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Reproductive biology of subalpin endemic *Minuartia nifensis* Mc Neill (*Caryophyllaceae*) from West Anatolia, Turkey

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Abstract

Reproductive biology of *Minuartia nifensis* Mc Neill (*Caryophyllaceae*), was investigated from 2001 to 2004 to determine the reasons for the restricted distribution of this endemic species, to that end, the environmental conditions and the reproductive biology were studied. In Flora of Turkey records, the entry for *M. nifensis* includes just one locality on the Nif mountain. As a result of field investigations, we found another locality, approximately 1 km to the southwest of the first one. Using GIS and sampling methods, these two localities together form an area of 1.2 km^2 and the number of individuals in all two localities was recorded as 3308. This species had been declared as endangered according to IUCN (1994) categories. Our studies led us to reccomend it as Critically Endangered (CR) B2ab(ii)+(iii) according to the IUCN (2001) categories. Moreover, for the first time, it was found in this study that this species also contains a hermafrodit flower and a female flower. The observations gathered in this study justify the fact that, the hermaphrodite individuals are self or insect pollinated and the female individuals are insect pollinated only. Pollination experiments also showed that all flowers are potentially able to develop fruits throughout the flowering period, suggesting that no abberation occurs in sporogenesis, fertilization or post-zygote processes. However, calculated seed/ovule ratio was low (24% in hermaphrodite flowers and 17% in female flowers) and low reproductive success in the field should be attributed to insufficient pollen transfer between anthers and stigmas.

Key Words: *Minuartia nifensis*, Reproductive Biology, Nif Mountain, Izmir, Turkey.

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Sub-alpin endemiği *Minuartia nifensis* Mc Neill (Caryophyllaceae)'in üreme biyolojisi, Batı Anadolu

Özet

Minuartia nifensis Mc Neill (*Caryophyllaceae*)'in, 2002-2004 yılları arasında gerçekleştirilen çalışma ile bu türün sınırlı yayılış nedenleri belirlenmeye çalışılmış, bu amaçla bitkilerin yaşadıkları çevre koşulları ile üreme biyolojileri araştırılmıştır. *M. nifensis* Mc Neill (*Caryophyllaceae*), Türkiye Flora'sında Nif Dağı'ndaki tek lokaliteden kaydedilmiştir. Arazi çalışmaları sonucunda, ilk lokalitenin yaklaşık 1 km güney batısında yeni bir lokalite tanımlanmıştır. GIS ve örnekleme metodları kullanılarak, bu türün yayılış alanı 1.2 km^2 ve alanda bulunan birey sayısı 3308 olarak hesaplanmıştır. Bu tür IUCN (1994) kriterlerine göre hassas olarak belirlenmiştir. Çalışmalarımız sonucunda tür, IUCN (2001) kriterlerine göre Kritik Tehlike altında (CR) B2ab(ii)+(iii) olarak önerilmiştir. Ayrıca, ilk defa olarak bu türün hermafrodit ve dişi çiçekli bireylerinin olduğu saptanmıştır. Araştırma sırasında elde edilen veriler, hermafrodit bireylerin kendikendine veya böcekle dişi bireylerinse sadece böcekle tozlaştığını göstermiştir. Tozlaşma denemeleri ayrıca, tüm çiçeklerin potansiyel olarak tohum oluşturabileceğini göstermiş ve sporogenez, tozlaşma ve zigot sonrası dönemde herhangi bir olumsuzluğun oluşmadığını göstermiştir. Tohum/ ovül oranı (hermafrodit çiçeklerde 24% ve dişi çiçeklerde 17%) ve üreme başarısı düşük bulunmuş, bunun da doğada anterler ve stigmalar arasındaki yetersiz polen transferine dayandığı düşünülmüştür.

Anahtar Kelimeler: *Minuartia nifensis*, Üreme Biyolojisi, Nif Dağı, Türkiye

1. Introduction

Turkey has one of the highest concentrations of endemic plant species, with about 30% of its plants being endemic, and a large proportion (95%) of these are categorized as endangered, rare or threatened. Detailed studies on the conservation biology of these species is essential to prevent extinctions and as stressed by Schemske et al. (1994) studies on the reproductive biology of such species may be useful for understanding their restricted distributions. For most of the threatened species that are not commercially exploited, the population size is rarely known, as such conservation biology of such threatened species need to be investigated Matsuda et al. (2000).

The *M. nifensis* found in Turkey, is one of the 16 members which belong to the *Xeralsine* subsection of the *Minuartia* section of the *Caryophyllaceae* family. The *M. nifensis* species was first discovered on the Nif mountain peak by Reino Alava in the year 1966. In 1969, Mc Neill who had been doing research on the *Xeralsine* subsection, categorized this species as a distinct endemic one that can be found only on the Nif mountain (Davis 1988). This species is very rare and known from type gathering only. As indicated by John Mc Neill, who conducted his PhD research on *Arenaria* and *Minuartia* in the mid 1950's in Southwest Asia and neighbouring areas, the taxonomy of this subsection, of which *M. nifensis* is a member, is extremely complicated and its area of existence within Turkey is still unclear. In his study, Mc Neill suspected that the reproductive biology of the group to which *M. nifensis* belongs was somewhat unusual and wondered about the possibility of agamospermy. To address the issues raised by Mc Neill, there is need for the investigation of the breeding system and the evolutionary status in Turkey as well as for the interrelationships among all species within the subsection, including the European and the five Caucasian species.

In 2000, the Turkish Association for the conservation of Nature declared *M. nifensis* as an endangered (En) species, despite the fact that ecological and quantitative data for this species was not available (Ekim et al. 2000). Given the low number of known populations and the lack of data concerning *M. nifensis*, a study to identify the status, distribution and ecological requirements of the species was undertaken.

Therefore, the aim of this study was;

(1) to determine the conservation status of *M. nifensis* by updating existing data and hence its IUCN status.

(2) to asses the dependence of the species on sexual reproduction through an investigation of its pollination ecology, pollen viability, stigma receptivity and seed productivity;

(3) to assess the future life history of existing populations by estimating possible reproduction rate through examination of seed viability.

2. Materials and methods

2.1. *The plant*

The material consisted of *M. nifensis* McNeill (Sect. Minuartia subsect. Xearlsine (Fourr) McNeill)in Notes R.G.B. Edinb. 29:327 (1969). It's an endemic perennial herb restricted to the peak of Nif Mountain in Western Anatolian part of Turkey, approximately 40 km East of Izmir (Fig. 1).

2.2. *Methods*

During the field investigations, GPS data was collected and analyzed with GIS programs to develop distribution maps. The number of individuals in a given population was recorded by using 50 meters long line transect. In each population 5 repititions were made. The experiments were carried out in the field at Nif mountain (1500 m asl). In order to determine whether other populations existed in the area, additional suitable habitats for this species in a two km radius around the known population were visited from 2001 to 2004 during the vegetation period from March to July of each year.

The pollination type in *M. nifensis* was followed for two consecutive years 2002 and 2003 during the months of May to July using 50 individual plants. These were chosen at random depending on the easiness to approach the site.

Five treatments were used for pollination experiments in the field: a) self pollination (flowers were bagged); b) wind pollination (anthers were removed); c) insect pollination (anthers were removed and flowers caged); d) cleistogamy (anthers were removed and flowers bagged); e) controls. Each treatement was applied to a group of ten randomly selected flowers of similar size from ten individual plants per year (n = total of 50 flowers per year). The selected flowers were examined three weeks following application of the treatments to observe the fruit/seed set. Pollen viability and stigma receptivity were investigated in two experiments carried out simultaneously from May to July, 2002. The flowering starts in May and continues till June. Our field observations revealed that the flowers of *M. nifensis* have a floral cycle of 7-8 days. Ten flowers were collected for each treatment as follows: a) three days prior to flower

opening (A-3); b) two days prior to flower opening (A-2); c) one day prior to flower opening (A-1); d) on the day flower opened (A). Each experiment was replicated twice, therefore a total of 80 flowers were used in two experiments. Since the number of individuals in the area was low we used a lower number of samples and the flowers during our experiments in order to safegaurd the reproductive potential of future populations. Each collected flower was placed separately in an ependorf tube, stored in a cool box and transported to the laboratory within two hours following collection. In the laboratory, each flower was dissected and the anthers and stigmas were removed. For the pollen viability tests, one anther per flower from each of the ten flowers corresponding to each of the four developmental stages (total 40 flowers), was stained with 1% tetrazolium bromide for 45 minutes at 35-37 ^{0}C. The stained anthers were examined under the microscope by randomly selecting 500 pollen particles per anther and examining whether they were stained or not (Firmage and Dafni 2001). Stained pollen particles corresponded to viable pollen, while those that had not been stained were considered not viable. For the stigma receptivity tests, the stigma of each flower from each of the ten flowers corresponding to each of the four developmental stages (total 40 flowers), was treated with a Perex (Merck 16206) solution (Dafni and Maues 1998). The stained stigmas were examined under the microscope and allocated to three categories according to the gradation of staining: a) orange (receptive); b) deep orange(more recptive); and c) red (highly receptive). Enzyme activity was estimated according to a colour scale prepared especially for this test.

For estimating seed production (or pollination) success, the seed to ovule ratio was calculated according to Bosch et al. (1998) in 40 randomly selected flowers collected in two batches of 20. The first batch was collected at the beginning of July 2002 and the second batch one week later. It was possible to count the number of seeds per flower in the laboratory because the seeds remain attached to the flower at the first stages of its formation. Lastly, the collected seeds were tested for their viability. A total of 20 randomly selected seeds were treated with 0.1 % tetrazolium chloride. Stained seeds were categorized as viable, semi-stained were viable but weak, and those that had not been stained were considered not viable. Germination test was applied to test the reliability of viability as well as dormancy in the seeds. Fresh seeds (2002) were left for germination on whatman paper in 9 cm petridishes using double distilled water in preset incubators at 5° , 10° , 15° C under 16/8 photoperiod. In another lot of seeds the seed coat was removed and these were divided into two groups, 50 and 100 ppm gibberellic acid and kinetin was applied in equal volumes to the two lots of ten seeds each. These were left for germination under the aformentioned conditions together with the lot without getting a hormonal treatment.

3. Results

M. nifensis is strictly endemic to the peak of Nif Mountain in Western Anatolian region of Turkey. Its known distribution area at the start of this study was limited to one population and it occupied an open site from 1350-1500 m, with an area of approximately 500 × 200 m, according to the records published in the Flora of Turkey and East Aegean Islands (Davis 1988). However, during our field studies in addition to this locality, another locality was recorded approximately 1 km to the southwest of the first one (Fig. 1). This location was also found in a zone extending between 1350-1500 m It was found to flourish on open sites, on gravelly habitats with shallow soils which usually cover calcareous rocks. The vegetation period is restricted to the months of April-July. The growth conditions outside these months are either too cold and snowy or too hot and dry for this plant. The distribution area of all known *M. nifensis*

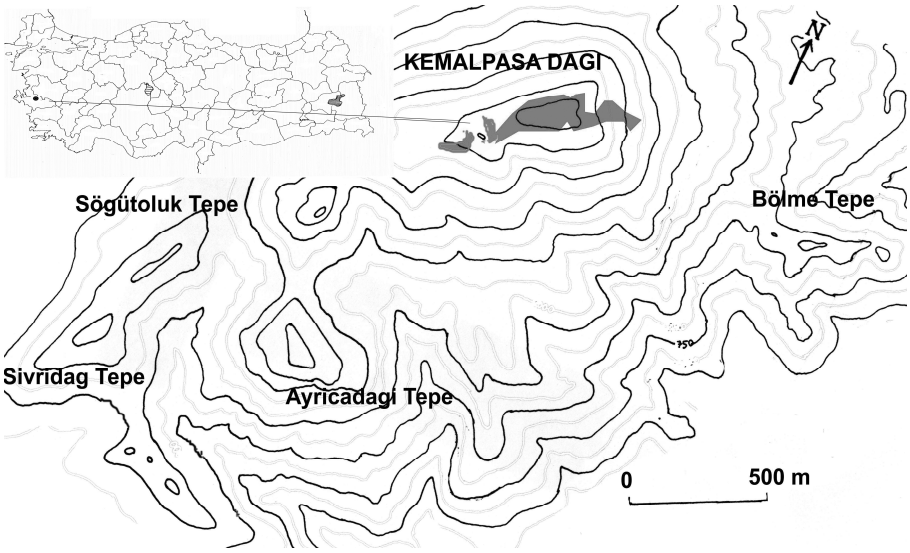

Figure 1. Geographic distribution of the two known populations of *Minuartia nifensis*.

locations is approximately 1.2 km^2 with a total of 3308 individuals (Table 1). According to this data and the IUCN (2001) categories, the endangered status of this species was recommended as CR B2ab(ii)+(iii).

The issues of problematic reproduction and the evolutionary status of the *Xeralsine* section which were first pointed out by Mc Neill, were verified by the observations on flower variations. Moreover, for the first time, it was found in this study that this species also contains a hermafrodit flower which carries 10 stamens and a female flower which carries 5 short 5 long sterile stamens. The hermafrodit flower has klavat stigmas, while the female flower has plumoz lobed stigmas but both flowers possess 3 lobes. As a result of the pollination experiments, all ten bagged, the ten whose anthers were removed and the ten control flowers developed fruits in hermaphrodite flowers while none of the other two categories developed any fruits, and in female flowers all ten control flowers developed fruits while none of the other three categories developed any fruits (Table 2). After the flower opening the climatic features were observed to play a great role in the pollen viability and stigma receptivity. These limitations prevent in getting reliable results in this connection.

Table 1. Population densities of *Minuartia nifensis*.

Population Number	Area (km$^{2)}$	Number of Individuals
1	1.2	2647
2	0.3	661

Table 2. Data recorded from the pollination experiments.

Treatment	Author's justification	Test of this treatment	Results (seed-set-hermaphrodite flowers)	Results (seed-set-female flowers)	Conclusion
A. flowers bagged	Self-pollination	Spontaneous selfing	100%	0%	Spontaneous selfing occurs; self-compatibility
B. Anthers removed	Insect pollination	Cross-pollination	100%	N/A	Pollen/pollinators limitation
C. Anthers removed + cage	Wind pollination	Wind pollination (depend on cage type)	0%	N/A	No wind pollination
D. Anthers removed + flowers bagged	Cleistogamy	Cleistogamy/ parthenogenesis	0%	N/A	No parthenogenesis
E. Control	Natural open pollination	Natural open pollination	100%	100%	High natural pollination success

The TTC tested average pollen viability was determined as; 74% three days before opening of the flowers, 92% two days before opening of the flowers, 88% one day before opening of the flowers and 85% when the flowers fully opened (Figure 2).

The results of the stigma receptivity tests in both flower types showed that the stigma receptivity starts increasing from two day before the opening of the flowers and reaches its peak when the flowers are fully opened. The stigma receptivity decreases after the fully opening of flowers. The average of enzyme activity for stigma receptivity, in hermaphrodite flowers, two and three days before the opening of flowers was 27 ppm, 80 ppm one day before the fully opening of the flowers and 130 ppm when the flowers are fully opened. In female flowers the stigma receptivity, two and three days before the opening of flowers was 27 ppm, 55 ppm one day before the fully opening of the flowers and 70 ppm when the flowers are fully opened. In accordance with the test applied, stigmas exhibited an increase in the enzyme activity one day before the opening of flowers, that reached its highest point when the flowers are fully open. As in the case of the pollen viability, data on the days after flower is opened are missing. The stigma receptivity declines after the fully opening of flowers (Figure 2).

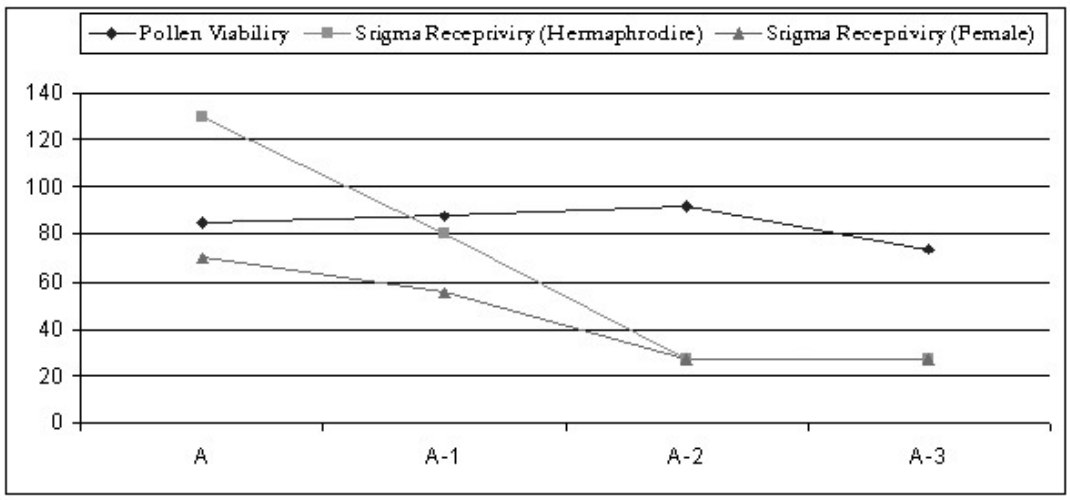

Figure 2. Average fluctuation of pollen viability and stigma receptivity in *Minuartia nifensis* flowers (A: Fully opened (n=10), A-1: One day before opening (n=10), A-2: Two days before opening (n=10), A-3: Three days before opening (n=10).

In order to note the successfullness of pollination forty flowers were examined and ovule/ seed ratio determined. However, calculated seed/ovule ratio was low (24% at hermaphrodite flowers and 17% at female flowers) (Fig. 3). According to the tetrazolium staining viability test, 24 percent of the seeds from hermaphrodite flowers and 17 percent from female flowers were viable. All of the seeds tested for germination showed 90-100 percent germination without any treatement.

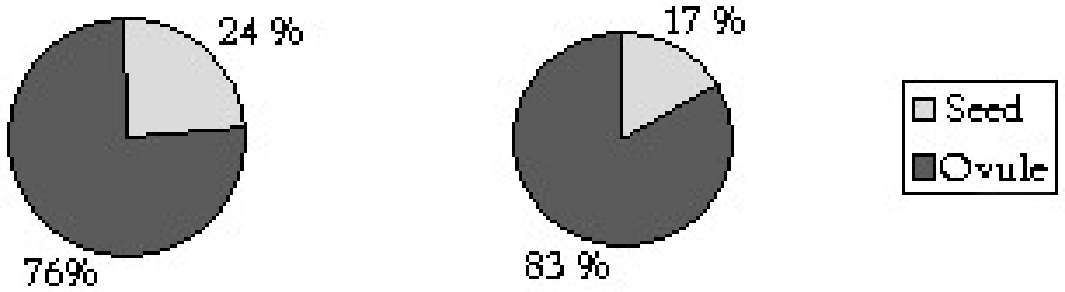

Figure 3. Seed/ovule ratio of hermaphrodite and female flowers.

4. Discussion

The studies undertaken here revealed that, stigma receptivity in *M.nifensis* starts to develop 2 days before the blooming and reached its peak when the flowers fully opened. Zhou et al. (1999), studied the pollination biology of *Paeonia jishanensis* and suggest that pollen viability, stigma receptivity and pollination time is an important indicator of pollination type. These findings depict that the hermaphrodite flowers of *M. nifensis* are self or insect pollinated and the

female flowers are pollinated only by insects. Studies on the conservation biology of *Ericocaulon cornickianum* (Watson et al. 1994), and the pollination biology of *Paeonia jishanensis* (Zhou et al. 1999), stress that seed productivity is not related to pollen quality. We also found that all flowers have the potential to produce seeds. Moreover, pollination experiments showed that all flowers are potentially able to develop fruits throughout the flowering period, suggesting that no abberation occurs in sporogenesis, fertilization or post-zygote processes in *Minuartia nifensis*, for pollination success.

When we examined the reproductive biology of the species, the failure in reproductive success was not related to pollen quality or stigma receptivity because both pollen viability and stigma receptivity were found to be high but the seed/ovule ratio of *M. nifensis* was very low in each flower type indicating low seed set. Reproduction by seed is a limiting factor in the establishment and maintenance of conservation genebanks and the low seed set rate for *M. nifensis* may indicate that the production of seedless fruit is probably involved in the problem of pollination. As stated by Burne (2003) successful pollination and thus fruit set is dependent on pollen and pollinator availability and because hermaphrodite flowers seed set rate is higher then female flowers, we think that the reason for low seed set in female flowers of *M. nifensis* is related with inadequtae viable pollen availability.

The reason for the threat of extinction in *M. nifensis* is human impact in its area of distribution. Such abiotic pressure causes the destruction of mature individuals. Additionally, the construction of fire alarm and radio station buildings together with activities of employees in the distribution areas, decrease the distribution areas and their quality. This is in agreement with the view put forth by Fahrig and Merriam (1994), who mention that human activities increasingly fragment natural habitats and greately alter the size, shape and spatial arrangement of habitats for wild species and these characteristics of habitats affect extinction rates and sizes of local populations as well as dispersal patterns of individuals among local populations.

The reproduction success of the species is low and according to Wolf (2001) the potential explanation for lower reproductive success of plants is the loss of alleles through genetic drift so as explained and demonstrated by Lamont (2001) this will affect the future of the population dynamics and the seedbank replacement capability. This, together with disturbance at the distribution area, leads to a decrease in the number of mature individuals, and hence, the populations of the species are declining over time.

In order to safegaurd the future populations we suggest a CR B2ab(ii)+(iii) endangered status for this species according to the IUCN (2001) categories, because the number of individuals is very low. Moreover, there is an urgent need for reducing the anthropogenic pressure because these greatly influence the habitat size and lead to a reduction in the number of individuals. Therefore, construction of new buildings around the fire station and antenna site should be prohibited and logistical activities should be implemented with great care.

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