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Conservation biology of *Asperula daphneola* (Rubiaceae) in Western Turkey

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Abstract: *A. daphneola* O.Schwarz (Rubiaceae*)* was originally recorded from a single location on Nif Mountain (Kemalpaşa) in western Turkey. This study was carried out from 2001 to 2004 to investigate the causes of this endemic species' restricted distribution. To that end, the environmental conditions and reproductive biology of the species were studied. As a result of field investigations, 4 additional locations were identified. These 5 localities (the peak of Nif Mountain, Alaca Mountain, Ayrıca Mountain, Bölme Hill, and another locality west of Nif Mountain) encompass an area of 2.5 km^2. The total number of individuals in these localities was estimated to be 7956. The species had been declared as vulnerable (Vu) according to the 1994 IUCN categories. Our studies led us to recommend it as critically endangered (CR) B2ab(ii)+(iii), according to the 2001 IUCN categories. Our study of its pollination ecology demonstrated that A. *daphneola* is strictly autogamous and that the timing of pollen viability and stigma receptivity are synchronous. The seed/ovule ratio was calculated as 1:1. The seed/ovule ratio and observed low seed germination percentage could be the result of autogamy. Autogamy is contributing to the decline of this species' population.

Key words: *Asperula daphneola*, conservation biology, Nif Mountain, Turkey

Asperula daphneola (Rubiaceae)'nın koruma biyolojisi, Batı Anadolu, Türkiye

Özet: *A. daphneola* O.Schwarz (*Rubiaceae)*, ilk olarak Türkiye'nin Batısında bulunan Nif Dağı'ndaki (Kemalpaşa) tek lokaliteden kaydedilmiştir. Bu türün sınırlı yayılış nedenleri, 2002-2004 yılları arasında gerçekleştirilen çalışma ile belirlenmeye çalışılmış, bu amaçla bitkilerin yaşadıkları çevre koşulları ile üreme biyolojileri araştırılmıştır. Arazi çalışmaları sonucunda, dört yeni lokalite (Alaca dağı, Ayrıca dağı, Bölme tepe ve bilinen ilk lokalite Zirve ve zirve'nin batısındaki diğer bir lokalite) tanımlanmıştır. Bu beş lokalite toplam olarak 2,6 km^2'lik bir alan oluşturmaktadır. Tüm bu lokalitelerdeki birey sayısı 7956 olarak tahmin edilmiştir. Bu tür, IUCN (1994) kriterlerine göre "Duyarlı" (Vu) olarak belirlenmiştir. Çalışmalarımız sonucunda tür, IUCN (2001) kriterlerine göre "Kritik Tehlike Altında" (CR) B2ab(ii)+(iii) olarak önerilmiştir. Tozlaşma ekolojisi üzerindeki çalışmamız *A. daphneola*'nın zorunlu otogam olduğunu ve polen canlılığı ile stigma olgunluğu zamanlamasının örtüştüğü göstermiştir. Tohum/ovül oranı bir olarak hesaplanmıştır. Tohum/ovül oranı ve bulunan düşük tohum çimlenme yüzdesi, türün birey sayısının azalmasının sebebi otogami olabilir. Otogaminin bu türün birey sayısının azalmasında payı vardır.

Anahtar sözcükler: *Asperula daphneola*, koruma biyolojisi, Nif Dağı, Türkiye

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Introduction

Compared to European and Mediterranean countries, Turkey has one of the highest concentrations of endemic plant species; about 33% of its plants are endemic. A large percentage (95%) of these are categorized as endangered, rare, or threatened. The Mediterranean Region of Turkey is considered a diversity hot-spot zone (Myers et al., 2000). Detailed studies on the conservation biology of such species are essential in order to prevent extinctions. Studies on the reproductive biology of such species could shed light on the probable causes of their restricted distributions (Schemske et al., 1994).

For most threatened species that are not commercially exploited, population size is rarely known; therefore, features related to the conservation biology of such threatened species need to be investigated (Matsuda et al., 2000). *Asperula daphneola* O.Schwarz is related to such species as *Asperula pulvinaris* (Boiss.) Heldr. ex Boiss. and *Asperula icarica* Ehrend. & Schonb, which belong to the *Cynanchicae* section located in the mountains of southern Greece. The distribution area of these species possibly fragmented before the last glaciation period (Verdier, 1963). Of these 3 species, only *A. daphneola* has been categorized according to the IUCN Red List Categories, and it is also included in Turkey's *Red Data Book* (Ekim et al., 2000). In 2000 the Turkish Association for the Conservation of Nature declared *A. daphneola* as a vulnerable (VU) species, despite the fact that ecological and quantitative data for this species were not available.

The primary aims of the present study were to:

i. Update existing data on the distribution of *A. daphneola* in order to determine its conservation status.

ii. Investigate *A. daphneola* pollination ecology, pollen viability, and stigma receptivity in order to determine its dependence on sexual reproduction.

iii. Estimate the *A. daphneola* reproduction rate via examination of seed viability in order to assess the future life history of existing populations.

Material and methods

The plant

The study material consisted of *A. daphneola* in Feddes Rep., 36: 139 (1934). It is an endemic perennial herb restricted to the peak of Nif Mountain (1500 m asl) in western Turkey, approximately 40 km east of İzmir (Figure 1). Five populations in this area were

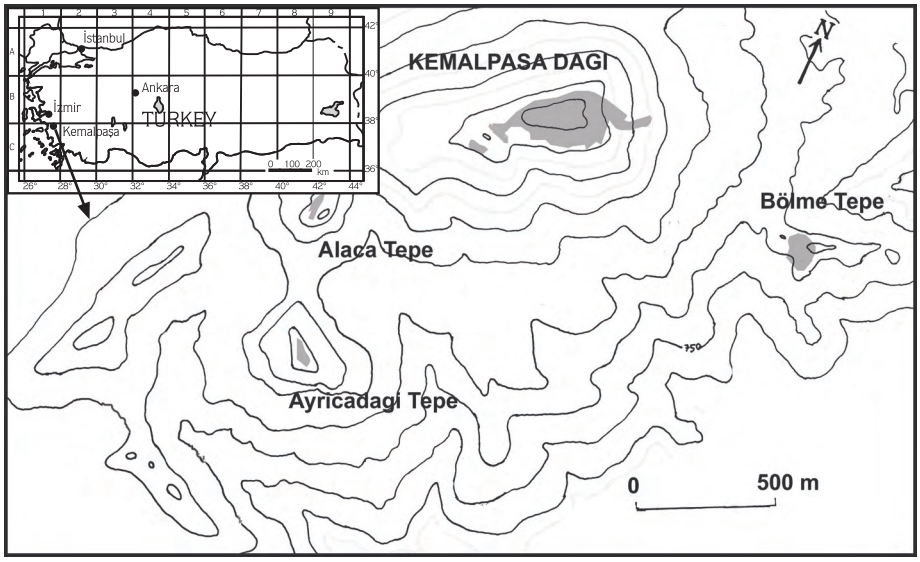

Figure 1. Geographic distribution of the 5 known populations of *A. daphneola*. Nif Mountain (1467 m), Bölme Hill (920 m), Ayrıca Mountain (1216 m), and Alaca Mountain (1276 m). Shaded areas on the map indicates the distribution areas of the species; the difference in elevation between any 2 gridlines is 100 m.

evaluated for the study. *A. daphneola* is a perennial cushion-forming, low-growing herb, with maximum height of 60 cm. It has imbricate leaves, producing up to 8 flowers in the axils of leafy bracts. The flowers lack a calyx, but corollas are bright pink, 10-12 mm long, and exhibit an autogamous syndrome. The stamens are epipetalous; usually there are 4, and rarely 5 or 6. Flowering begins in May and continues until June. It is strictly endemic to the peak of Nif Mountain in western Turkey. At the start of this study its known distribution area was limited to 1 population in an area of approximately 500 × 200 m.

Methods

In order to determine whether additional populations existed in the area, probable suitable habitats in an approximately 2-km radius of the previously known population were systematically searched several times during the vegetation period of March-July each year from 2001 to 2004. During the field investigations GPS data were collected and analyzed with GIS programs, and distribution maps were developed. The number of individuals in a given population was estimated using a 50-m line transect, which was repeated 5 times for each of the 5 populations.

The *A. daphneola* pollination type was monitored for 2 consecutive years, in May and July of 2002 and 2003, based on 50 individual plants. The plants were randomly chosen at sites where access and field-study conditions were suitable. To determine which pollination type was operating on the species we used 5 different treatments on the flower buds of intact plants 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 treatment was applied to a group of 10 randomly selected flowers of similar size from 10 individual plants per year (n = 50 flowers per year). The treated and control flowers were monitored and examined for 3 weeks following application of the treatments in order to observe the fruit/seed set.

The field observations showed that *A. daphneola* flowers have a floral cycle of 7-8 days. Pollen viability and stigma receptivity were investigated in 2 experiments carried out simultaneously from May to July 2002. Ten flowers were collected for each experiment as follows: a) 3 days prior to flower opening (A-3); b) 2 days prior to flower opening (A-2); c) 1 day prior to flower opening (A-1); d) day of flower opening (A). A total of 80 flowers were used in the 2 experiments. Since the number of individuals in the area was low, we used the lowest possible number of samples and flowers during our experiments to avoid possible negative influences on the reproductive potential of the populations. Each collected flower was separately placed in an eppendorf tube, stored in a cool box, and transported to the laboratory. In the laboratory each flower was dissected and the anthers and stigmas were removed. For the pollen viability tests, 1 anther from each of the 10 flowers corresponding to each of the 4 developmental stages (total 40 flowers) was treated with 1% tetrazolium bromide for 45 min at 35-37 °C. The treated anthers were examined under a microscope to determine if they were stained; 500 pollen particles per anther were randomly selected. Those pollen grains that were stained were considered as viable pollen, while those that were not stained were accepted as inviable (Firmage and Dafni, 2001).

For stigma receptivity testing the stigma of each flower (total of 40 flowers, 10 from each of 4 developmental stages) was treated with Perex (Merck 16206) solution. The stained stigmas were examined under a microscope and assigned to 1 of the following 3 categories based on the gradation of staining: a) orange (receptive); b) deep orange (more receptive); and c) red (highly receptive). As a measure of stigma receptivity, average enzyme activity was estimated based on a colour scale prepared especially for this test (Dafni & Maues, 1998).

To estimate fertilization (or pollination) success the seed/ovule ratio was calculated according to Bosch et al., (1998) using 40 randomly selected flowers collected in 2 batches of 20. The first batch was collected at the beginning of July 2002 and the second batch 1 week later. It was possible to count the number of seeds per flower in the laboratory because the seeds remain attached to the flower during the first stages of its formation.

Lastly, the collected seeds were tested for viability. In all, 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 were not stained were considered inviable.

The germination test was conducted to determine the reliability of seed viability and dormancy. Current year seeds (i.e. 2002) were placed for germination on Whatman paper in 9-cm petri dishes using double distilled water in incubators preset to 5 °C, 10 °C, and 15 °C under a 16/8 h photoperiod. In another group of seeds the seed coats were removed and the seeds were divided into 2 groups; 50 and 100 ppm of gibberellic acid and kinetin was applied in equal volumes to the 2 groups, each which contained 10 seeds. These were left to germinate under the aforementioned conditions together with a group that was not hormonally treated.

Results

According to the records published in the *Flora of Turkey and the East Aegean Islands* (Davis, 1982), the first known population grew at an open site 1500 m asl; however, during our field studies 4 new locations of this taxon were recorded (Figure 1). These populations were located in an elevation zone extending from 1350 to 1500 m asl. *A. daphneola* was flourishing at open sites on gravelly areas with shallow soils that usually cover calcareous rocks. The vegetation period in the study area was restricted to the months of April-July. Growth conditions beyond these months were either too cold and snowy, or too hot and dry for the species. The species is distributed in 5 localities. These localities are the peak of Nif Mountain, which covers 1.5 km^2 and included 1786 individuals, Alaca Mountain (0.4 km^2, 888 individuals), Ayrica Mountain (0.4 km^2, 2652 individuals), Bölme Hill (0.1 $km^{2,}$ 1750 individuals), and another locality west of the peak of Nif Mountain (0.2 km^2, 880 individuals); total area was 2.5 km^2 and total number of individuals was estimated to be 7956.

Pollination experiment results show that all 10 bagged and 10 control flowers developed fruits, while none of the other 3 flower categories developed any fruit. Average pollen viability was also determined (Figure 2).

Stigma receptivity test results show that stigma receptivity started no more than 3 days before the flowers started to open and that enzyme activity was about 180 ppm during this stage. Receptivity reached its peak 1 day before the flowers fully opened and enzyme activity was 400 ppm. Enzyme activity was only 320 ppm when the flowers were in full bloom (Figure 2).

Stigma receptivity declined after the flowers fully opened. In accordance with the applied test, stigmas started to activate enzymes 2 days before the flowers opened. This activity reached its peak 1 day before the flowers opened. Average pollen viability was 78% 3 days before the flowers opened, 80% 2 days before the flowers opened, 89% 1 day before the flowers opened, and 72% the day flowers were fully opened.

To assess fecundity 40 flowers were examined and the ovule/seed ratio was 1:1, indicating 100%

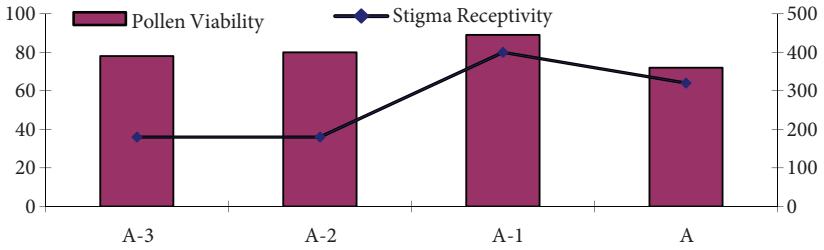

Figure 2. Trends in pollen viability and stigma receptivity (as measured by enzyme activity, ppm) in *A. daphneola* flowers.
A: Fully opened (n = 10); A-1: 1 day before opening (n = 10); A-2: 2 days before opening (n = 10); A-3: 3 days before opening (n = 10).

fertilization success. No differences were observed between the populations. From the 40 flowers 40 fruits were produced, and all contained seeds. Altitude and plant size had no effect on the fruit set. According to the tetrazolium staining viability test, 27% of the seeds were alive, 7% were weak, and 66% were dead. Although the seed/ovule ratio was 1:1 (indicating high seed set), *A. daphneola* seed viability was very low. At 5 °C none of the seeds germinated, but at 10 °C and 15 °C seed germination was as high as 20%. In addition, the germination rate was only 10% in the group of seeds with intact testae that had kinetin applied to them. It was 30% in the group of seeds with testae removed and hormone applied at 10 °C and 15 °C (Figure 3).

Discussion

Endemic species with a restricted geographic distribution have become a major concern for biologists faced with ensuring their conservation (Navarro & Guitian, 2002). Potential causes of rarity may be low population size, habitat specificity, and/or narrow endemicity (Prober & Austin, 1990). Rare plants are especially vulnerable to environmental and demographic stochastic events and, hence, extinction. Thus, management of such plant species requires prior knowledge of their abundance, spatial distribution patterns, breeding biology, and genetics, as well as the factors affecting seeds, seedling survival, and establishment (Schemske et al., 1994).

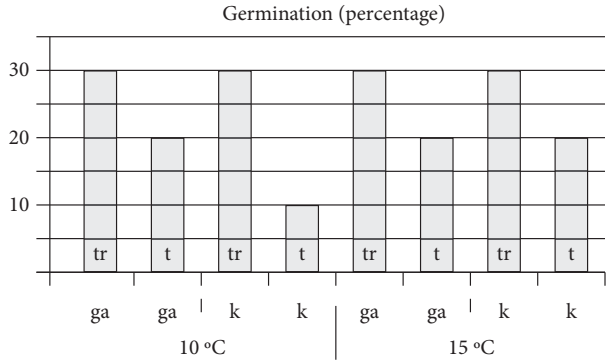

Figure 3. Germination behaviour of *A. daphneola* seeds under various treatments.
[tr: testa removed; t: testa intact; ga: giberellic acid (50 ppm); k: kinetin (100 ppm)].

Zhou et al. (1999) studied the pollination biology of *Paeonia jishanensis* T.Hong & W.Z.Zhao and suggest that pollen viability, stigma receptivity, and pollination time are important indicators of pollination type. Our experiments with bagged *A. daphneola* flowers indicate self-compatibility and the results show that the species is autogamous. Self-incompatibility is further supported by low germination rates and by the results of the seed viability tests. In both the germination experiments and viability tests, the low percentage of fertility could be attributed to selfing.

Studies on the conservation biology of *Eriocaulon coernickianum* Van Heurck & Müll. Arg. (Watson et al., 1994) stress that seed productivity is not related to pollen quality. Our *A. daphneola* reproductive biology results also show that the reduced reproductive success of the species was not due to low pollen quality or low stigma receptivity, as we observed that both pollen viability and stigma receptivity were high and synchronous during the flowering period. Furthermore, the *A. daphneola* pollination results indicate that there were no problems with sporogenesis, pollination, or zygote formation.

As reported by Gibbs (2001), inbreeding leads to homozygosity, which subsequently reduces genetic diversity within populations. Our hypothesis is that low seed viability in *A. daphneola* is due to seed sterility, which is a result of selfing; therefore, during the establishment and maintenance of conservation gene banks, and during the reintroduction processes additional measures should be taken to raise new seedlings from seeds.

One reason that *A. daphneola* is at risk of extinction appears to be the impact of humans throughout its distribution range. Such anthropogenic pressures as construction of a new forest-fire watch tower caused the destruction of mature individuals in the study area. Additionally, the construction of a radio broadcasting facility, together with the activities of employees in the distribution areas have led to degradation and decline of the species' distribution range. This is in agreement with the view held by Fahrig and Merriam (1994), that human activity increasingly fragments natural habitats, and greatly alters the size, shape, and spatial arrangement of wild species habitats. Such alterations to the habitats of

species accelerate extinction rates and reduce the size of local populations, as well as disturb dispersal patterns of individuals among local populations.

The reproduction success of this species, as measured by germination percentage, was low. According to Wolf (2001), a potential explanation for lower reproductive success of plants is loss of alleles and, therefore, loss of heterozygosity via genetic drift and/or inbreeding. As reported by Lamont (2001), such genetic attributes influence the future of population dynamics in plants, as well as seed bank replacement capability. All these factors, together with disturbance and fragmentation of the distribution area, leads to a decrease in the number of mature individuals and, hence, the species' populations are declining.

As an initial step to safeguard future populations we suggest, based on the IUCN (2001) categories, that *A. daphneola* should be listed as critically endangered [CR B2ab(ii)+(iii)]. Moreover, there is an urgent need to reduce the present and future anthropogenic pressure on the natural range of the species, as such pressure greatly reduces habitat size and the number of individuals in each habitat. Construction of new buildings around the forest-fire watch towers and radio broadcasting facilities should be prohibited, and logistical activities should be implemented with great care to encourage the return of new individuals at the related sites.

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References

Bosch M, Simon J, Molero J & Blanche C (1998). Reproductive biology, genetic variation and conservation of the rare endemic dysploid *Delphinium bolosii* (*Ranunculaceae*), *Biol Conserv*, 86: 57-66.

Dafni A & Maues MM (1998). A rapid and simple procedure to determine stigma receptivity, *Sex Plant Reprod*, 11: 177-180.

Davis PH (1982). *Flora of Turkey and the East Aegean Islands*, Vol. 7, Edinburgh: Edinburgh Univ. Press, UK.

Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z & Adıgüzel N (2000). *Turkey Plants Red Data Book*, Turkey's Nature Protection Association, Ankara, Turkey.

Fahrig L & Merriam G (1994). Conservation of fragmented populations. *Conserv Biol* 8: 50-59.

Firmage DH & Dafni A (2001). Field Tests for Pollen Viability: A Comparative Approach, Proc. 8th Pollination Symposium, *Acta Hort*. 561.

Gibbs PJ (2001). Demography versus habitat fragmentation as determinants of genetic variation in wild populations, *Biol Conserv* 100: 15-20.

IUCN (2001). *IUCN Red List Categories and Criteria: Version 3.1*. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK.

Lamont BB, Marsula R, Enright NJ & Witkowski ETF (2001). Conservation requirements of an exploited wildflower: modelling effects of plant age, growing conditions and harvesting intensity. *Biol Conserv* 99: 157-168.

Matsuda H, Yahara T & Kaneko Y (2000). Extinction risk assessment of threatened species. *Popul Ecol* 42: 3-4.

Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB & Kent J (2000). Biodiversity hotspots for conservation priorities. *Nature* 403: 853-858.

Navarro L & Guitian J (2002). The role of floral biology and breeding system on the reproductive success of the narrow endemic Petrocoptis viscosa rothm. (Caryophyllaceae). *Biol Conserv* 103: 125-132.

Prober S & Austin MP (1990). Habitat peculiarity as a cause of rarity in Eucalyptus paliformis. *Austr J Ecol* 16: 189-205.

Schemske DW, Husband BC & Ruckelshaus MH (1994). Evaluating approaches to the conservation of rare and endangered plants, *Ecology* 75(3): 584-606.

Verdier J (1963). *Kemalpasha Mountain Survey (Izmir)*, Journal of Quarry Technical Research Institute 61: 37-39.

Watson LE, Uno GE, McCarty NA & Kornkven AB (1994). Conservation biology of rare plant species, *Eriocaulon koernickianum* (Eriocaulaceae), *Am J Bot* 81(8): 980-986.

Wolf A (2001). Conservation of endemic plants in serpentine landscapes. *Biol Conserv* 100: 35-44.

Zhou SL, Hong DY & Pan KY (1999). Pollination biology of *Paeonia jishanensis* T.Hong & W.Z. Zhao (*Paeoniaceae*), with special emphasis on pollen and stigma biology, *Bot J Linn Soc* 130: 43-52.