Turk J Bot
36 (2012) 677-682

doi:10.3906/bot-1104-4

Research Article

Reproductive success of the rare endemic *Orchis galilaea* (Orchidaceae) in Lebanon

Nisrine MACHAKA-HOURI[1,2], Mohammad Souheil AL-ZEIN[3],
Duncan Brian WESTBURY[1], Salma Nashabe TALHOUK[2,*]

[1]School of Agriculture, Policy & Development, The University of Reading - UK

[2]Department of Landscape Design and Ecosystem Management, and IBSAR, Nature Conservation Center for Sustainable Futures, American University of Beirut, Beirut - LEBANON

[3]Department of Natural Sciences, School of Arts and Sciences, Lebanese American University, Beirut - LEBANON

Received: 04.04.2011 ● Accepted: 05.06.2012

Abstract: The biology and ecology of *Orchis galilaea* Schltr., a species endemic to Lebanon, Palestine, and Jordan, is poorly studied, a fact that hinders present and future management and conservation efforts concerning this species. In this paper, we report findings of a field investigation that assessed the impact of altitude, population density, and plant size on the reproductive success of *O. galilaea*. The results revealed that plant size and population density were significantly correlated with reproductive success while altitude was not. This study is part of ongoing research on the ecological responses of *O. galilaea* and provides a baseline for understanding the conservation potentials for this rare endemic species.

Key words: Altitude, population density, plant size, fruit set, deceptive pollination, conservation

Introduction

Semi-natural habitats of the Mediterranean region, a recognised biodiversity hotspot harbouring more than 50% of endemic plant species, are subject to increasing threats. Hence, there is a need to step up efforts to generate baseline data that enable monitoring, conservation, and management of plant species, particularly rare and endemic ones (Quézel, 1985; Heywood, 1995; de Montmollin & Strahm, 2005).

In line with this goal, the present study investigated *Orchis galilaea* Schltr., an Eastern Mediterranean geophyte endemic to Lebanon, Jordan, and Palestine. *O. galilaea* flowers from March to May; it has 2 elongated round tubers, a rosette of 4 to 5 pale green lanceolate leaves, and a spike of 15 to 90 densely arranged, small, white to yellowish-green to pinkish-purple nectarless flowers (Bino et al., 1982; Kretzschmar et al., 2007). The plant is relatively tall compared to its congeners, its height usually ranging from 30 to 70 cm (Bino et al., 1982). *O. galilaea* belongs to the Orchidaceae, one of the largest families of flowering plants, represented in Lebanon by 10 genera and 40 to 90 species. It is 1 of at least 20 species in the genus *Orchis* L. occurring in Lebanon

* E-mail: ntsalma@aub.edu.lb

(Post, 1932; Mouterde, 1983; Tohmé & Tohmé, 2007; Haber & Semaan Haber, 2009). Like many rare orchid species, *O. galilaea,* is threatened with extinction (Kretzschmar et al., 2007). Key factors affecting this and many orchid populations are climate change, habitat destruction, overharvesting (especially for *salep* production in the Eastern Mediterranean), and a decline in natural pollinators resulting from the use of insecticides (Koopowitz, 2001; Vaasa & Rosenberg, 2004); therefore, efforts to promote its conservation, including proper monitoring and management, must be prioritised (Işık, 2011).

Orchis galilaea grows at altitudes ranging from 100 to 1130 m, in full sun to mid-shade, on calcareous, often stony, dry to moist substrates (Delforge, 2006). Bino et al. (1982) examined the pollination ecology of *O. galilaea* on Mount Carmel, Northern Palestine, and affirmed that it adopts a sexually deceptive pollination strategy. Instead of producing nectar as a reward for visiting pollinators, it generates a musk-like scent that mimics the pheromones secreted by female *Lasioglossum marginatum* (Brullé, 1832) (= *Halictus marginatus* Brullé) bees, thus attracting male *Lasioglossum marginatum* bees to its flowers.

Understanding the biology and ecology of species allows predictions of population behaviour in response to external influences, and is a fundamental component of threatened species management (Hutchings, 1987; Kéry & Gregg, 2004). As part of ongoing work on *O. galilaea* in Lebanon, this study investigated the effect of altitude, plant density, and plant size on the reproductive success of this species. We hypothesise that reproductive success of *O. galilaea* is correlated with plant size, population density, and altitude.

Materials and methods

Study sites

In 2010, 6 populations of *Orchis galilaea* were selected following frequent field visits that were conducted to locate the species in natural and semi-natural habitats in the region of Mount Lebanon and Greater Beirut. The targeted populations occurred in different habitat types, along the western slopes of Mount Lebanon at altitudes ranging from 370 m to 1140 m above sea level (a.s.l.) (Table 1).

Each target site was visited at least twice during the flowering and fruiting season between March and May. At each site, 1 to 3 belt-transects, each 10 m × 30 m, were laid down and marked by metal rods at the beginning and end of each transect. To take into account the clustered distribution of the populations, which consisted of 31 to 147 individuals, the number of transects selected in each site varied as indicated in Table 1.

Population density

The total number of flowering individuals of *Orchis galilaea* was recorded for each transect at each of

Table 1. The 6 *Orchis galilaea* study sites in the Chouf district, Mount Lebanon Province, Lebanon, indicating the village, habitat type, altitude, GPS location, and number of transects.

Site number	Village	Habitat type	Altitude	GPS location	No. of transects
1	Burjein	Mixed woodland	397 m	33°39.647′N 35°28.516′E	3
2	Chhim	Oak woodland	370 m	33°38.124′N 35°27.816′E	2
3	Gharifeh	Oak woodland	682 m	33°38.718′N 35°33.086′E	1
4	Maaser	Garrigue	1140 m	33°39.938′N 35°39.449′E	2
5	Boutmeh	Garrigue	1074 m	33°39.901′N 35°38.587′E	2
6	Khraibeh	Abandoned terraces	1120 m	33°39.506′N 35°38.873′E	3

the sites. Population density was estimated as the density of flowering individuals per transect (Hansen & Olesen, 1999; Jacquemyn et al., 2002). The mean population density for all transects at each site was used as an estimate of the population density at each site.

Plant size

Twenty plants (in bud or in bloom) were randomly selected at each of the study sites. Each plant was assigned a unique number and marked using wooden tags, inserted into the soil 2 cm west of the plant with about 1 cm of the tag left above ground (Kéry & Gregg, 2003; Coates & Duncan, 2009). When flowering ceased, total plant height, spike length, and number of flowers per tagged plant were recorded as measures of plant size (Hansen & Olesen, 1999). Selected plants that were damaged before flowering bud opening (a total of 4 plants) were excluded from the analysis.

Reproductive success

A fruit in the Orchidaceae can be safely assumed to be the product of successful pollination, as agamospermy is rare in the family (Neiland & Wilcock, 1995) and, according to Bino et al. (1982), *Orchis galilaea* is not autogamous. Since fruit set is the most common measure of reproductive success (Proctor & Harder, 1994), we estimated fruit set by determining the percentage of flowers that successfully developed into fruits on each tagged plant at each site (Kindlmann & Jersakova, 2005), and then calculated the mean for each site (Jacquemyn et al., 2002).

Effect of altitude

To investigate the effect of altitude on the reproductive success of *Orchis galilaea*, the study sites were selected at different altitudes (Table 1). According to Delforge (2006), *O. galilaea* grows in areas ranging from 100 to 1130 m a.s.l. However, the orchid was not observed at altitudes lower than 300 m a.s.l. The rarity of the orchid, the difficulty of Lebanon's topography, and the lack of security in some areas were limiting factors to the selection of the study sites.

Statistical analysis

Descriptive statistics were analysed using Microsoft Excel (Microsoft, 2007). Prior to analysis, data for total plant height, spike length, number of flowers, reproductive success, altitude, population density, and pooled reproductive success were tested for normality using the Kolmogorov-Smirnov test. We investigated the relationship between reproductive success and altitude, population density, and plant size using PASW Statistics 18 (SPSS Inc., 2009). Correlations between total plant height, spike length, number of flowers, and reproductive success were tested using Spearman's index. However, correlations between altitude, population density, and pooled reproductive success were tested using Pearson's index.

Results

Population density

The population density of *Orchis galilaea* ranged from 0.04 to 0.16 individuals per square metre and averaged 0.08 ± 0.02 individuals per square metre (mean ± standard error of the mean).The results for all study sites are shown in Table 2.

Table 2. Population density, total height, and inflorescence length of *Orchis galilaea* at selected sites at Mount Lebanon, Lebanon.

Site code	Population density (number of plants per square metre)	Average total height per plant (cm)	Average inflorescence height per plant (cm)
Site 1	0.04	30.63 ± 1.4	7.49 ± 0.5
Site 2	0.06	26.82 ± 1.8	5.85 ± 0.6
Site 3	0.10	34.32 ± 1.5	9.03 ± 0.4
Site 4	0.07	28.61 ± 1.5	6.92 ± 0.7
Site 5	0.06	27.95 ± 1.5	7.28 ± 0.5
Site 6	0.16	26.45 ± 1.5	6.03 ± 0.6

Plant size

Plant size was assessed based on measurements taken from a total of 117 plants at the 6 study sites. Individual plant height ranged from 13 cm to 46 cm, with an average plant height of 29.03 ± 0.7 cm. The individual spike length ranged from 2.5 to 15 cm and averaged 7.08 ± 0.2 cm. The number of flowers ranged from 10 to 53 flowers per plant, with an average of 22.5± 0.9. The results for plant size are shown for all study sites in Table 2.

Reproductive success

The average number of flowers per plant per site ranged from 18.0 ± 1.3 (Site 2) to 27.6 ± 2.4 (Site 3), while the average number of fruits per plant per site ranged from 2.8 ± 0.9 (Site 2) to 7.2 ± 1.4 (Site 5) (Table 3). The per-site reproductive success of *O. galilaea* ranged from 7.6% (Site 6) to 32.2% (Site 5).

Reproductive success was significantly correlated with total plant height (Spearman's rho = 0.453, $P < 0.01$) and spike length (Spearman's rho = 0.502, $P < 0.01$). However, no significant correlation was found between reproductive success and the number of flowers per plant. Furthermore, reproductive success seems to be inversely related to population density as the linear regression explains 55% of the variation in reproductive success. However, this correlation was not significant (Figure 1).

Effect of altitude

Altitude was not significantly correlated with reproductive success (Figure 2). In fact, the reproductive success of *Orchis galilaea* was lowest (7.59%) at Khraibeh (Site 6), which is at an elevation of 1120 m a.s.l., and highest (ca. 30%) at both sites 1 (Burjein; 397 m a.s.l.) and 5 (Boutmeh; 1074 m a.s.l.), despite a difference of about 700 m in elevation between the 2 sites.

Discussion

The biology and ecology of *Orchis galilaea* in its natural range in general and in Lebanon in particular are poorly known. To the best of our knowledge, this is the first reported study on this orchid since Bino et al. (1982).

Our findings revealed 21.3% reproductive success of *Orchis galilaea* in Lebanon, lower but comparable to the reproductive success of *O. galilaea* (27.5%) reported by Bino et al. (1982). This is within the reported range for nectarless orchids (11.5% in the tropics to 41.4% in the temperate southern hemisphere) (Neiland & Wilcock, 1998), which typically have a lower reproductive success than nectariferous ones. Vandewoestijne et al. (2009) reported that the reproductive success of European orchids is on average 27.7% for nectarless species and 63.1% for nectariferous ones. The low reproductive success of this orchid, compared to the reproductive success of nectariferous orchids, may explain its rarity and consequently its susceptibility to extinction (Jacquemyn et al., 2005). Low reproductive success observed in orchids suggests that these plants are pollinator limited. This fact was demonstrated in many studies through hand-pollination experiments (Wilcock & Neiland, 2002; Tremblay et al., 2005).

An important factor that is found to limit the reproductive success of nectarless orchids is high population density, as pollinators learn to avoid the non-rewarding orchids the more often they

Table 3. Reproductive success of *Orchis galilaea* at selected sites at Mount Lebanon, Lebanon.

Site Code	Average flowers per plant (flower per plant)	Average fruits per plant (fruit per plant)	Reproductive success (%)
Site 1	24.7 ± 1.9	6.6 ± 1.0	30.0
Site 2	18.0 ± 1.3	2.8 ± 0.9	14.1
Site 3	27.6 ± 2.4	5.1 ± 0.9	18.8
Site 4	21.0 ± 2.1	3.6 ± 0.6	18.3
Site 5	20.6 ± 2.1	7.2 ± 1.4	32.2
Site 6	23.0 ± 1.9	2.2 ± 0.9	7.6

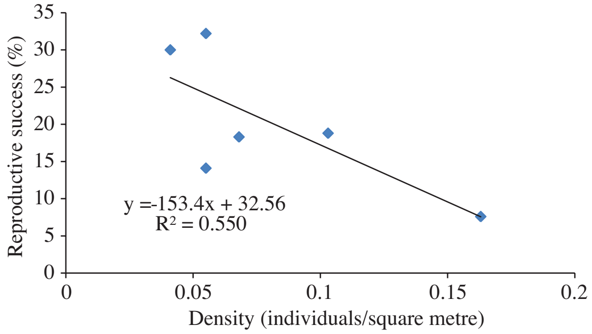

Figure 1. The effect of flowering plant density on reproductive success in *Orchis galilaea* recorded at selected sites at Mount Lebanon, Lebanon.

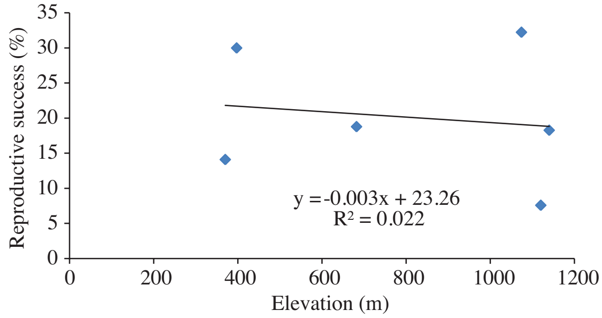

Figure 2. The effect of elevation on reproductive success in *Orchis galilaea* recorded at selected sites at Mount Lebanon, Lebanon.

encounter them (Vandewoestijne et al., 2009). This may not be uncommon among deceptive orchids as pollination by sexual deception is known to be advantageous at low population densities (Peakall & Beattie, 1996). In our study, the population density of *O. galilaea* did seem to have a negative effect on its reproductive success; however, this effect was not statistically significant (Figure 1).

Plant size was found to have a significant effect on reproductive success of *O. galilaea,* with 2 parameters, total plant height and spike length, showing significant correlation with reproductive success. In contrast, the number of flowers per plant was not significantly correlated to reproductive success. This is in accordance with the conclusion reached by Kindlmann and Jersakova (2005) that reproductive success of terrestrial orchids is independent of the number of flowers per plant in many populations and years. It is thought that the reason for having taller plants and larger inflorescences is to increase visibility in terms of visual and/or olfactory cues, thus attracting more pollinators (O'Connell & Johnston, 1998; Vandewoestijne et al., 2009).

Previous studies of the effect of altitude on seed set in plants have yielded inconsistent results rendering generalisation very difficult (Gugerli 1998). In our study, the reproductive success of *O. galilaea* was found to be independent of the site altitude, as there was no significant correlation between altitude and reproductive success. Differences in reproductive success among the 6 study sites must therefore be related to pollinator availability, plant performance, and plant community composition, in addition to population density.

The findings of this study suggest that population density should not be used as an indicator of species vitality since it was found to be negatively correlated with the species' ability to reproduce. Furthermore, since the average reproductive success of *O. galilaea* was found in the range of the pollinator-limited nectarless orchids, we think that pollination limitation is likely to be the main limiting factor. Accordingly, provisions for conservation will need to further understand factors affecting pollinators and pollination; research is in progress to address these questions.

References

Bino R, Dafni A & Meeuse A (1982). The pollination ecology of *Orchis galilaea* (Bornm. et Schulze) Schltr. (Orchidaceae). *New Phytologist* 90: 315-319.

Coates F & Duncan M (2009). Demographic variation between populations of *Caladenia orientalis*—a fire-managed threatened orchid. *Australian Journal of Botany* 57: 326-339.

Delforge P (2006). *Orchids of Europe, North Africa and the Middle East.* London: A & C Black.

Gugerli F (1998). Effect of elevation on sexual reproduction in alpine populations of *Saxifraga oppositifolia* (Saxifragaceae). *Oecologia* 114: 60-66.

Haber R & Semaan Haber M (2009). *Orchids of Lebanon.* Beirut: Terre du Liban.

Hansen I & Olesen J (1999). Comparison of reproductive success in two orchids: the nectarless *Dactylorhiza majalisss* and the nectar-producing *Gymnadenia conopseasl. Nordic Journal of Botany* 19: 665-671.

Heywood V (1995). The Mediterranean flora in the context of world biodiversity. *Ecologia Mediterranea* 21: 11-18.

Hutchings MJ (1987). The population biology of the early spider orchid, *Ophrys sphegodes* Mill. II. Temporal patterns in behaviour. *The Journal of Ecology* 75: 729-742.

Işık K (2011). Rare and endemic species: why are they prone to extinction? *Turkish Journal of Botany* 35: 411-417.

Jacquemyn H, Brys R & Hermy M (2002). Patch occupancy, population size and reproductive success of a forest herb (*Primula elatior*) in a fragmented landscape. *Oecologia* 130: 617-625.

Jacquemyn H, Brys R, Hermy M & Willems JH (2005). Does nectar reward affect rarity and extinction probabilities of orchid species? An assessment using historical records from Belgium and the Netherlands. *Biological Conservation* 121: 257-263.

Kéry M & Gregg K (2003). Effects of life state on detectability in a demographic study of the terrestrial orchid *Cleistes bifaria*. *Journal of Ecology* 91: 265-273.

Kéry M & Gregg K (2004). Demographic analysis of dormancy and survival in the terrestrial orchid *Cypripedium reginae*. *Journal of Ecology* 92: 686-695.

Kindlmann P & Jersakova J (2005). Floral display, reproductive success, and conservation of terrestrial orchids. *Selbyana* 26: 136-144.

Koopowitz H (2001). *Orchids and Their Conservation.* London: Batsford.

Kretzschmar H, Eccarius W & Dietrich H (2007). *The Orchid Genera* Anacamptis, Orchis *and* Neotinea*: Phylogeny, Taxonomy, Morphology, Biology, Distribution, Ecology and Hybridisation.* Bürgel: Echino Media.

Microsoft (2007). Microsoft Excel [computer software]. Redmond, Washington, U.S.A. www.microsoft.com.

Montmollin B de & Strahm W (2005). *The Top 50 Mediterranean Island Plants: Wild Plants at the Brink of Extinction, and What Is Needed to Save Them.* Gland, Switzerland and Cambridge, UK: World Conservation Union.

Mouterde P (1983). *Nouvelle Flore du Liban et de la Syrie: tome 3*: Beyrouth, Dar El-Machreq SARL.

Neiland MRM & Wilcock CC (1995). Maximisation of reproductive success by European Orchidaceae under conditions of infrequent pollination. *Protoplasma* 187: 39-48.

Neiland MRM & Wilcock CC (1998). Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany* 85: 1657-1671.

O'Connell LM & Johnston MO (1998). Male and female pollination success in a deceptive orchid; a selection study. *Ecology* 79: 1246-1260.

Peakall R & Beattie AJ (1996). Ecological and genetic consequences of pollination by sexual deception in the orchid *Caladenia tentactulata*. *Evolution* 50: 2207-2220.

Post G (1932). *Flora of Syria, Palestine and Sinai.* Beirut: American Press.

Proctor H & Harder L (1994). Pollen load, capsule weight, and seed production in three orchid species. *Canadian Journal of Botany* 72: 249-255.

Quézel P (1985). Definition of the Mediterranean region and the origin of its flora. *Plant Conservation in the Mediterranean Area* 9: 24.

SPSS Inc. (2009). PASW Statistics 18, Release version 18.0.0. SPSS Inc. Chicago, IL. www.spss.com.

Tohmé G & Tohmé H (2007). *Illustrated Flora of Lebanon.* Beirut: National Council for Scientific Research.

Tremblay RL, Ackerman JD, Zimmerman JK & Calvo RN (2005). Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. *Biological Journal of the Linnean Society* 84: 1-54.

Vaasa A & Rosenberg V (2004). Preservation of the rare terrestrial orchids in vitro. *Acta Universitatis Latviensis* 676: 243-676.

Vandewoestijne S, Rois A, Caperta A, Baguette M & Tyteca D (2009). Effects of individual and population parameters on reproductive success in three sexually deceptive orchid species. *Plant Biology* 11: 454-463.

Wilcock C & Neiland R (2002). Pollination failure in plants: why it happens and when it matters. *Trends in Plant Science* 7: 270-277.