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Biology and conservation of *Coptis teeta* Wall. – an endemic and endangered medicinal herb of Eastern Himalaya

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Summary

Medicinal plants are a valuable resource for regional economic development in the tropics, and the Eastern Himalaya in particular harbours many such species. Extensive deforestation and over-exploitation in this region have brought several species to the brink of extinction, and *Coptis teeta* is such an endangered species; yet scientific information for its conservation is lacking. Investigations on the distribution range, demography, ecology, cytology, reproductive biology and population genetic structure of *C. teeta* were carried out; it was found to be endemic to a small area, to occupy a very narrow habitat and to be highly dispersed with very small population sizes. Edaphic factors were found to have played a vital role in ecological preference, natural distribution and evolutionary divergence of the species. The species exhibits a 'K' strategy, high male sterility, low reproductive success and efficiency, inadequate seed dispersal, and little genetic variability. A combination of these genetic hurdles and external threats in the form of habitat disturbance and over-exploitation for commercial purposes could result in its extinction. The species was found to have highly specific microsite requirements that cannot be met in other habitats. It is argued that *in situ* conservation measures would be the best strategy for the continued survival of this species. For effective management of the species it is recommended that its habitat be declared a protected area with the active cooperation of local inhabitants including the sharing of benefits of conservation.

Keywords: medicinal plant, endangered species, ecology, conservation biology, Eastern Himalaya

Introduction

The Himalaya, one of the 'hot spots' of biological diversity (Myers 1988), is a vast wooded mountainous landmass. It extends over 3000 km from the Karakoram ranges (27°–36° N latitude) in the west, to the Assam/Burma border in the east (72°-91° E longitude). Of the 7000 endemic species found in India, over 3000 are confined to the Himalaya (Chatterjee 1940); there are no other recent estimates available. The region is known to be a natural reservoir of medicinal plants; several important and traditionally-used medicinal plants have been exploited on a commercial scale from this region for centuries and recently there has been a growth in the demand for drugs of herbal origin (Jain & Rao 1983). Most native medicinal plants have vanished from their natural populations and many have been reduced to very small population sizes which are inaccessible to plant gatherers and poachers. Many plant species of medicinal value are also vanishing because of habitat destruction. A high percentage of endangered taxa consist of plants having medicinal properties (Qureshi & Kaul 1970).

The Eastern Himalaya, particularly the Arunachal Pradesh range, is one of the major centres of plant diversity (Nayar 1996). Although a large number of these Himalayan plant species are being utilized by the pharmaceutical industry, few scientific studies have yet been carried out for them and no efforts have been made towards commercial cultivation of the vast majority of these medicinal plants. Instead, these medicinal herbs are threatened by over-exploitation, several anthropogenic factors, and environmental disruption. In the Eastern Himalaya and northeastern hill regions of India, the practice of shifting cultivation called *jhum*, and deforestation for timber extraction, have been the most significant factors in habitat loss of a number of plant species (World Conservation Monitoring Centre [WCMC 1992]).

Coptis teeta, locally known as *Mishmi teeta*, is a well-known herbal drug endemic to the Mishmi Hill ranges of Arunachal Pradesh of northeastern India (Mudgal & Jain 1980; Pandit & Babu 1993). As a result of over-exploitation of its rhizomes for medicinal purposes the plant has entered into the Indian Red Data Book (Nayar & Sastry 1990), yet scientifically the plant remains a little-known species and an understanding is needed for its effective management (Lande 1988). Studies such as the monitoring of the genetic variation of endangered species provide useful data to achieve the goal of conservation (Falk 1992). The need for conservation of medicinal plants is being recognized by international bodies such as WHO and IUCN (Akerele *et al*. 1991). Since a large number of medicinal plants are found in the tropics and are used by the traditional communities, there is a great need for undertaking surveys and assessing the species at risk, followed by demarcation of areas to be set aside for *in situ* conservation (Frankel *et al*. 1995). Besides the in situ conservation practices, other strategies, such as the founding of new populations, have also

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been reported to be useful in the case of *Amsinckia grandiflora* (Pavlik *et al*. 1993) by using data on the microsite choice, maximum allelic diversity and demography. Identifying crucial demographic and risk factors is considered a particular challenge for biological conservation (Frankel *et al*. 1995). The investigations on *C. teeta* reported here were undertaken to: (1) establish the conservation status of the species populations; (2) ascertain the microsite choice of the species; (3) estimate the extent of genetic diversity in the species; (4) assess the extent and role of abiotic and biotic pressures on the survival of the species; and (5) develop suitable strategies for conservation of the species. The multidisciplinary approach was pursued because such information is required for successful conservation.

Methods

Sampling sites

The Mishmi hills are bound by the Abor and Miri hills in the west, by Tibet in the north and east and in the south by the ranges of Patkai Bum. The general elevation in these areas varies from 610 m to 5233 m. The regional topography encompasses lofty snow-covered peaks, precipitous slopes, wooded and open valleys, deep areas cut by turbulent rivers and their tributaries, and extensive river beds having stabilized islands and massive floodplains in the foothills. One of the striking features of the Mishmi hills is the abrupt rise of mountains above narrow and deep valleys. These valleys are divided by two main river systems, namely the Dibang and Lohit, which are fed by several perennial streams originating from a number of glaciers located in the north. The wooded slopes along the catchments of these rivers form the habitat of *C. teeta*.

On the basis of information on the type locality of *C. teeta* (Hooker 1872; Mudgal & Jain 1980), expeditions to the Mishmi hills in Arunachal Pradesh were undertaken and surveys were extended to similar habitats in the entire hill range as well as the adjoining hill ranges. These surveys were conducted in different seasons during 1986–90. The sites where the species was encountered were marked and a distribution map was prepared. This map represents almost the entire distribution range of the species, although the authors came across *C. teeta* specimens in the herbarium at Botanical Survey of India, Shillong (ASSAM), indicating the site of collection as the adjoining Abor Hill ranges, whereas our surveys in the Abor hills could not confirm this.

A total of 13 natural populations were sampled from 13 sampling sites (Fig. 1) belonging to five localities in the two ecozones. The two ecozones and the 13 sites differed in habitat features (Table 1). The western ecozone comprised three localities, namely Mayodia (four sites), Chinipani (two sites) and Achuli (one site) (Fig. 1). Similarly, the eastern ecozone comprised three localities, namely Burfu-Supliang (two sites), Kamlaiglat (one site) and Chingwinty (three sites) (Fig. 1).

Ecology

To define habitat and niche of the species, latitude, altitude, mean annual rainfall, and soil characteristics like texture, pH and organic matter of the 13 sampling sites were studied. In addition, the type and nature of the community as evinced by the major floral associates, forest storeys, and the type of canopy, and also the nature and magnitude of disturbance factors operating in each population, were recorded.

Analysis of soil samples

Each population was sampled at two or three spatially isolated locations, 40–50 m apart, along a transect. Soil samples each weighing 500–600 g were collected from the rhizosphere (6 cm soil depth) of plants at the sampling locations along the

Table 1 Habitat characteristics of the 13 natural populations of *C. teeta*. Site codes: Mayodia MCT1, MCT2, MCT3 and MCT4, Chinipani CCT5 and CCT6, Achuli ACT7; Burfu-Supliang BCT8 and BCT9, Kamlaiglat KCT10, Chingwinty CCT11, CCT12 and CCT13.

Ecozone	*Population code*	*Latitude*	*Altitude (m)*	*Annual rainfall (cm)*	*Mean temperature*		*Relative humidity*	
					Max. (°C)	*Min. (°C)*	*Max. (%)*	*Min. (%)*
WEST	MCT1	28° 24′	2798	375	16	−7	75	45
	MCT2	28° 24′	2700	375	19	−6	73	38
	MCT3	28° 24′	2550	380	21	−5	85	42
	MCT4	28° 24′	2900	375	15	−8	70	45
	CCT5	28° 42′	2350	378	20	−4	85	50
	CCT6	28° 42′	2480	378	20	−4	85	50
	ACT7	29° 0′	2800	370	15	−6	80	45
EAST	BCT8	28° 12′	2900	382	14	−7	85	45
	BCT9	28° 12′	2800	386	16	−4	90	50
	KCT10	28° 0′	2900	390	16	−4	85	50
	CCT11	27° 48′	2700	396	18	−3	85	50
	CCT12	27° 48′	2780	396	17	−4	80	45
	CCT13	27° 54′	3100	390	16	−6	85	40

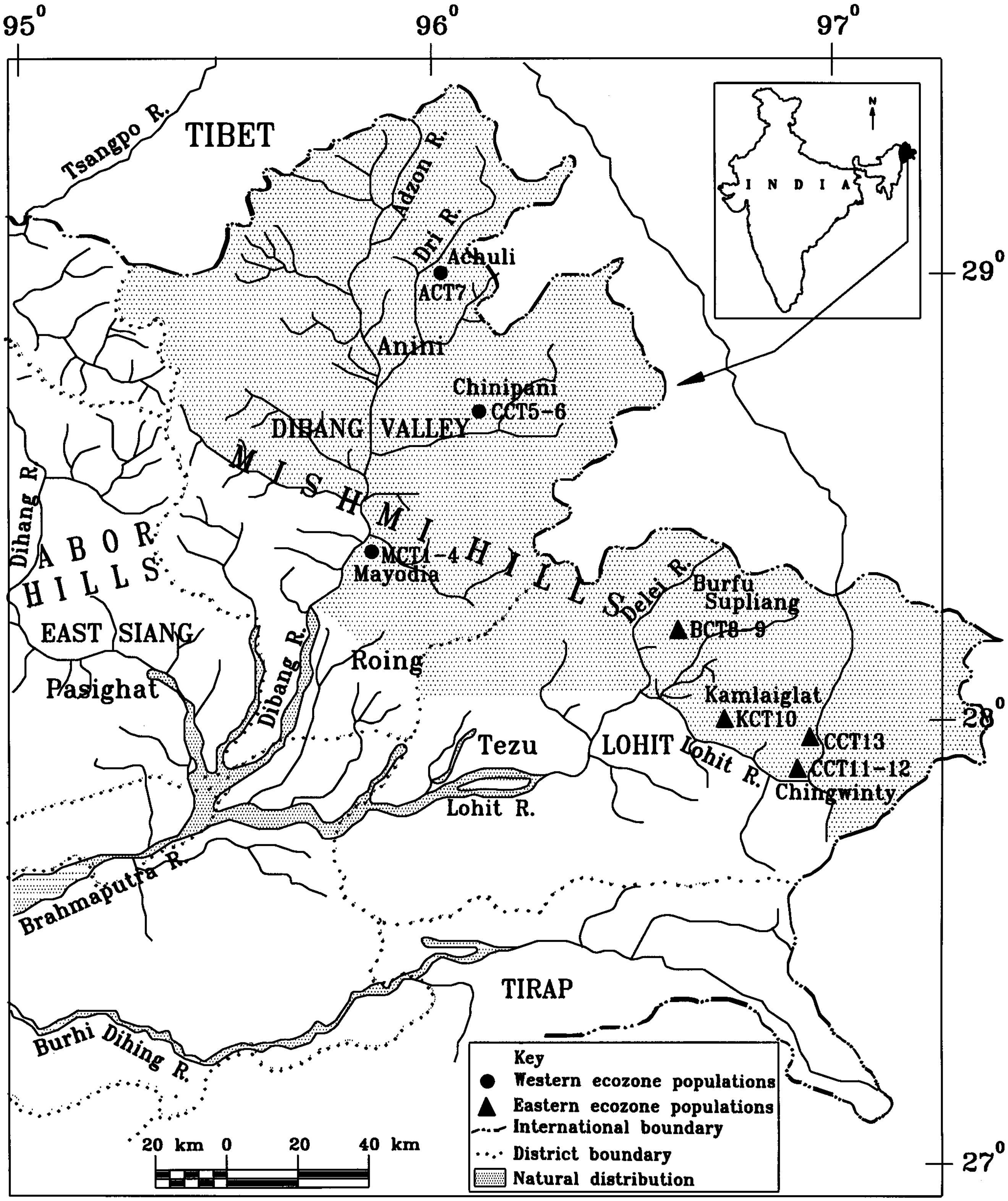

Figure 1 Map showing the area surveyed and sampling sites of natural populations of *C. teeta*. The sampling sites are represented by the serial numbers listed in Table 1.

transect in each site; the samples were air-dried and packed in perforated polythene bags, which were stored at room temperature (25 ± 2°C) until analysis.

Soil texture analysis and classification were based on the method of Piper (1944). Soil pH was determined for three replicates per sample. A soil suspension was prepared by stirring a mixture of air-dried soil (sieved through No. 8 mesh) and deionized water: 20% (w/v), for about 20 min. Soil pH was measured by the electrometric method using an Orion Digital pH meter (Model 901) and Orion pH electrode (Orion Research Inc. USA). Walkly and Black's Rapid Titration Method (see Jackson 1973) was used to determine soil organic matter.

Natural and anthropogenic factors responsible for habitat disturbance were recorded in each natural population; the factors were expressed as values ranging from 0–4. These values represent qualitative estimates of changes in the physical features of habitat, and the intensity of human activities around each natural population, and not the actual quantification of these factors. The habitat disturbance was assessed by recording visible changes in landscape features, soil depth and moisture, canopy cover, and presence of weedy elements. The anthropogenic pressures causing the habitat changes were valued in terms of density of human settlements, intensity of agriculture practices, number of domestic cattle grazing, magnitude of extraction of rhizomes and the extent of road building activity.

Reproductive biology and breeding systems

To understand the reproductive biology and the breeding strategies adopted by *C. teeta*, observations were made on 15 individuals in each population on the appearance of the first inflorescence in an individual, number of flowers per individual, number of carpels per flower, and number of ovules and seeds per flower. The seed/ovule ratio was calculated.

Field pollinations were carried out in 15 individuals of each of the natural populations during February and March 1986–88 simply to determine the breeding behaviour of the species. Selfing was assessed by simply bagging the flower buds until fruit formation had occurred. For crossing, the buds were emasculated and pollen from another individual was dusted on the receptive pistils as indicated by the sticky surface of the stigma. These hand-pollinated flowers were kept bagged until the formation of fruits.

The pollen fertility was tested in 15 individuals in each population by the acetocarmine stainability test. The fresh pollen grains from dehisced anthers of freshly-opened flowers as well as those fixed in 70% alcohol were used for the tests. Pollen grains were stained by incubating in a few drops of 2% acetocarmine for 1–2 h at room temperature (25 ± 2°C). After mounting, the slides were scanned under a compound light microscope. Turgid, larger-sized and stained pollen were counted as fertile, whereas shrivelled, small-sized unstained pollen grains were taken as sterile.

Seed biology

The manually- and naturally-pollinated carpels of 15 individuals from each population were observed for fruit and seed development. The mode of dehiscence of the fruit and the mechanism of seed dispersal were recorded. The seed surface of 10–15 seeds from each population was scanned under a compound light microscope to reveal any specialized structures or features that contribute to seed dispersal.

The extent of seed germination and seedling establishment was recorded for the natural populations of *C. teeta* by making direct observations in the permanent plots demarcated in each sampling site. An area with 20–30 individuals in each population was permanently marked and observations were made every season for four years. Fifty seeds from each population were sown in their respective sites and observed for germination and establishment. The time and duration of seed germination were recorded. The seedlings were counted and kept under observation in the subsequent years. Seedlings which formed the leaves and rhizomes and survived for 3–4 years and entered the reproductive phase were recorded as established.

Population genetic structure

Fresh leaves from 6–10 individuals, 20–30 m apart along a transect, in each population were used in the analysis of isozymes. Fresh leaf tissue weighing 500 mg was crushed and homogenized by a pre-chilled pestle and mortar and made into a slurry by adding 3 ml of chilled tris-HCl buffer (0.1M, pH 7.5). The slurry was filtered and centrifuged at 12 500 rev min^{-1} for 15 min at 0°C. The clear supernatant was decanted into sample vials which were stored in a deep-freeze at −20°C until used. Polyacrylamide gel electrophoresis was used to study enzyme polymorphism in the natural populations of the species. Disc electrophoresis as outlined by Davis (1964) and Ornstein (1964) was followed with slight modifications. For localization of enzymes on gels, methods described by Scandalios (1969) and Santamour and Demuth (1980) were followed and the gels were scanned on a UV-Vis spectrophotometer.

Conservation measures

As part of a conservation strategy, translocation studies were carried out to assess the possibility of multiplying *C. teeta* within and outside its native distribution range. Thirty to fifty plants from each population were transplanted to two sites; site 1 was similar and near to the natural habitat, but at a lower altitude (1900 m), and site 2 was in Shillong, Meghalaya (1500 m), and far away from the natural distribution range of the species. At each site the transplanted individuals were grown under different sets of habitat conditions. At site 1, one set of individuals was planted on a well-drained open slope in the immediate vicinity of an oak-rhododendron forest and a second set was planted inside the forest stand. Similarly, at the Shillong site, one set of plants

Table 2 Soil analysis of the 13 natural populations of *C. teeta*. Texture categories: sc = sandy clay; slg = sandy loam with gravel; sl = sandy loam.

Population	*Texture*	*pH*	*Organic matter (%)*
MCT1	sc	3.7	26.77
MCT2	sc	3.9	25.32
MCT3	slg	4.7	22.80
MCT4	sc	3.8	32.10
CCT5	sc	5.0	25.90
CCT6	sc	4.0	22.70
ACT7	sc	4.3	22.09
BCT8	sl	4.2	21.70
BCT9	slg	4.0	23.91
KCT10	sl	4.2	22.68
CCT11	sl	5.0	18.30
CCT12	slg	4.8	20.70
CCT13	slg	4.3	22.60

was grown under the shade of a *Pinus kesiya* stand, while another was grown under the shade of a mixed broad-leaved forest. The third and fourth sets at Shillong were grown in pots and beds in the botanic garden of the Botanical Survey of India.

Statistical methods

Means, standard deviations and standard errors were calculated, and one-way nested analysis of variance (ANOVA) was used to test significance of the variation amongst the populations. The statistical techniques were based on Sokal and Rohlf (1969).

Results

Geographical distribution

All the populations studied were restricted to between 2480 m and 3100 m except CCT5 (Table 1), which was located at a slightly lower elevation of 2350 m. The natural populations occurred in two ecozones, namely the western ecozone (28°24′–29° N) and the eastern ecozone (27°48′–28°12′ N) (Fig. 1, Table 1).

Habitat, community structure and niche

The species was mostly confined to steep slopes in moist temperate, broad-leaved oak-rhododendron climax forests, but some of the populations (MCT3 and CCT11) were found to inhabit seral communities of these (e.g. bamboo brakes). In general, these forests were 2–3 storeyed with closed canopies; very little sunlight reached the forest floor. The western ecozone populations (MCT1–4, CCT5–6 and ACT7), with *C. t. teeta*, were located at higher latitudes and received less rainfall (370–380 cm; Table 1); the eastern ecozone populations, with *C. t. lohitensis*, were mostly at lower latitudes, but received comparatively higher rainfall (382–396 cm; Table 1). The maximum and minimum temperatures and the relative humidity did not vary much between the western and eastern ecozone; there was, however, a general trend of comparatively lower mean temperatures in the western ecozone and higher relative humidity in the eastern ecozone (Table 1).

The soil texture in the western ecozone populations was mostly a sandy clay type with the only exception being MCT3, which was a sandy loam with gravel type (Table 2). In contrast, the eastern ecozone soils were of sandy loam as well as of sandy loam with gravel (Table 2). All the populations had soils which were distinctly acidic (pH 3.7–5.0; Table 2). The soils of western ecozone populations, however, recorded lower pH 3.7, 3.8 and 3.9, for MCT1, MCT4 and MCT2, respectively, than other populations. In general, the western ecozone populations had higher organic matter contents than the eastern ecozone populations (Table 2). The highest organic matter level (32.1%) was recorded in the soil of the MCT4 population of the western ecozone (Table 2). The only population of the eastern ecozone which recorded higher soil organic matter (23.91%) than some of the western ecozone populations was BCT9. The lowest organic matter level (18.30%) recorded for both ecozones was found in the soil of the CCT11 population in the eastern ecozone (Table 2).

C. teeta occurred in the Montane Wet Temperate forests, sub-type 'East Himalayan Wet Temperate forests' of the 'Lauraceous and high level oak' types (Champion & Seth 1968). These forest types were composed of woody elements such as *Quercus pachyphylla*, *Q. lamellosa*, *Castanopsis tribuloides*, *Magnolia campbellii*, *M. griffithii* and several species of *Rhododendron*. The ground of these forests was covered with several layers of leaf litter and humus, which along with high moisture content gave a bog-like appearance to the forest floor. Members of Ranunculaceae, Rosaceae, Saxifragaceae, Lamiaceae, Apiaceae and Liliaceae were found in abundance in and around these forests. It is amongst these groups of plants that the populations of *C. teeta* were found confined, mostly to higher precipitous slopes (50°).

Habitat loss

The prominent natural factor which was observed to bring about changes in the habitat structure of some of the natural populations during the field surveys was land erosion caused by rains and landslides (Table 3). The natural processes leading to habitat modifications varied from being absent to being very high in different natural populations of *C. teeta*. A high degree of instability was recorded for populations MCT3 and BCT9 as a result of erosion, due to a combination of rains and landslides (Table 3). In contrast, a high degree of stability could be seen in the populations CCT5, CCT6 and ACT7. Anthropogenic pressures leading to habitat disturbance were highest in BCT8 and MCT3 (Table 3), where human settlements, resource exploitation and road building activities were prevalent. Resource exploitation by way of extraction of rhizomes was the prominent pressure-factor in western ecozone

Table 3 Natural and anthropogenic factors operating in different natural populations of *C. teeta*. Factors: 0 = Nil, 1 = Low, 2 = Moderate, 3 = High, 4 = Very high. The values have been expressed as estimates of visible habitat change brought about by natural processes and anthropogenic activities in each population.

Population	*Natural*		*Anthropogenic*				
	Rain	*Landslide*	*No. of settlements*	*Agriculture*	*Grazing*	*Resource exploitation*	*Road building*
MCT1	2	1	1	0	1	1	2
MCT2	2	2	1	0	1	2	2
MCT3	4	3	1	0	1	3	3
MCT4	1	0	1	0	0	1	2
CCT5	1	0	0	0	1	2	0
CCT6	1	0	0	0	1	2	0
ACT7	2	1	1	1	2	2	0
BCT8	2	2	3	2	2	2	1
BCT9	4	3	2	1	1	1	0
KCT10	4	2	1	1	1	1	0
CCT11	2	2	1	1	1	1	0
CCT12	2	1	2	1	1	1	0
CCT13	2	1	2	1	1	1	0

populations. The combination of natural and anthropogenic factors was highest in MCT3 and BCT8, while these were lowest in CCT5 and CCT6. One of the major casualties of these destabilizing agencies was the loss of habitat of *C. teeta* and several other endemic and/or economically important plants, including medicinal plants.

Reproductive biology and breeding systems

The scapes emerged out from the leaf sheaths mostly during January–February and within 20–30 days elongated to a height of 5–6 cm. The young flower buds matured within a period of 8–10 days after elongation of the peduncle. Anthesis of the mature buds occurred in the late morning hours or around noon and the flowers remained fresh for 12–20 days after anthesis.

The mean number of flowers per plant varied from 1.9 to 3.3 (Table 4) and this tended to be higher in the western ecozone as compared to the eastern ecozone. The 'F' ratio for between the populations was statistically non-significant (Table 5). The number of carpels per flower varied from 8.6 to 11.5 (Table 4); between the populations means were statistically non-significant (Table 5), but the eastern ecozone populations showed lower values than western ecozone populations (Table 4).

The means of the pollen fertility varied from 21.3 to 68.3%; the eastern ecozone populations showed less than half of the values reported for the western populations (Table 4). The differences in means between populations were highly significant at $P < 0.01$ (Table 5). No insects were found to visit the flowers and no other pollinators were observed; the controlled pollination experiments revealed that the species is essentially self-pollinated, but is also amenable to cross-pollination.

Seed biology

The seed-ovule ratio, in general, was low, ranging from 0.1 to 0.2 (Table 4). The normal seeds appeared turgid and heavier

Table 4 Reproductive capacity (means ± SE, n = 15) of different populations of *C. teeta*.

Population	*Flowers per plant*	*Carpels per flower*	*Pollen fertility (%)*	*Seed/ovule ratio*
MCT1	3.1 ± 0.4	10.9 ± 1.7	60.3 ± 3.6	0.2
MCT2	2.8 ± 0.3	10.8 ± 0.4	55.0 ± 3.3	0.2
MCT3	2.6 ± 0.3	10.5 ± 0.4	60.6 ± 2.6	0.2
MCT4	3.3 ± 0.3	11.5 ± 0.2	64.3 ± 3.1	0.2
CCT5	2.6 ± 0.3	10.7 ± 0.2	60.0 ± 2.1	0.2
CCT6	3.1 ± 0.3	11.0 ± 0.3	63.6 ± 2.2	0.2
ACT7	3.2 ± 0.4	11.1 ± 0.4	68.3 ± 2.3	0.2
BCT8	2.4 ± 0.4	08.7 ± 1.0	29.6 ± 5.2	0.2
BCT9	2.4 ± 0.5	09.0 ± 1.0	22.3 ± 4.1	0.1
KCT10	1.9 ± 0.4	08.6 ± 0.7	21.3 ± 3.9	0.2
CCT11	2.5 ± 0.4	08.7 ± 1.0	29.3 ± 3.7	0.1
CCT12	2.5 ± 0.4	08.8 ± 1.0	28.0 ± 3.4	0.2
CCT13	2.2 ± 0.4	08.6 ± 1.0	32.6 ± 4.0	0.2

Table 5 One-way analysis of variance of different reproductive traits of *C. teeta:* ** significant at $p < 0.01$ ns non-significant.

Trait	*Source of variation*	*df*	*SS*	*MS*	'F'
Number of flowers per plant	Between populations	12	32.34	2.69	0.98^{ns}
	Within populations	182	497.20	2.73	
	Total	194	529.54		
Number of carpels per flower	Between populations	12	244.08	20.34	2.45^{ns}
	Within populations	182	1511.60	8.30	
	Total	194	1755.68		
Pollen fertility	Between populations	12	340.4	28.36	7.81**
	Within populations	182	661.4	3.63	
	Total	194	1001.8		

than the unviable, shrivelled and light seeds which floated in water; both the western and eastern ecozone populations produced these two types of seeds.

In the natural populations, the seeds germinated during February–March or after the snow melted. In western populations, the maximum germination recorded was 32% in the population ACT7. The seeds of eastern populations, in general, showed very poor germination (4–10%); sets of seeds at BCT9 and CCT11, however, showed no germination. Under natural conditions, seedlings varying from two–three months to one year old were observed in the vicinity of mother genets in all western populations; for some of the eastern populations, no seedling was observed in the field (BCT9, KCT10 and CCT11), but for other eastern ecozone populations, one or two seedlings were recorded near two–four mother plants each. The plant showed a long-generation life-cycle. The seedlings emerged out of the soil in early spring (February–March) and remained in cotyledonary stage for a period of five–seven months (until June–August). After first leaf development, the seedlings remained at this stage for about one year. In the second year of growth, the seedlings developed a second leaf, sometimes with persistent cotyledonary leaves. The rhizome started thickening during the later part of the second year's growth, with a conspicuously organized shoot bud. In the third year of growth, a proper rosette of four–six leaves developed which contributed significantly to the further growth of rhizome and its increased biomass.

Genetic variability and population size

The electrophoretic variation at three enzyme loci studied showed that there was no genetic polymorphism and all the loci were homozygous, suggesting genetic homogeneity of the populations. One locus for esterases and two loci each for the phosphatases and peroxidases were recognized.

The apical bud or shoot tip was observed to form the regenerative organ in the rhizome of *C. teeta*. The rhizome contained axillary buds at each node in *C. t. teeta* populations, but only the apical bud developed either into a ramet or an inflorescence. In stoloniferous *C. t. lohitensis* populations, the apical as well as axillary bud developed into a ramet bearing a stolon or an inflorescence. In this way, a single individual of *C. t. lohitensis* produced a number of tillering shoots (ramets). Clone fragmentation by natural means was occasional in the natural populations; a few fragments disconnected from mother plants were observed in the neighbourhood, but their frequency was low (< 1%).

The natural populations of *C. teeta* were highly isolated by geographic barriers, such as rivers, mountain peaks and long distances, and their sizes were small. The dispersal of propagules was limited in the species. The seeds lacked any special features on their surfaces to allow them to travel longer distances.

Conservation measures

Our personal interviews with the local inhabitants revealed that, in the past, they harvested the rhizomes in such a way that the portion bearing the shoot tip or apical bud was replanted in the soil and only the remaining non-regenerative portions of rhizomes were harvested. We also found out that the rhizomes were harvested only during specific days of a month and only after flowering and fruiting was completed. Traditional harvesting methods have declined and clandestine extraction year-round has developed; the increasing number of migrant labourers in the region has further added to the problem of over-exploitation of the species.

The results from the experimental studies on translocation of the species showed that the plants grown on open slopes at site 1, though in the vicinity of natural habitat, did not become established and exhibited 60% mortality during the first year; by the second year, all individuals had died. Similar results were observed for the individuals transplanted to site 2, in Shillong, under the *Pinus kesiya* stand. The best results, approximately 90% survival, were shown by the individuals grown at site 1 inside the forest stand. The survival rate in mixed broad-leaf forest at Shillong was also approximately 55%, especially in the plants where soil moisture was high, but these plants lasted for only three–four years. The individuals transplanted in pots and beds at the botanical garden also showed a high survival rate because of constant care. *C. t. lohitensis* populations did very well at the botanic garden at Shillong exhibiting almost zero mortality at the end of three years. Most of these transplanted individuals not only survived, but recorded normal multiplication of the ramets and flowered. At the end of the fourth year, most of these individuals died and the survival rate declined to 4–5%.

Discussion

Habitat, community structure and niche

Investigations on the habitat, ecology and niche specialization of a species are of paramount value, not only to unravel the underlying ecological diversity associated with range and pattern of distribution, but also to provide much needed information on the biotic and abiotic requirements of the species. This information is a prerequisite for re-introduction of a species faced with the threat of disappearance (Wilson 1985).

The distribution pattern of *C. teeta* in a small area of the Mishmi Hill ranges suggests that the species is restricted to a very narrow range of higher altitude; the latitudinal gradient is also rather narrow (Table 1). The geographic and other physical features of the habitat and its edaphic characteristics appear to have played a major role in the distribution pattern of the species. For instance, highly acidic soils with high levels of organic matter are a specific microhabitat requirement of *C. teeta*, although the eastern populations can thrive in relatively lower levels of organic matter in the soil (Table 2). The 2–3 storey temperate evergreen forests with closed canopy and stable microclimate, together with the specific structure and composition of the plant community evidently help *C. teeta* to thrive. Except for some populations (MCT3 and CCT11) that grow in bamboo brakes, all other populations are confined to climax type forests. The drastic changes in the habitat conditions, such as temperature and soil, both upwards and downwards on the slopes rule out any vertical movement of the species to increase its distribution range. These observations suggest that *C. teeta* has a specific micro-site choice, which in turn explains its rarity. Some authors have expressed plant rarity in terms of interaction of three factors, namely geographic range size, habitat specificity and local population size (Kruckeberg & Rabinowitz 1985); the rarest species show small geographic ranges, narrow habitat specificity, and small population size, which is the case in *C. teeta*.

The distribution pattern of a species can be altered by natural and anthropogenic processes through habitat modification; the magnitude of anthropogenic pressures on the natural populations of *C. teeta* is cumulatively high (Table 3). The major factors that have direct impact on the survival of the species are its over-exploitation in the wild and road building activities in the habitat involved (Table 3). The threat of depletion posed by continued exploitation of medicinal plants is well documented (Cunningham 1990). Habitat loss and habitat degradation have been shown to be the major causes of plant species decline, extirpation or extinction (Angermeier & Karr 1994; Noss & Cooperrider 1994). *C. teeta* is faced with such threats and is vulnerable to extinction and has been classified here as endangered in the same way as defined by Holsinger and Gottlieb (1991).

Reproductive biology and breeding systems

It is evident that *C. teeta* balances sexual and asexual modes of reproduction. *C. t. teeta* populations mostly produce genets from sexual reproduction, while *C. t. lohitensis* populations produce ramets through vegetative reproduction. As a result, the diversity within the population of genets is low because of their low birth rate. The clonality however ensures a high birth rate of genetically uniform individuals. There are reports that such multiple reproductive strategies produce genotypes of immediate fitness through asexual reproduction, as well as those variants born out of sexual reproduction, which have adaptive value in unpredictable environments (De Wet & Stalker 1974; Asker 1979).

The generally-low pollen fertility indicates the presence of pre-fertilization barriers in sexual reproduction (Table 4). The low pollen fertility in eastern ecozone *C. t. lohitensis* populations is attributable to the meiotic abnormalities in the pollen mother cells (Pandit 1991; Pandit & Babu 1993). The insufficiency of viable pollen leads to imbalanced or unequal sex ratio in the natural populations of *C. teeta*, ultimately resulting in limited seed output. Earlier workers have shown that an imbalanced sex ratio, arising from non-availability or limited availability of pollen, makes more functional females than functional males available in a population (see Frankel *et al*. 1995), and this limits build-up of an effective population size and also increases probabilities of extinction particularly in small populations (Norse 1993). Studies of Schoen and Stewart (1987) on *Picea glauca* have also indicated that variation in male fertility had a marked effect on its effective population size.

As shown by our results, *C. teeta* is essentially an inbreeding species as a result of its reproductive strategy, but also of a compulsion induced by the micro-climatic conditions in which it occurs. Selfing is favoured by the species possibly due to non-availability of pollinators such as insects. Insects do not visit the flowers of *C. teeta* evidently because they are non-showy, small and offer no reward such as nectar. Because *C. teeta* is a forest floor herb in closed canopy dense forests, wind is almost non-existent and cannot play any significant role as a pollinator. As a result, it seems that the species has resorted to inbreeding, which leads to genetic uncertainties and relatively lower levels of heterozygosity; these factors have been reported to pose a threat to the survival of species (Shaffer 1987; Frankel *et al*. 1995).

In *C. teeta*, the female reproductive efficiency, as shown by the seed/ovule ratio, was found to be low (Table 4); this suggests that the sterility factors along with other restrictive controls operate not only in pollen production but also at various stages in female reproduction, namely ovule formation and seed development. The mean number of seeds maturing within the individual fruits (seeds/ovule ratio) is often referred to as brood size (Wiens 1984). The brood size in *C. teeta*, in general, was low (Table 4). The low brood size in perennial herbs has been reported to be a result of outcrossing, which in turn results in genetic polymorphism and segregation of lethals and sublethals (Wiens 1984). Our results on female reproductive efficiency of *C. teeta* do not lend support to Wiens' (1984) observations. In our opinion, it is not just the breeding systems, but several other factors, including developmental, genetic and ecological factors that regulate the fertility/sterility of an individual. The present studies are indicative of the presence of a high genetic load perpetuated through limited outcrossing, and inbreeding, and clonality, which all contribute to the low sexual reproductive potential of *C. teeta* (see also Piper *et al*. 1984; Charlesworth *et al*. 1990). Our results are also in conformity with those reported by other workers such as Menges (1990) in an endangered perennial herb, *Pedicularis furbishiae*, which include small neighbourhood size, low niche width, selfing and inbreeding, genetic uncertainty and phenotypic plasticity.

Genetic variability and population size

Biochemical studies in plants at the population level have revealed a bewildering genetic diversity at enzyme loci, and the electrophoretic variations at these loci have been widely used for a variety of studies including the elucidation of genetic structure of populations and species (Hamrick *et al*. 1979; Gottlieb 1981, 1982). Studies at the protein level, mostly using isozymes, have greatly assisted and augmented various classic studies of ecotypic divergence (Frankel *et al*. 1995). The studies of Hamrick and Godt (1989) on allozyme data of 450 taxa have shown that average gene diversity of widely-distributed species was double that of narrow endemics. Similarly, self-pollinated species were half as polymorphic as outbreeding ones; inbreeding species had more population divergence (Hamrick & Godt 1989). Isozyme work on natural populations of *C. teeta* indicated there was little genetic polymorphism in the species; there is in fact substantive genetic homogeneity at the few loci looked at. The basis of this genetic homogeneity in *C. teeta* can be attributed to extremely limited crossing-over during meiosis and a high degree of asexuality in the species. Our studies have also shown that lower individual fitness may result from lower genetic diversity in small populations (Mitton & Grant 1984), leading to greater risk of extinction.

A number of recent studies have established that a positive correlation exists between diversity and population size (Van Treuren *et al*. 1991; Ellstrand & Elam 1993). Demographic uncertainty in finite populations has been shown to arise due to variation in their survival and reproductive success (Menges 1991). The contribution of sexual reproduction to increasing the population size of *C. teeta* is rather limited; the number of seedlings observed in the natural populations was extremely low. In many populations of *C. t. lohitensis*, seedling recruitment was rare or even absent. The effective population size in the species was found to be maintained by a relatively higher birth rate of genets in *C. t. teeta* and higher multiplication of ramets of each genet in *C. t. lohitensis* populations. This phenomenon, however, does not help the populations either to increase genetic diversity or to extend their distribution range and exploit new habitats. This restriction imposed by the asexual mode of reproduction in *C. teeta* can also lead to an increased risk of extinction.

Conservation strategy

Traditional harvesting practices

The observations on the mode of rhizome harvesting by the local inhabitants suggest that their methods and cultural practices were the best ways by which the species could be utilized on a sustainable basis. It is also interesting to note that rhizomes were harvested by the local people only during auspicious days of a month and also after the plant had completed the sexual cycle. This mode of collection ensured that sexual reproduction could take place and allowed the generation of some variation in otherwise genetically-homogeneous populations. We believe that the indiscriminate harvesting of the 1970s and 80s was largely responsible for the decline of the species before a ban on its extraction from the wild was enforced in the mid-1980s. Some authors have argued that over-exploitation is a more selective threat to species survival than is habitat loss. This is so because habitat loss is a threat to a wide range of taxa, and may not be focused on one species, while over-exploitation selectively poses problems to targeted taxa (Reid & Miller 1989) such as medicinal plants, orchids and timber trees. *C. teeta* has been the target of such a threat of over-exploitation, which has seriously jeopardized its survival chances (Pandit 1991).

Genetic management of populations

One of the ways to reduce the risk of extinction in natural populations which are restricted to small areas and numbers is to use genetically-heterogeneous samples, such as crosses between several sites (Barret & Kohn 1991; Pavlik *et al*. 1993). Such measures have proved successful in species such as *Hymenoxys acaulis* in which mixing of self-incompatible populations enhanced its out-crossing potential (DeMauro 1993). However, genetic management of populations of an endangered plant species like *C. teeta* requires enormous investment of time, money and expertise (see also Holsinger & Gottlieb 1991). Besides, reservations have been expressed by several workers about wide crosses involving sub-species, as would be the case in *C. teeta*. One of the reasons behind the apprehension is that the offspring of these wide crosses could succumb to outbreeding depression (Rieseberg 1991). Thus wide crosses involving divergent ecotypes, such as in *C. teeta*, might best be avoided (see Frankel *et al*. 1995); other management options such as assisting the species to build its population size by reducing external threats such as overharvesting may prove to be more practical and helpful at this stage.

Transplantation strategy

It is obvious from the observations on transplantation studies that the edaphic and other ecological requirements have significant bearing on the survival of the species. It is therefore necessary to secure its original habitats because *ex situ* conservation measures may not succeed. Earlier workers have shown that translocation of an endangered species to suitable new habitats within its native range is a possible means of recovery (Frankel *et al*. 1995). Such studies need to be carried out in *C. teeta* over a longer period of time than the present one to come out with a suitable ex situ conservation strategy. Until that is done, the in situ conservation of the species remains the only choice.

In-situ conservation

As a conservation management policy it may be necessary to declare the habitat of *C. teeta* a protected area. The presence of a number of other important and endangered plant species in the habitat could form a reasonable justification and basis for bringing it under a protective regime. At the same time, the traditional rights of local inhabitants would have to be borne in mind; a cooperative conservation management programme might therefore be launched to ensure that there are no conflicting interests between conservationists and the local inhabitants. The local population would be required to regulate and control activities such as grazing of domestic cattle, extension of agriculture, particularly jhum, and the conservation authorities would have to share with the local people benefits (McNeely & Thorsell 1991) which might accrue such as from the regulated sales of the plant products, and eco-tourism.

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