International Journal of Botany

ISSN: 1811-9700

International Journal of Botany 6 (4): 371-382, 2010
ISSN 1811-9700

Factors Contributing to Critically Endangered Status of *Aquilegia nivalis* Falc ex Jackson- an Alpine Endemic Angiosperm in the Kashmir Himalaya, India

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Abstract: Five populations of *Aquilegia nivalis* were regularly surveyed during 2004-2006 for the study of phenological events, population size, recruitment and mortality of its individuals besides reproductive ecology. The study revealed that its perennating organs start sprouting in the first week of June, followed by flowering of individuals in the 3rd week of June and seed formation in the last week of September. Due to the herkogamous and dichogamous nature of its flowers, *A. nivalis* is an out-breeder. The number of individuals in its populations ranges from 2.87 ind./$m^2\pm0.12$ to 10.28 ind./$m^2\pm0.57$. Due to small size of its populations, very few (0.37 ind/$m^2\pm0.15$ to 2.86 ind./$m^2\pm0.14$) individuals reach the reproductive stage. Furthermore, 10.66%±4.19 to 18.72%±10.31 of individuals are damaged by herbivores in various populations. These factors limit the availability of compatible mates in the populations and contribute to low-insect visitation frequency (0.03±0.00 to 0.14±0.02; n=18), low pollen viability (46.50±1.93; n=3) and consequentially very low fruit (0%±0 to 70%±15.28) and seed set (0%±0 to 60.8%±15.85). All these factors, in conjunction with hostile habitat conditions and enhanced anthropogenic pressures, contribute to the present threat status of this endemic species.

Key words: Population size, reproductive individuals, herkogamy, seed set, refuge sites

INTRODUCTION

Narrow-endemic species are by definition rare and, therefore, inherently threatened (Ceballos *et al*., 1998; Myers *et al*., 2000; Debski *et al*., 2002; Vamosi and Vamosi, 2005). In view of the unprecedented increase in habitat destruction (IUCN, 2002), the spread of invasive alien species (IUCN, 2002), over-exploitation of economically useful species (Lavergne *et al*., 2005; Colling and Matthies, 2006) and other components of global environmental change (Holsinger, 2000), the endemic and rare species, in particular, are increasingly threatened with extinction (Pitman and Jorgensen, 2002; Johnson *et al*., 2004), as in majority of the cases they have least colonizing abilities and generative strategies, like effective vegetative reproduction and persistent seed-bank. Consequently, such species comprise of relatively small populations that are subjected to demographic fluctuations (Holsinger, 2000). Low frequency of flowering during late stages of their life-history (Matsumura and Whashitani, 2002; Lavergne *et al*., 2005), reduced pollinator visitation (Suttle, 2003), altered foraging behavior (Evans *et al*., 2004), production of few seeds with low viability (Meney *et al*., 1997), low propagule dispersal potential (Wolf and Harrison, 2001) and very low germination of propagules, such as seeds (Cerabolini *et al*., 2004; Dar *et al*., 2009), make these species particularly prone to extinction. In fact, several studies (Houghton, 2001; Root, 2003; Parmesan and Yohe, 2003) have documented increased extinction of endemic species due to current unprecedented wave of global environmental changes (Woodward, 2002; Beaumont and Hughes, 2002; Bakkenes *et al*., 2002; Malcolm *et al*., 2006).

Although reproductive ecological studies are of pivotal importance in conservation and restoration of such threatened species, only a few studies describe the limits to reproduction at all stages from flower production to dispersal of germinable seeds (Massey and Whitson, 1980; Pavlik and Manning, 1993). Consequently, the study of factors that critically affect reproductive success needs to be considered in the design of conservation and management strategies (Godt and Hamrick, 1995; Hamrick *et al*., 1991; Bosch *et al*., 1998; Navarro and Guitian, 2002; Neel, 2002). It is because reproductive ecological studies provide information about the nature and origin of a species, its adaptations, hybridization and systematics (Ornduff, 1969; Anderson *et al*., 2002; Neal and Anderson, 2005) as well as about several life-history

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traits (Kaye, 1999; Herrera, 2000; Rey and Alcantara, 2000; Vila and Lloret, 2000; Traveset *et al*., 2003; Gulias *et al*., 2004) and the relationship of reproductive ecology with demography, breeding behavior and population genetics. Reproductive biology is direly required to identify the key bottlenecks in the wide distribution of such species, so as to devise strategies for their successful conservation (Wiens *et al*., 1989; Navarro and Guitian, 2002).

It is in this backdrop that we attempted to identify the intrinsic and extrinsic factors that have led to the present threat status of *Aquilegia nivalis* in the Kashmir Himalaya. We examined population size, fate of individuals, extent of herbivory, phenotypic variability in traits across populations, number of reproductive individuals, pollen viability, breeding behavior, insect visitation frequency and seed set. This information would be greatly helpful in formulating effective recovery and restoration strategies for this and similar other narrow endemic species.

MATERIALS AND METHODS

Study area: Five study sites in the Kashmir Himalaya, (Table 1) were chosen to study the reproductive ecology of *Aquilegia nivalis*. The Kashmir Valley is spread over an area of about 16,000 km^2., located between 33°.20' and 34°.50′ N latitudes and 73°.55′ and 75°.35′ E longitudes. Its vegetation is predominantly temperate, changing to sub-alpine and alpine higher up in the mountains. Dhar and Kachroo (1983a) reported an overall percentage of 31.38 endemic dicots in Kashmir Himalaya. However, Dar and Aman (2003), reported only 8% endemic taxa in the Kashmir Valley, which constitute about 3% of the total Indian angiosperm endemics. Nevertheless, it needs to be emphasized that Kashmir Himalaya constitutes only 0.48% of the total landmass of India, is geologically younger and, among adjoining regions, has the least area per endemic taxon. A significant proportion (40%) of these Kashmir Himalayan endemics are endangered due to a multitude of factors and several of them are now listed in the Red Data Books at the regional, national and international levels (Dhar and Kachroo, 1983b).

Study species: Aquilegia nivalis Falc ex Jackson (Columbine, 'Zao Neil') is a perennial herb that belongs to the family Ranunculaceae and exists in small and patchy populations in moist shady soil or in open alpine meadows among rocks or, rarely, in rock crevices on very steep, least stable, rocky slopes in slightly hard or pebbled soil at an altitude of 3,000-3,800 m (Table 1). Plant

Table 1: Habitat characteristics of the study sites supporting six different populations of *Aquilegia nivalis* in the Kashmir Himalaya

	Study sites				
Characters	Apharwat (Gulmarg)	Harmukh range (Gangabal)	Khillanmarg (Gulmarg)	Sarsoon (Gulmarg)	Thajwas (Sonamarg)
Altitude	3250 m	3450 m	3350 m	3350 m	3300 m
Composition	Steep slope in the immediate vicinity of Gondola with thick layer of soil upon a rock cover which at many places emerge and form part of surface of this site	Very steep rocky slope, composed of big sedimentary rocks with thin and small soil patches among these big rocks	Steep slope, composed of a few longer patches of soil with small as well as big sedimentary rocks through out	Very steep rocky slope, composed of thin pebbled soil patches between small as well as big rocks throughout	Extremely steep and very narrow slope, composed of small pebbled soil layer on rocky slope, some of which emerge on surface
Soil	Deep, moderately hard, moist, mixed with little sand, light-brown to dark coloured	Less deep, pebbled, hard, moderately-moist, mostly mixed with good amount of sand, dark-coloured	Thick soil layer with little sand, loose, moderately moist, light-brown to dark coloured	Thin, relatively hard and moderately moist, sandy, light-brown	Less deep, pebbled, moderately-hard and moist, sandy, light-brown to dark coloured
Species dwells	Soil patches between big as well as small rocks, but mostly among *Rhododendron hypenantheum* shrubs	On small soil patches among or under big rocks as well as small rocks	Mostly among *Rhododendron hypenantheum* shrubs or in small spaces under and between big rocks	Open small soil patches, among or under big as well as small rocks or rarely among *Rhododendron hypenantheum* shrubs	On open soil patches, among or under, moderately big as well as small rocks
Exposure	Well-exposed to partially shaded	Well-exposed	Well-exposed to partially shaded	Well-exposed to partially shaded	Well-exposed
Stability	Less stable, constant narrowing by the erosion via along side gorge and further threatened by construction activities of GDA (Gulmarg Development Authority)	Least stable, subjected to very frequent landslides and continuous rolling of rocks	Relatively stable due to dominance of *Rhododendron hypenantheum*, but unheld massive rocks pose a big threat	Least stable, subjected to frequent landslides, trampling by army and ponies	Least stable due to its very steep nature and devoid of any soil supporting trees or shrubs except a few *Salix* spp., landslide prone patch
Grazing	Very frequent	Less frequent	Frequent	Very frequent	Frequent

(9.01±2.69) cm (n = 15) tall; stem solitary or, rarely, a few (1.73±1.16) (n=15). Lower (cauline) leaves a few (6.86±2.97) (n = 15), petiolate, (5.25±2.01) x (3.24±0.66) cm (n = 15), bipinnate. Upper (spike) leaves usually absent, much reduced, if present. Flowers solitary or a few (1.53±1.12) (n = 15) per plant, dark-blue to violet, 2-5 cm across; sepals 5, petaloid, ovate, free, acute, (2.4±0.44) x (2.37±0.34) cm (n = 15), dark-blue to violet (Fig. 1a); petals 5, small, (2.66±0.39) x (1±0.28) cm (n = 10), longer than broad, linear to obovate, obtuse, violet; each petal has a (0.82±0.10) cm (n = 15) long, tubular, straight or (rarely) curved conic-obtuse spur (Fig. 1b). Stamens many (43±8.67) (n = 15), whorled, (0.78±0.29) cm (n = 15) long, anthers basifixed. Carpels usually 5-9, (2.19±0.27) cm (n = 15) long; ovary (0.86±0.19) cm (n = 15) long; style (1.26±0.31) cm (n = 15) in length, with a pinhead-shaped stigma. Fruit follicle/capsule, (3.13±0.29) x (2.30±0.24) cm (n = 10) (Fig. 1c, d). Seeds per capsule variable, 30.55±5.35 to 69.2±5.49, broadly ovate, flattened, hard, solid, 1.5 -2 x 1-1.5 mm, very smooth, dark- blue.

Variability in vegetative and reproductive traits: In order to record the extent of variation in different traits across the studied populations, 10 randomly-selected, healthy individuals were tagged and examined weekly throughout the growing season to record the onset and duration of various vegetative phenophases (sprouting, seed germination), sexual phases (bud formation, anthesis, pattern of anthesis), flowering period, type of dichogamy, etc. Characteristics of floral parts (pedicels, sepals, petals, stamens, carpels) were determined using magnifying lenses (10x, 20x) and dissection microscope. Attributes, such as plant height, rhizome length, number of leaves, length and breadth of the largest and smallest leaves, flower number, percentage fruit set and number of seeds per plant were recorded for the calculation of reproductive output of different populations.

Population density and fate of individuals: To comprehend why populations of this species squeeze and is each individual able to survive and contribute to

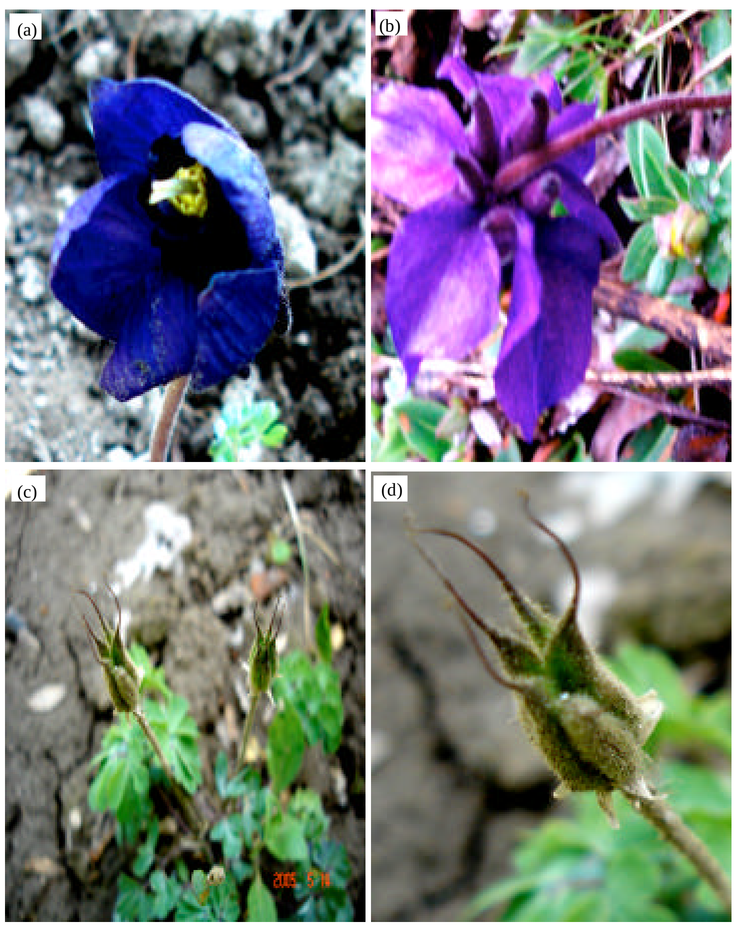

Fig. 1: (a) Flower after anthesis, (b) Spurs of flower; (c) and (d) Fruits/Capsules

population growth, demographic data for *Aquilegia nivalis* was collected during 2004-2006 from a total of 40 quadrats at 5 selected sites in high- altitude localities (Gulmarg, Naranag and Sonamarg) in the Kashmir region. These quadrats were established across five populations immediately after the melting of snow in the month of May (sprouting/seed germination) to study population density (numbers/m^2) and fate (survival and reproduction) of individuals. The seedlings, after attaining distinctive appearance, were marked using colour-paint following Khushwaha *et al*. (1981). Seedlings were distinguished from sprouted individuals on the basis leaf texture, colour and presence of bristles. The marked seedlings were again counted on the subsequent census to record mortality and survival of the individuals. The seedlings without colour marking on each sampling date constituted the new recruitments, which, after counting, were marked with the colour-paint in the permanent quadrats to distinguish between the already recruited and new recruits on the successive sampling dates. Marked plants in quadrats were monitored from sprouting to senescence throughout the growing season and the census was conducted after every 15 days. The life-stage of individuals (reproductive and vegetative), mortality by herbivory, part herbivored and other related details were also noted.

Breeding behavior: Eighteen plants were selected randomly from the Apharwat and Khillanmarg populations with 1-2 floral buds each and were subjected to pollination experiments. Three randomly selected plants in each of these populations were then subjected to one of the following controlled hand-pollination treatments (Kearns and Inouye, 1993) in their natural environment: (1) unemasculated flowers tagged for open pollination (2) emasculated and bagged to prevent pollination (3) emasculated and hand-pollinated with self pollen (4) emasculated and cross pollinated with pollen from different individual, (5) unemasculated and bagged before anthesis to enforce selfing and (6) emasculated flowers left open to pollinate. These treatments were used to test for open pollination, apomixis, geitonogamy, cross-compatibility, autogamy or self-compatibility and other breeding characteristics of the species. Emasculations, done with the help of blunt ended forceps, were performed at an appropriate time (just prior to floral opening or immediately after flower opening for appropriate treatments). Pollination experiments were performed between 9:00 am to 1:00 pm when flowers open and mature anthers are about to disperse their pollen. Fully mature pollen laden anthers were gently tapped to shower pollen on the receptive stigma. Butter paper bags with fine pores were used for bagging purposes. The bags were removed after the stigmas became completely non-receptive (approx. 5 days). The resulting fruits from these treatments were collected and number of fruits and number of seeds (wherever formed) per fruit was determined at the end of growing season.

Insect visitors to flowers: Floral visitors to *Aquilegia nivalis* were monitored in two populations located at Khillanmarg and Sarsoon Post. In a 10×10 m area, 36 observations of 30 min apiece during different times of the day (from 8 am to 6 pm) were conducted for 6 days by one or more observers. This totaled to about 18 h of observation. Number of visits an insect made, time it spent on one flower, number of flowers it visited in a single visit and the number of plants it visited were recorded. To standardize these observations, we calculated the number of floral visitors per flower on hourly basis as the total number of visitors observed divided by the number of flowers. An insect landing anywhere on the inner petals, pistil, or stamen was treated as one insect visit. Visitation rate was calculated as the total number of visits made to each population divided by the number of flowers to yield number of insects per flower per hour. Representative floral visitors were trapped, anaesthetized with NaCN and scrutinized for pollen load on their body parts. The pollinator was identified at the Department of Zoology, University of Kashmir, Srinagar, Jammu and Kashmir, India.

Stigma receptivity: To record the initiation and longevity of receptivity, stigmas at different developmental stages were fixed in FAA (formaldehyde: acetic acid: alcohol) (Tangmitcharoen and Owens, 1997) for 3-4 h and later transferred to 70% alcohol for storage. The stigmas were stained with aniline blue in lactophenol (Hauser and Morison, 1964) and scanned under light microscope (×400) for pollen deposition. Number of non-germinated, number of germinated and total number of pollen grains on the stigma surface was determined to obtain percentage of germinated pollen grains on sigma surface. The stigmas with germinated pollen grains were considered as receptive. The initiation of receptivity and its longevity was also recorded. The morphological features associated with the receptive and non-receptive stigma vis-à-vis anther developmental stages were noted.

Pollen viability and abundance: Fresh pollen collected from Apharwat, Khillanmarg and Thajwas populations were checked for their viability and longevity of viability. By using aniline blue in lactophenol (Hauser and Morison, 1964), the percentage of stained pollen in random but non-overlapping microscopic fields (three replicates for each staining treatment) was determined (Bernardello *et al*., 2004) using an Olympus microscope at ×100 magnification. The mean pollen abundance was determined for this species by selecting five reproductive plants and calculating pollen number per anther, flower and plant.

Pollen ovule ratio: Pollen ovule ratio was determined for three populations (Apharwat, Khillanmarg and Sarsoon Post) using 10 randomly selected reproductive plants. The ovary of each flower was dissected and the number of ovules determined with the help of dissection microscope. We also calculated total number of pollen grains per anther for same floral buds (crushing one anther in ten drops of water and after through stirring of the mixture pollen count in one drop and its multiplication by ten gave the mean total pollen count per anther). Total number of pollen grains in one floral bud was worked out. Pollen/ovule ratio per flower was then calculated (Cruden, 1976).

Reproductive output: Ten randomly selected and tagged reproductive individuals from five populations were periodically inspected to record data on plant height, number of flowers, percentage fruit set and number of seeds per plant for the calculation of reproductive output.

Statistical analysis: The data for demography and extent of herbivory aspects of *Aquilegia nivalis* was recorded from 10 replicate quadrats for each population. It was converted to numbers/m^2 and then subjected to statistical treatment so as to workout mean and standard error for each parameter in each population. Also mean and standard error for different vegetative and reproductive traits were worked out from 10 plants in each population. The breading experiment details have been worked out for 18 plants in total. The mean and standard error for stigma receptivity and pollen viability is based on three replicates. Basic statistics, such as trait means and variances and Analysis of Variance (ANOVA) were calculated using SPSS 10.

RESULTS

Phenology: The overall growing season is short, although, it varies by a few days in the studied populations. *Aquilegia nivalis* sprouts in week or so after the snow melts when temperatures increase in the last weak of May to mid June (Table 2). The seed germination occurs in the second half of June. Initiation of floral bud formation can be noticed 12-14 days after the sprouting of perennating organs. The seed formation starts from the month of July and continues up to September.

Vegetative and reproductive traits: The various vegetative and reproductive attributes worked out in the studied populations (Fig. 2) reveals that, the Sarsoon population showed highest values (12.54±0.57) cm for plant height and number of leaves/plant (9.3±2.32) and lowest corresponding values (7.81±0.27) cm and (5.7±0.72) were recorded for Apharwat population. The number of flowers showed variation between populations in the

Table 2: Chronology of various phenophases in *Aquilegia nivalis* based on 10 tagged individuals in each population studied in the Kashmir Himalaya

	Population				
Features	Apharwat (Gulmarg)	Harmukh range (Gangabal)	Khillanmarg (Gulmarg)	Sarsoon (Gulmarg)	Thajwas (Sonamarg)
Initiation of sprouting of permanent organs	Ist weak of June	Last weak of May	Last weak of May	2nd weak of June	Ist weak of June
No. of days for which prouting continues	115	125	130	110	120
Initiation of seed germination	4th weak of June	2nd weak of June	3rd weak of June	4th weak of June	3rd weak of June
No. of days for which seed germination continues	65	75	78	60	75
End of sprouting phase	Last weak of September Ist weak of October		Ist weak of October	Ist weak of October	Last weak of September
Initiation of floral bud formation	3rd weak of June	2nd weak of June	2nd weak of June	4th weak of June	3rd weak of June
No. of days for which floral bud formation continues	74	70	85	65	72
Initiation of flower anthesis	Ist weak of July	Ist weak of July	4th weak of June	2nd weak of July	Ist weak of July
Days taken by flower to anthesce	up to 15	up to 15	up to 15	up to 15	up to 15
Pattern of floral anthesis	Apical	Apical	Apical	Apical	Apical
No. of days for which a flower persists	10-12	10-12	10-12	10-12	10-12
Initiation of seed formation	2nd weak of July	2nd weak of July	1st weak of July	3rd weak of July	2nd weak of July
No. of days for which plant continues to produce seeds	75	75	80	65	75
End of seed formation	Last weak of september	Last weak of september	Last weak of september	Last weak of september	Last weak of september
End of sexual phase	Last weak of august	Last weak of august	Last weak of august	Last weak of august	Last weak of august

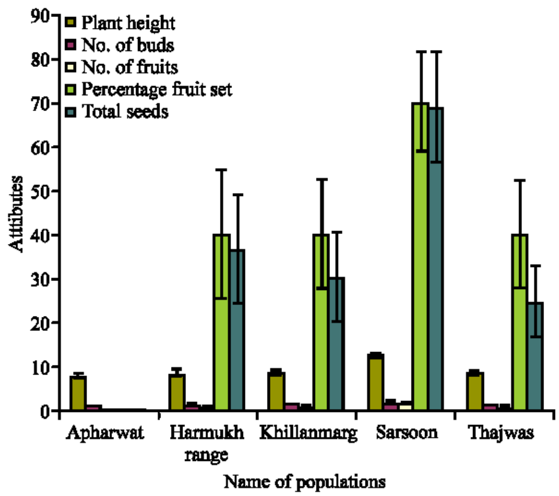

Fig. 2: Vegetative and reproductive attributes (Mean±SE) in various populations of *Aquilegia nivalis* in the Kashmir Himalaya

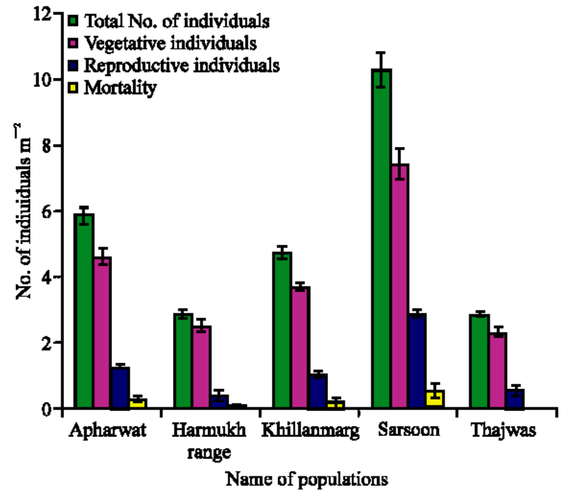

Fig. 3: Comparative demographic details (Mean±SE) of *Aquilegia nivalis* at various populations in the Kashmir Himalaya

respect that greater numbers of individuals in some populations bear more than one flower per plant as against the usual pattern of one flower per plant in most of the reproductive individuals in other populations. The values for flower number depict that it was highest (1.9±0.23) for Sarsoon population followed by Khillanmarg (1.5±0.16) and, lowest (1.1±0.1) for Apharwat population. Consequently, the maximum number of fruits (1.2±0.29) and seeds set was (60.8±15.85) was recorded for Sarsoon population and lowest for Apharwat population. Thus, significant variations were noted across populations studied for plant height ($p\leq 0.000$), number of leaves ($p\leq 0.457$), number of flowers ($p\leq 0.026$), fruit set ($p\leq 0.013$) and seed set ($p\leq 0.006$).

The Apharwat population, besides having small population size and small number of reproductive individuals with most of those bearing only one flower each, was frequently visited by herbivores throughout the growing season damaging the flowers, leaves and the fruits. In the Sarsoon population, the relatively greater number of reproductive individuals with many bearing more than one flower, showed a relatively greater reproductive out put above and beyond the prevalence of herbivory.

Population density and fate of individuals: The patchy populations of this species were found to consist of a small number of individuals with majority being vegetative and a smaller number being in reproductive phase. From the perusal of demographic details (Fig. 3) it can be observed that the population density varied significantly ($p\leq 0.000$) across studied populations and was highest (10.28 ind. m^{-2} ±0.57) at Sarsoon population and least (2.85 ind. m^{-2}±0.08) at Thajwas population. The number of vegetative individuals varied significantly ($p\leq 0.000$) and numbered highest (7.42±0.48) in Sarsoon populations and lowest (2.3±0.14) in Thajwas population. The number of individuals entering the reproductive phase is a big constraint in this species and showed significant ($p\leq 0.000$) variation across the studied populations. The highest number (2.86±0.14) of reproductive individuals was recorded in Sarsoon population and least (0.37±0.15) in Harmukh range population. The overall proportion of individuals suffering mortality is low, yet it showed significant ($p\leq 0.017$) variation across the studied populations. The mortality of individuals was recorded to be highest (0.53±0.21) in Sarsoon and least (0±0) in Thajwas population. The reproductive individuals suffer the least mortality although mostly their leaves and flowers are browsed by herbivores.

Herbivory: Herbivores (cattle) damage individuals of this species by browsing petiolate leaves in vegetative individuals and leaves as well as large flowers and fruits in reproductive individuals. The herbivory affected every studied population and varied insignificantly ($p\leq 0.903$), thus changing the configuration of the vegetative and reproductive individuals in the studied populations. The extent of herbivory determined in the present study in different population in the sampled quadrants (Fig. 4) shows that the highest number of individuals herbivored occurred in Sarsoon (1.22±0.50) and least (0.42±0.17) in Thajwas population. The number of herbivore damaged vegetative and reproductive individuals varied

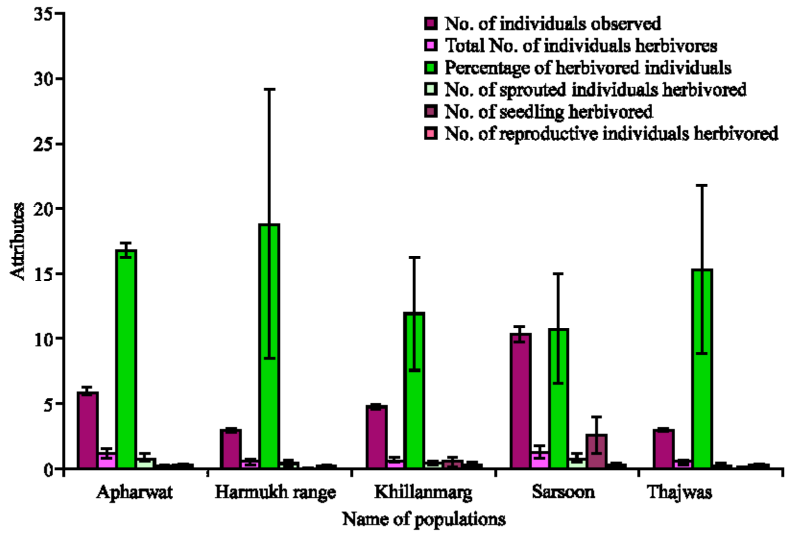

Fig. 4: Extent of herbivory in various populations (Mean±SE) of *Aquilegia nivalis* in the Kashmir Himalaya

insignificantly ($p \leq 0.380$) and ($p \leq 0.878$), respectively, across the studied populations.

Heterostyly: The hermaphrodite flowers in *Aquilegia nivalis* are showy, nectariferous and large. The stamens are many and smaller than the carpels. Carpels are many and free with the style placed higher above the anthers. The stigma matures earlier than the anthers even when the latter are yet to dehisce, hence flowers of this species exhibit both dichogamy as well as herkogamy.

Stigma receptivity: The stigma elongates and reaches up to the tips of petals before anthesces. The stigma and anthers are separated in space as well as time, the former can be found without any pollen even up to 3 days before anthesces (in bud conditions). On the day of anthesis. the stigma is fully developed and starts to become receptive but possess less (21.33±3.28) deposited pollen grains and the least (8.79±4.40) percent of germinated ones. The number of deposited pollen grains as well as the percentage of germinated pollens starts increasing thereafter. The percentage of germinated pollen grains reaches (11.92±7.24) one day after anthesis and highest (42.91±4.83) two days after anthesis. Hereafter the number of deposited pollen grains as well as their percentage germination starts declining gradually and reaches even up to (10.39±5.19) on the fourth day after anthesis. Hence the stigma receptivity gradually increases from the day of anthesis and reaches its peak on the 2nd day of anthesis.

Breeding behavior: Bagging experiments revealed that individuals of this species produced seeds upon cross-pollination as well as on pollen transfer between flowers on the same individual which develop asynchronously. No seeds were formed either under apomictic or autogamous conditions (Fig. 5). The percentage of fruit set was highest (64.28±17.97) in unaltered or unemasculated flowers tagged for open pollination followed by those in manually cross pollinated flowers (57.14±17.00) then in geitonogamous (50±22.36) and emasculated flowers tagged for open pollination (42.85±17.00). The breeding experiments established that this species exhibits a facultative xenogamous breeding system. The slight disparity in the percent fruit set in different treatments may be a result of the damage and removal of pollen reward although the all important nectar rewards were still unaltered.

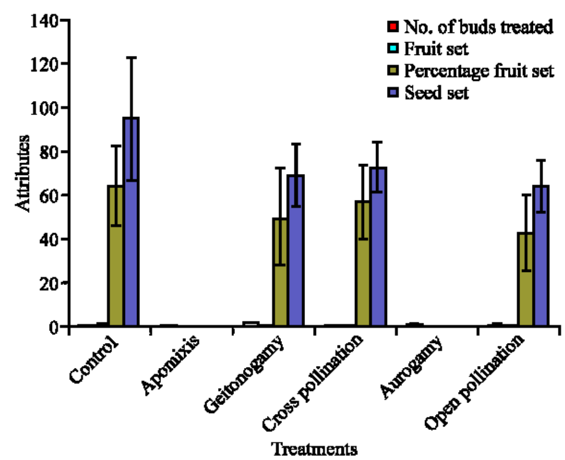

Fig. 5: Breeding behavior of *Aquilegia nivalis* (Mean±SE) in the Kashmir Himalaya

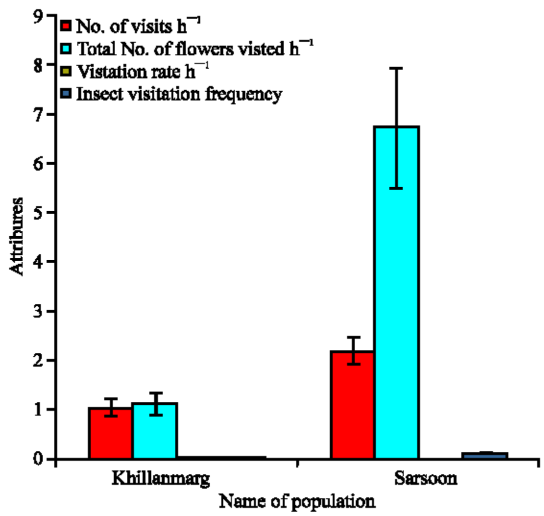

Fig. 6: Insect visitation frequency (Mean±SE) at some population of *Aquilegia nivalis* in the Kashmir Homalaya

Insect visitors to flowers: In *Aquilegia nivalis* only one insect species (*Bumbus* sp.) of Bombideae was found foraging its flowers. This *Bombus* sp. is of bigger size corresponding to the larger flower size of this species. The pollen load on the abdomen and hind legs of the insect was high. Observation pertaining to the visitation behavior of the insect species revealed that the insect, after hovering, lands initially on the inner petals or on closely held style fascicle for a very short while. It thereafter angles downwards to anthers while brushing its abdomen and upper portion of hind legs against the stigmas. The insect generally visits 1-3 flowers in one bout in the same population. On each visit, the insect spends at most 10-15 seconds on each flower. The individuals of *Bumbus* sp. visited the flowers more frequently between 9 am to the noon and then again from 3:30 pm till 6 pm. The overall visitation rate and insect visitation frequency is considerably low in the two population studied. The frequency of visitation increased with an increase in the number of flowering individuals and the number of flowers in a population. Higher values for the number of visits h^{-1} (2.22±0.27), number of flowers visited h^{-1} (6.72±1.22), visitation rate h^{-1} (0.05±0.00) and visitation frequency (0.14±0.02) occur for Sarsoon population than the corresponding values in Khillanmarg (Fig. 6).

Pollen viability and pollen ovule ratio: The number of flowers per plant is usually one and rarely more. The flowers of this species have many anthers and many ovules in a polycarpellary apocarpus ovary. The mean pollen viability is (46.50±1.93) (n = 3). The recorded data shows that the values of the P/O ratio are highest (4684.20±299.44) for Sarsoon followed by Apharwat (4173.58±125.76) and relatively low (4016.78±453.08) for the Khillanmarg population.

DISCUSSION

Aquilegia nivalis never forms large continuous populations in a range; instead they occur as isolated, fragmented patches in restricted intimidating habitats. These populations comprised smaller number of individuals at almost all the study sites. The individuals are mostly thinly placed, except in a few patches where they may occur relatively closely.

The presence of inadequate proportion of reproductive individuals in its populations appears to have serious consequences, manifested in the form of less availability of mates, pollen limitation, very low insect visitation-frequency, seed abortion, low seed set; and, hence, negligible contribution to population growth and expansion. Kirchner *et al.* (2005) stated that low population size, or low population density, can lead to an Allee effect- a threshold density, population size, or combination thereof, below which pollinators no longer visit flowers. Many other studies carried out in plant populations have shown that low densities are associated with reduced pollination success (Allison, 1990; Feinsinger *et al.*, 1991; Kunin, 1997) and reduced seed set (Roll *et al.*, 1997; Bosch and Waser, 1999). Furthermore, studies have also established that large population size had a positive effect on pollination (Aizen and Feinsinger, 1994), seed or fruit production (Luijten *et al.*, 2000; Hackney and McGraw, 2001; Brys *et al.*, 2004), or on both (Groom, 1998; Hendrix and Kyhl, 2000).

Herbivores take a heavy toll on the populations of this species, as they browse both vegetative as well as reproductive individuals. Hence, even in its refuge sites (high-altitudes), populations of this species face herbivory pressure. The herbivores, besides damaging leaves of vegetative and reproductive individuals, destroy the beautiful, nectariferous and conspicuously large, flowers of this species; hence further diminishing the number of available mates and other attributes. Consequentially, it has been worked out that in animal-pollinated flowering plants, floral and seed predators and herbivores can dramatically limit seed production (Ayre and Whelan, 1989; Escarre *et al.*, 1999), seedling survival and recruitment (Louda, 1982) and ultimately the population growth (Ehrlen, 1996).

The floral architecture, such as presence of dichogamy (protogynae), herkogamy, nectariferous spurs and showy appearances affect the cross compatible-mode of pollination in this species, which is further evidenced by high pollen/ovule ratio. The pollen transfer was found to be mediated by one insect species (*Bumbus* sp.), which transfers pollen between its individuals and also between flowers of the same individual. This pollen transfer is predominant within population and less frequent across populations. The pollinator visitation is affected by the density of the reproductive individuals and, hence, by the abundance of flowers and floral reward. In this species, the already restricted number of reproductive individuals is further hampered by the herbivore damage which makes its populations face less insect visitation and hence further exaggerates the already serious problem of pollen limitation due to few compatible mates. The pollinators visiting flowers of this species show less priority for its flowers due to the abundance and availability of flowers of other plant species. Similar conclusions from other studies support the argument that low population or patch size may affect the attraction of pollinators, because small populations or patches may be less apparent and offer lower pollen and nectar rewards (Rathcke, 1983; Sih and Baltus, 1987). As a consequence, flowers will be less visited, resulting in lower seed set (Agren, 1996; Groom, 1998).

The transferred pollen quantum in *Aquilegia nivalis* is much diluted as the insect species forages on other species more preferentially than flowers of this species. Such situation again leads to pollen limitation, which is further complicated as a result of low pollen viability in this species, established by the viability and *in vitro* germination tests. Similar conclusions have been reported by other workers, stating that when too few individuals of a particular species occur in an area with many other flowering species, it leads to dilution of conspecific pollen in the pollen load carried by generalist visitors and deposited on stigmas (Feinsinger *et al*., 1991; Kunin, 1993; Aizen and Feinsinger, 1994). All these factors ultimately manifest in the form of seed abortion and hence extremely scarce seed set. This has been put forth by many other researchers that trait of primary importance in regulating the abundance and distribution of a plant species is its capacity for seed production in nature (Byers and Meagher, 1997; Eriksson and Jakobsson, 1998). Also, some studies have established that restricted endemic species generally have lower maternal fertility than their widespread congeners (Lavergne *et al*., 2004), while others have suggested that ecological factors limit the reproductive success of rare or endemic plants (Murray *et al*., 2002).

The scant populations with patchy distribution, sparse occurrence of reproductive individuals and their further damage due to herbivory, limited presence of compatible mates, less pollen viability, the least insect visitation and consequentially, very low fruit- and seed-set constrain this species to be restricted in its occurrence. The matter gets further complicated by the presence of limited germination microsites, extremely low seed germination percentage evidenced by the occurrence of meager proportion or total absence of seedlings from most of its populations. Besides, the prevalence of within population pollen transfer, less adoptability of individuals, seed abortion, least seedling survival and inability of majority of the individuals to enter reproductive phase, hints towards the probable occurrence of inbreeding depression in this species. All these factors, in conjuncture with the hostile environmental conditions and speedy anthropogenic pressures, contribute to the present threat status of this species and may further render these refuge populations out of present asylum and hence not even available for recovery and restoration.

Conservation implications: Like other endangered endemic plant species, the high-altitude *Aquilegia nivalis* has over the period of time been forced to find refuge in the hostile environmental conditions, where it has to sustain amidst habitat fragility, habitat destruction, trampling, herbivory and extraction. Possible reasons behind the low pollen viability, hence pollen limitation and low seed set, meiotic behavior, prevalence of inbreeding depression in this species need further indepth study. Recovery and restoration measures are desirable in earnest for this species and may include, besides protecting existing sites and managing habitat conditions to promote large population sizes, measures to multiply the individual from its robust rhizome which does not happen in nature but appears a possibility as the plant produces more off shoots (not separate individuals) in resource-rich habitats from the same rhizome. This, besides other possible means, should be an effective and efficient way to salvage out this species from extinction, because the seed set and seed germination is the major stumbling block in the growth and expansion of this species.

ACKNOWLEDGMENTS

Thanks are due to the Head, Department of Botany, University of Kashmir, Srinagar for providing all the necessary facilities and encouragement required to accomplish the present study.

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