerance to drier environment, it also flourished in shrub lands, open ground and along the forest edges in the buffer zone of KBR.

*Paris polyphylla* thrives in cool temperate (2,400-2,800 m a.s.l. elevation) forests in moderate to deep shade condition and is found most commonly in soft humid and humus rich sandy loam and weak acid soil and on moderate slopes of  $10^{\circ}-30^{\circ}$ .

*Panax bipinnatifidus* occurred at an elevational range of 3,300 m to 3,700 m a.s.l. It is a typically shade-preferring plant whose normal growth and development is ensured exclusively under forest shade and is found in 5°-25 ° slope. It preferred well drained although sufficiently moist weak-acid soils rich in mould. In KBR, *Panax bipinnatifidus* 

thrived in the *Abies densa* dominated coniferous forests and was found in cool microclimate conditions, and on litter- rich/moss-matted well drained soils. The habitat distribution model successfully predicted the potential habitat distribution of the three study species. This was evident from the high AUC output of 0.91, 0.99 and 0.97 for *Swertia chirayita, Paris polyphylla* and *Panax bipinnatifidus*, respectively. The contribution of vegetation to the overall habitat model prediction (as seen by EVI values) was maximum in all the three species. It was 89.3% in *Swertia chirayita*, 68% in *Paris polyphylla* and highest proportion of 94.9% in *Panax bipinnatifidus* habitat models. Among different EVI layers, maximum contribution was evidently by EVI 145 representing spring season with 37.5%, followed by EVI 289 which corresponds to autumn season. For *Panax bipinnatifidus*, EVI 097 corresponding to early spring had maximum contribution (20.6%) to the predictive distribution pattern. Pre-monsoon vegetation index (EVI 145) with 18% contribution and monsoon data (EVI 209) with 18.4% contribution had equal importance to the model prediction. These were followed by other fewer contributors such as early monsoon EVI 161 (13.6%) and post monsoon EVI 273 (13.2%).

The contribution of physiographic parameters (elevation slope and aspect) as predicted by DEM was much lower in all cases with 10%, 32% and 5.1% in *Swertia chirayita*, *Paris polyphylla* and *Panax bipinnatifidus*, respectively. Among physiographic variables, slope (7.4%) was a strong determinant for the distribution of *Swertia chirayita* followed by elevation with 2.8% and least by aspect with 0.5%. On the other hand, elevation had high importance in the distribution of *Paris polyphylla* with 27.3%, followed by aspect (3.6%) and slope (1.1%). Elevation also had more importance (4.6%) in *Panax bipinnatifidus*, whereas, slope and aspect contributed only 0.2% and 0.3%, respectively. Overall, the potential habitats of all the three species were confined towards the southern part of KBR. For *Swertia chirayita*, the distribution of potential habitats was more patchy and dense, while for *Paris polyphylla* and *Panax bipinnatifidus*, it showed a restricted range. For all the species, the occurrence of potential habitats in the northern KBR was relatively less.

#### **Population Dynamics**

For studying population dynamics, the populations of the three species were systematically surveyed by walking 50 transects of 50 m x100 m dimension along the accessible trekking path. All the populations encountered were mapped. A population was defined as an individual patch separated by a distance of at least 100 m from the nearest neighbor patch. Patch isolation was measured as the mean distance between the two nearest populations. To estimate the area occupied by the population, the outermost plants of a population were marked on a map, and the area of the convex polygon defined by these plants was determined using Geographical Information System (GIS) software ArcMap 9.3. The sub-populations were categorized according to the habitats of occurrence. *Swertia chirayita* populations occurred in four habitats or group type (henceforth, referred to as group) *viz.*, continuous forest (CF), forest fragments (FF) and shrubland populations (SH). Populations of *Paris polyphylla* and *Panax bipinnatifidus* occurred in two groups *i.e.*, CF and FF.

Population size of the study species was estimated during peak flowering season. Matured individuals were counted in the case of *Swertia chirayita*. For the two perennials *Paris polyphylla* and *Panax bipinnatifidus*, the population size was determined by counting the total number of individuals including all stages in a life cycle. This was chosen because unlike *Swertia chirayita*, all the life cycle stages of these two species grow simultaneously during the growing season. For populations, estimates were based on the total number of plants was counted, while for larger populations, estimates were based on the total number of reproductive individuals per unit area. This was determined through laying 3-10 replicated quadrats of 1 m x 1 m size depending on the population size and area of occupancy. The population size of each patch was extrapolated by multiplying the area of each patch with the number of reproductive individuals per unit area of that population.

The population growth rate was calculated using the formula:  $\lambda = N_{(t+1)}/Nt$ ; where N<sub>t</sub> is the population size at time t and N<sub>(t+1)</sub> is its size after time t. Populations with  $\lambda > 1$  indicates an increase in population size;  $\lambda = 1.0$  shows no growth whereas,  $\lambda < 1.0$  signifies a decline in

growth. To initiate demographic studies, the life cycle of the three species was divided into discrete life-stages based on preliminary survey prior to conducting detailed demographic studies. Demographic studies on the three species were conducted for four years beginning 2005. For *Swertia chirayita*, the complete demographic data pertaining to three generations were studied (2005, 2006 and 2007). While for long-lived *Paris polyphylla* and *Panax bipinnatifidus*, a 4-years demographic census was completed during 2005 and 2008. The study was performed in permanent plots which were established in each population (n= 72 plots for *Swertia chirayita*, n= 44 plots for *Paris polyphylla* and n= plots 28 *Panax bipinnatifidus*). For *Swertia chirayita*, the plot size was 1 m x 1 m and each plot was demarcated by putting boundary structures made of bamboo. For *Paris polyphylla* and *Panax bipinnatifidus*, the plot size was 2 m x 2 m, and the plants were identified, tagged and counted during successive censuses. The populations of the two latter species were very sparse *i.e.*, 0.39 ±0.03 plants/m<sup>2</sup> for *Paris polyphylla* and 0.22 plants/m<sup>2</sup> for *Panax bipinnatifidus*. Demographic census therefore, was based on the number of individuals that was recorded in each of the plots.

Micro-environment and soil factors were measured during pre-monsoon, monsoon and post monsoon period. Climatic variables such as light intensity, relative humidity and air temperature were recorded at 1m above ground level at each population of the three species at five hourly intervals during the day of measurement. Each parameter was measured at ten random points adjacent to the demographic plots of all populations. Light intensity was measured by a Digital Luxmeter (TES-1332A), while air temperature and relative humidity were measured by a Thermo hygrometer TH–103 (Mex–therm). The mean values for the microclimatic parameters were used for comparing the variables among the populations of each of the three species.

Soil samples were collected on a seasonal basis for two annual cycles during 2006 and 2007. One composite sample for each population patch was prepared by mixing soil samples collected from the five replicate points in each patch. Soil temperature was measured using a

digital portable soil thermometer (Multi–Thermometer). Soil pH was estimated by a digital portable pH meter. Edaphic variables *viz.*, soil moisture content (SMC), water holding capacity (WHC), soil organic carbon (SOC), total Kjeldahl nitrogen (TKN), available phosphorus (P) and exchangeable potassium (K) were estimated in the laboratory.

The metapopulation of Swertia chiravita consisted of 18 populations. These were classified into 4 demographic groups based on their similarity of habitat of occurrence viz., subtropical continuous forest (CS), temperate continuous forest (CFT), sub-tropical forest fragment (FF) and shrubland (SH). Seven populations occurred in CFS, 3 each in CFT and FF, and 5 were located in the SH. The mean patch size of populations was 2.57 ha in CFS, 1.55 ha in CFT, 0.47 ha in FF and 1.32 ha in SH. The total patch areas were 17.95 ha in CFS, 466 ha in CFT, 1.4 ha in FF and 6.6 ha in SH. The mean plant density was  $37/m^2$ ,  $14/m^2$ , 5/m<sup>2</sup> and 25/m<sup>2</sup> in CFS, CFT, FF and SH, respectively. Average patch isolation of populations in each group was 0.29 ±0.13 km in CFS, 0.54 ±0.05 km in CFT, 0.28 ±.22 km in FF and  $0.31 \pm 0.16$  km in SH. All the three cohorts of the populations exhibited concave survivorship curve. A notable decline in population was observed during the transition from seedling stage to vegetative-rosette stage, and the individuals exhibited higher survival during latter stage *i.e.*, from vegetative-aerial to reproductive stage. Overall, the populations in CFS and CFT had better survivorship than those in FF and SH. However, significant difference was observed only in CFS and FF (survivorship test; log-rank test  $\chi^2$  = 4.24, p= 0.039; Gehan-Breslow-Wilcoxon test = not significant).

For *Paris polyphylla*, the metapopulation consisted of 11 populations of which 7 were in CF and 4 in FF. The mean size of the population patches was 2.85 ha in CF populations and 1.06 ha in FF. The total patch size of the populations in CF was 19.95 ha and that in FF it was 4.24 ha. The average density of plants was  $3/m^2$  in CF while in FF it was only  $1.4/m^2$ . The mean patch isolation was  $0.75 \pm 0.25$  km in CF populations and  $0.44 \pm 0.17$  km in FF populations. The survival of seedlings exhibited an identical pattern in all the populations where the annual mortality was more or less uniform except in two populations *i.e.*, YCF and
ANCF population of CF where all seedlings died in 2007. Similarly, seedling survivorship in FF populations also had similar pattern. Five out of 7 CF populations had seedlings survival till 2008 while in FF, 2 out of 4 populations had seedlings that survived till 2008. However, the overall seedling survival till 2008 in CF was 3.9% while in FF it was 5.9%. Survival of adult individuals depicted a sharp decline during the first year, followed by low mortality in subsequent years and for BCF1 population, it was constant till the year 2008. ANCF had no adult survival and so was in FFF population. There was however, no significant difference in the survivorship between CF and FF populations.

Panax bipinnatifidus metapopulation was represented by only 7 populations which included 4 in CF and 3 in FF. The mean patch size of the population was 3.72 ha in CF and 1.2 ha in FF. There was a total of 14.88 ha patch size in CF populations and 3.59 ha in FF populations. The mean plant density was  $017/m^2$  and  $0.06/m^2$  in CF and FF, respectively. The mean patch isolation of CF population was  $0.32 \pm 0.01$  km and that of FF populations it was  $1.26 \pm 0.7$ km. The survivorship curve for seedlings was concave and consistent for all populations. The one-leaved and three-leaved stages had better survivorship in all populations, and there was approximately only a linear increase in mortality during the study period. Two-leaved stage on the other hand, represented a transient stage with fewer individuals, and was encountered in only one population of CF i.e., DCF1 and only 4.7% survived till the year 2008. The occurrence of four-leaved stage was also very rare and found only in DCF2 and KCF1 populations of CF. 100% mortality was observed in KCF1 during the first year of census *i.e.*, 2006 while in DCF2, mortality was observed in the first year, after which, it remained constant till the third year, and declined in the fourth year with 16.7% survival. There was significant difference in the survivorship curves between CF and FF population groups (log-rank test  $\chi^2$ = 3.859, p= 0.04; Gehan-Breslow-Wilcoxon test  $\chi^2$ = 4.277, p= 0.04).

# **Effect of Micro-environment**

Different micro-environmental factors were correlated with different stages of the populations of the three species using multiple regression analyses. For *Swertia chirayita*,

large rosettes density was significantly correlated with three factors *viz.*, soil moisture content, TKN and soil temperature. Vegetative aerial density was significantly correlated with soil moisture content, while reproductive plant density was correlated to soil pH and available phosphorus. On the other hand, the annual growth rate was significantly correlated with soil moisture content and TKN.

In *Paris polyphylla*, seedling density was correlated with pH, TKN, available phosphorus and light intensity while adult plant density was significantly correlated with pH and light intensity. Reproductive plants density was significantly correlated with soil temperature, and annual growth rate of populations with relative humidity.

In *Panax bipinnatifidus*, seedling density was significantly correlated only with soil exchangeable potassium while annual growth rate of population was significantly correlated with soil water holding capacity and pH.

#### **Population Viability Analysis**

The viability analysis used two different model scenarios *i.e.*, M1 base model and M2 alternate model to simulate the future of the species based on demography data of the populations of the three study species. The PVA presented the demographically structured models. The individual populations of each of the study species were classified into distinct groups called population groups, based on their habitat of occurrence wherein they share similar micro-environmental conditions and hence had similar demography.

The PVA for the three species involved two basic analyses (i) Deterministic analysis which included two important measures *viz.*, finite rate of increase and elasticity analysis and, (ii) Stochastic analysis which included metapopulation occupancy and extinction risk analysis. The threat status of the three species was assessed as per IUCN criteria and management options were explored to suggest conservation measure of the species.

The projection matrix model was used for metapopulation analyses of the three species. Field demographic data was used to estimate the vital rates for each stage. Vital rates considered in this study were survival, and fecundity rates. Stage abundance (column vector of the matrix, n<sub>t</sub>) was defined as the total population size in each population at each life cycle 189 stage. This was enumerated by determining density per unit area in each population through sampling in 50 1 m x 1 m quadrats for each life cycle stage. The density so obtained was multiplied by the respective patch sizes of each population to obtain the total stage-specific population size. Vital rates for stage elements were calculated as the average changes over a period of six months for *Swertia chirayita*, and one year for *Paris polyphylla* and *Panax bipinnatifidus*.

Survival rate is the proportion of individuals that survived from a previous stage to the next. Therefore, survival rate for stage i (S<sub>i</sub>) was calculated as:  $S_i = N_j/N_i$  where  $N_i$  and  $N_j$  are the number of individuals at stage i and j, respectively. Fecundity (F) was calculated using the average number of seedling per flowering or reproductive plant in each population. Fecundity was the only entry in the matrix that did not represent a probability, rather, it represented the average number of seedlings a single flowering plant produced in a given year; Fecundity (F) =  $S_{(t+1)}/A_t$ ; where,  $A_t$  was the number of adults at time t and  $S_{t+1}$  was the total number of seedlings recruited at time t+1. Stage-based transition matrices were built from field data collected over 4 years period *i.e.*, 2005, 2006, 2007 and 2008.

Projection was carried out by a series of multiplication of the matrix (A) until a stable configuration was reached. The finite rate of increase ( $\lambda$ ) was calculated as: Finite rate of increase ( $\lambda$ ) =  $n_{t+1}/n_t$ . Sensitivity determines how much various life-history stage transitions affect the population dynamics by examining how changes in a particular stage affect the magnitude of the leading eigen value. Sensitivity ( $S_{ij}$ ) =  $w_i/W$  where, wi was the population vector at the i<sup>th</sup> generation and W was the sum of all population vector. Elasticity was a measure of proportional effect, *i.e.*, the effect that a change in a given matrix element had as a proportion to the change in that element; Elasticity (Eij) = (aij/ $\lambda$ ) Sij where, Eij was the elasticity value and represented the proportion of  $\lambda$  due to transition  $a_{ij}$ .

Demographic data was used to construct a stage structured projection matrix model (basemodel) for each group. Two different matrices designated as M1 (base-model) and M2 matrices (alternate model was used to account for biotic interference) were used separately for each population groups. The logic of using the two model matrices was to compare the population performance in two contrasting scenarios *i.e.*, with disturbance in natural conditions (M1) and without disturbance (M2). The stochastic population dynamics was modelled using Monte Carlo based software RAMAS Metapop version 5.0 (Akçakaya, 2005). Parameters included in the Model were vital rates, demographic and environmental stochasticity, and initial population structure/stage abundance. Since no catastrophic event disturbed the populations during the 4 years of study period, changes in population size most ikely reflected true environmental and demographic stochasticity. Environmental stochasticity was modelled through introducing random fluctuations in stage-specific fecundities and survivals. Demographic stochasticity was modelled using binomial distribution. In the first set, all simulation was run with 1000 replications until time to extinction was achieved. In the second set, a threshold population size (N<sub>e</sub>) was set for *Swertia chirayita*. While for *Paris polyphylla* and *Panax bipinnatifidus*, the threshold size was set at Ne= 100 each.

## Deterministic Analysis

The asymptotic growth rate ( $\lambda$ ) of all the three species fell below 1.0 depicting a general decline in population. For *Swertia chirayita*, the population groups in the descending order of  $\lambda$  was CFS> SH> CFT> FF. For *Paris polyphylla*, it was greater in CF than FF by 32%. The CF populations of *Panax bipinnatifidus* on the other hand, had marginally greater  $\lambda$  (1% higher) than that of FF. The trend of  $\lambda$  in yearly matrix differed among the three species. For *Swertia chirayita*, the overall growth pattern in the base model (M1) was better in the transition matrix of second year for all populations, except for CFT group which although had lower growth rate than other groups, had a relatively steady increase in  $\lambda$  over the three years period. In *Paris polyphylla* too, the  $\lambda$  value in CF increased in the second year matrix and declined in the third year. Nevertheless, both the second and third year matrices yielded a growth in population with  $\lambda$ > 1.0. The FF matrices had gradual decline in  $\lambda$  in the second year matrix. *Panax bipinnatifidus* had 191

constant  $\lambda$  in CF populations but differed marginally among the yearly matrices of FF and showed a small increase with the years. The M2 yearly matrices had the similar trend with M1 matrices for all the three species and with an observed increase in  $\lambda$  as expected for all groups. This was caused by the reduced vital rates in one of the matrix elements due to increased in the number of survivors in the preceding stages. There was significant variation in  $\lambda$  between the groups of *Swertia chirayita* in both M1 and M2 matrix (F = 75.26, p = 0.00 in M1 and F = 32.78, p = 0.00 in M2).

ANOVA and the confidence interval (CI) of mean  $\lambda$  showed that there was significant difference in  $\lambda$  between the groups of *Swertia chirayita* and it was due to the difference of growth rate in CFS and SH from CFT and FF. For *Paris polyphylla*, significant variation in  $\lambda$ between CF and FF was observed only in M1 matrix model (F= 12.91, p= 0.02) although partial overlapping in confidence interval was seen in M1, and total overlapping in M2 was observed. *Panax bipinnatifidus* showed total overlapping of confidence intervals between  $\lambda$ of CF and FF populations, hence there was no significant variation. Three demographic processes contributed to the finite rate of increase of *Paris polyphylla* and *Panax bipinnatifidus i.e.*, fecundity (F) growth (G) and survival (L). For *Swertia chirayita*, the contribution of growth was more in all the habitat groups both in M1 and M2 matrices since growth elasticities were higher in all groups compared to fecundity. *Paris polyphylla* exhibited a slight variation in the elasticities values. Survival had high elasticity values in all populations, but highest values were noted in FF populations in both M1 and M2 models. While in CF populations, growth had more elasticity in both the demographic scenarios.

### Stochastic Risk Analyses

The model predicted a continuous decline in the course of time, both in terms of metapopulation size and number of occupied habitat patches for *Swertia chirayita* and *Panax bipinnatifidus*. On the other hand, *Paris polyphylla* metapopulation exhibited a stochastic trend with some growth at least during a few time steps in the future, usually a characteristic feature of a stable population. A very large decline was expected in *Swertia chirayita* and

*Panax bipinnatifidus* during the next few years both in M1 and M2 scenarios. The trajectories for *Swertia chirayita* and *Panax bipinnatifidus* stabilized after the sharp decline, and although in a stochastic simulation the projection tends to lead to an almost deterministic decline in the future in both M1 and M2 scenarios. For *Paris polyphylla* however, the M1 predicted the similar trend in the initial time steps of the simulation but became more undulating as metapopulation size stabilized. M2 model of *Paris polyphylla* typically had an unpredictable trajectory with growth and decline in most time steps.

In *Swertia chirayita*, the persistence of all 18 populations was projected to maintain for the next 4 years. However, the curve depicted a sharp decline in the number of patches, and only 50% of which would remain extant within 12 years and most of which would remain are populations of CFS habitat. The metapopulation occupancy of *Paris polyphylla* also showed similar trend and approximately 50% of the population would be extant by 25<sup>th</sup> year, while in M2 model, the reduction by half the number of populations would not occur before 40 years and some patches may remain till 100 years. In the case of *Panax bipinnatifidus*, the decline in number of patches occurred at regular pace in both M1 and M2 unlike *Swertia chirayita* and *Paris polyphylla*. Model prediction showed that approximately 50% of the population would remain extant till 12<sup>th</sup> and 13<sup>th</sup> year in M1 and M2 scenarios. In *Swertia chirayita*, maximum time that a population would remain extant was 56 time step or 27 years in M1, and 81 time steps or 40 years in M2 (T-FE2 population of CFS habitat) while lowest time a population would persist was 7 years (T-FF of FF habitat).

In *Paris polyphylla*, the average time the population would persist through time was predicted at 30 ( $\pm$ SD 15.6) years in M1 and 45 ( $\pm$ SD 25.5) years in M2 scenarios. The CF populations had 41 ( $\pm$ SD 8.9) years in M1 and 62 ( $\pm$ SD 12) years in M2 models. The FF populations had persistence time of 13 ( $\pm$ SD 2.4) years in M1 and 15 ( $\pm$ SD 2.9) years in M2 models. The sub-population BCF1 of CF had the most persistence time with 52 and 77 years in M1 and M2 models, respectively. While BFF of FF had the least persistence time with 11 and 13 years in M1 and M2 models, respectively.

The average persistence of populations of *Panax bipinnatifidus* on the other hand, was 12 ( $\pm$ SD 25.5) years in M1 and 13 ( $\pm$ SD 3.4) years in M2. Habitat-wise, the persistence of CF populations was 14 ( $\pm$ SD 2.4) years in M1 and 15 ( $\pm$ SD 2.5) years in M2, and that of FF populations was 9 ( $\pm$ SD 1.6) years in M1 and 10 ( $\pm$ SD 1.8) years in M2 models. Among all populations, DCF had highest persistence time of 18 years in M1 and 19 years in M2 scenarios. Whereas, PFF of FF was projected to have 7 and 8 years persistence in M1 and M2 models, respectively.

The probability of extinction of *Swertia chirayita* was different for different groups. The extinction probability curves revealed a more or less identical shape for FF, CFT and SH population groups in both M1 and M2 models. However, the CFS and metapopulation extinction curves were relatively smoother in M2 model than in M1 model. CFT and FF were predicted to have the shortest quasi-extinction (Q<sub>E</sub>) and extinction (E) times. CFS had significantly longer persistence and a relatively extended range of predicted extinction. In M1 model, the quasi-extinction time was 25 years, and in M2 model it was 54 years while times to extinction were 48 and 88 years, respectively. The overall metapopulation had a quasi-extinction time of 25 years in M1 model while in M2 projection it was 46 years. The trend revealed an approximate doubling of persistence time in M2 scenarios in comparison to M1 scenarios in CFS and SH groups and metapopulation. Thus, groups with lower risk *i.e.*, SH and CFS had a greater persistence time range *i.e.*, up to 90 years, whereas, high risk groups *i.e.*, CFT and FF were predicted to be extinct within 20 years. Therefore, across the scenarios and population groups, the species is facing a projected extinction within 100 years.

The risk curves for *Paris polyphylla* exhibited a wide disparity in isolated scenario between FF populations and that of CF where the latter was highly skewed while the former was relatively smooth. However, the risk of CF populations and metapopulation was almost uniform throughout. This was seen both in M1 and M2 scenarios. Additionally, the prediction between M1 and M2 model showed wide differences although the pattern was

similar. The risk in M1 of CF was 0.86 while in the metapopulation scenario it was 0.97. In M2 the CF was predicted to have 0.43 risk probability and 0.42 for the entire metapopulation. The range of extinction was only shown for FF populations as it had probabilities of quasi-extinction and time to extinction well within 100 years. In the isolated scenario it had a time to extinction range from  $Q_E=20$  to E=36 years and in the M2 from  $Q_E=33$  to E=46 years.

*Panax bipinnatifidus* had similar pattern of prediction with skewed curves in the isolated scenarios as well as metapopulation scenarios. Contrastingly, the risk curves in both M1 and M2 model did not show much variation as that of the other two species. In the isolated scenario the FF populations had an extinction risk ranging from Ne= 10 to E= 25 in M1 model, and Ne= 11 to E= 28 in M2 model. CF had  $Q_E= 16$  to E= 33 in M1, and  $Q_E= 22$  to E= 39 years in M2. Metapopulation in M1 had extinction risk that ranged from  $Q_E= 17$  to 38 years and  $Q_E= 23$  to E= 42 years in M1 and M2 models, respectively.

Threat status of the study species was determined. *Swertia chirayita* and *Paris polyphylla* were vulnerable, while *Panax bipinnatifidus* was endangered. Management options of the two perennial species *i.e.*, *Paris polyphylla* and *Panax bipinnatifidus* was explored and introduction of approximately 10,000 plants for 3 years is required to bring down the risk of *Panax bipinnatifidus* from endangered to vulnerable category. While for *Paris polyphylla*, introduction of approximately 75,000 plants for three years is expected to bring down the species to lower risk category and approximately 2,00,000 individuals of *Paris polyphylla* is required to be introduced to reach its Minimum Viable Population (MVP) size.

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