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Reproductive success of lizard orchid (*Himantoglossum*) species under different land uses

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1. BACKGROUND AND OBJECTIVES OF THE RESEARCH

Introduction

The family Orchidaceae is one of the largest among the flowering plants (Dressler 1993, Pridgeon *et al.* 2005, Swarts & Dixon 2009). Ecological specialization has resulted the diversity of the Ochidaceae, but these species are highly threatened. (Cribb *et al.* 2003). Despite their world-wide dispersion and extreme plasticity, the majority of these species has key conservation importance (Kull & Hutchings 2006, Swarts & Dixon 2009).

In the last few decades the biodiversity crisis has affected orchids as well (Jacquemyn *et al.* 2005, Kull & Hutchings 2006). Several species have adapted to secondary habitats, meadows and pastures, and now the existence of orchids is tied to their survival. Changes in land use affect orchids on the one hand with the loss of habitats (Kull & Hutchings 2006), and on the other hand due to the decrease in the number of pollinating insects (Molnár V. 2011). From a conservation point of view, the survival of the most endangered species and the yield of insect-pollinated cultivated plants are linked through the presence of pollinating insects and the pollination crisis poses a global threat to biodiversity and also directly to food supply (Aguilar *et al*. 2006, Klein *et al.* 2007).

Deception is not rare among the orchids, it is estimated that as much as one-third of orchid species use various forms of floral deception to attract pollinators (Dafni 1984, Ackerman 1986, Jersáková *et al.* 2006). It is known that nectarless orchids usually suffer from low visitation rates and they have a lower reproductive success than rewarding orchids (Dafni & Ivri 1979, Gill 1989, Neiland & Wilcock 1999, Harder & Johnson 2008). But low fruit set in deceptive orchids should not be regarded as a failure according to Gill (1989), it can be a complex pollination strategy (Jersakova *et al.* 2006).

The long-term survival of populations is significantly influenced by the success of seed production (Hegland *et al.* 2009). This is especially true for species that reproduce sexually. Reproductive success is an important measure of plant fitness, which can ensure the survive the given population (Kindlmann & Jersáková 2006).

Several of publications have been written on the reproductive success of orchids species and the influencing factors, but the information available is often contradictory. However, little information is available on the factors influencing the reproductive success in the case of deceptive lizars orchids and how the surrounding vegetation affects the reproductive success of that orchids. The evolutionary relationships of the genus *Himantoglossum* have been intensively researched (Sramkó *et al.* 2014; Bateman *et al.* 2017, Niketic *et al.* 2018), but there are still unclear questions. The genus has particular conservation importance in Europe. Two *Himantoglossum* taxa are Natura 2000 species and they are strictly protected plants in Hungary, while *H. calcaratum* subsp. *calcaratum* is restricted geographically, it is a Balkan endemic taxa.

The aim of the research

The aim of the research was to investigate the reproductive success of three lizard orchid taxa. Studies focus primarily on populations of the *Himantoglossum adriaticum* Baumann, however, some case studies have provided information for cross-border populations as well as for relatives of the taxa: *Himantoglossum calcaratum* (G.Beck) Schltr. subsp. *jankae* (Somlyay, Kreutz & Óvári) Bateman, Molnár & Sramkó and *Himantoglossum calcaratum* (G.Beck) Schltr. subsp. *calcaratum*.

In the dissertation, the author presents her research on the reproductive success of *Himantoglossum* species through three case studies. The first case study focuses on the background factors with “Data to the management of the Adriatic lizard orchid populations in Hungary” title. It is important to know which factors influence the reproductive success of orchids, especially in the case of endangered species, so the author addresses the second and third case studies to "Honeybee (*Apis mellifera*) mediated increased reproductive success of a rare deceptive orchid" and “Factors influencing the reproductive success (plant size and surrounding vegetation) of lizard orchid species”.

The studies were conducted through the following objectives between 2013 and 2017:

- The research was aimed at the spatial and temporal comparative study of the *Himantoglossum adriaticum* populations, based on the assessment of flowering individuals. The aim was to detect changes, to describe the flowering and reproductive characteristics of the species and to compare them by population.
- The land use provides important information, therefore the aim of the author was to establish differences in land use and habitat aspects between the populations.

- The aim of the dissertation was to present the importance of the honeybee in the reproductive success of the Adriatic Lizard Orchid, and also to investigate whether there was a correlation between individual reproductive success and distance from beehives.
- It had been reported, that the height of plants or the number of flowers influences the reproductive success, so the aim of the research was to investigate the effect of size variables of *Himantoglossum* species on fruit set.
- According to Sabat and Ackerman (1996), flowering time was the most important trait affecting fruit set, so one of the objectives was to investigate flowering phenology.
- The Nearest Neighbour Distance (NND) can be also affect the reproductive success in most nonrewarding orchids as conspecific density (Jacquemyn *et al*., 2002; Tremblay *et al.*, 2005; Machaka-Houri *et al*., 2012), therefore, the studies targeted the effect of the conspecific density as well.
- The research aimed at the investigation the role of the surrounding vegetation in the pollination, as it is known that the fruit set of specimens growing in the shade is lower compared to those flowering in the woodless, sunny area (Bódis 2017, Jacquemyn *et al.* 2010).
- Among high-density heterospecific rewarding plants, a plant that occurs with low conspecific density competes for pollinator attention (Ratchke 1983, Bell *et al.* 2005). Alternatively, facilitation can occur and individuals receive increased visitation (Paulus 2005, Johnson *et al.* 2003, Juillet *et al.* 2007), thus, the aim of the author was to investigate the effect of color and morphological similarity on the reproductive success.

2. MATERIALS AND METHODS

1. Data to the management of the Adriatic lizard orchid populations in Hungary

In the first case study was involved all four largest Hungarian populations of the Adriatic Lizard Orchid (Kőszeg, Nagytevel, Sümeg, Keszthely), where we counted the flowering individuals in Hungary between 2013 and 2017. Altogether 1903 inflorescences were tagged to record the height of the flowering stalk, the length of inflorescence and the number of flowers and fruits. There were quantify fruit set as the proportion of flowers that developed fruits; to evaluate the reproductive success.

During statistical analyzes we used one- and two-way analysis of variance, Tukey test, and Kruskal-Wallis test (SPSS 13.1). Land use of each site have been compared based on the Corine land cover (Copernicus program). The analyses were performed in ArcGIS 10.2 program.

2. Honeybee (*Apis mellifera*) mediated increased reproductive success of a rare deceptive orchid

In the second case study eight populations of *Himantoglossum adriaticum* H. Baumann were studied in Hungary and Croatia in 2013. It was examined reproductive success to determine the significance of the honeybee (*Apis mellifera* L). An apiary was located on the study site at Nagytevel (Hungary) coinciding with the flowering time of *Himatoglossum* orchids. The reproductive success of populations were compared to data on the fructification rate of the same species collected in earlier years using the same methodology. For the comparison of the populations, the author used the data set of Judit Bódis collected between 1992 and 2011, covering a total of 905 specimens.

To compare the reproductive success of different populations we employed a generalized linear model (GLM) with quasibinomial error structure. This approach was necessary because fruit set (the measure of reproductive success) is a binomial variable (it takes values between 0 and 1) and samples were not uniformly distributed within this interval (in several populations most plants had fruit sets close to 0); quasibinomial models take into account such overdispersion in the data. The relationship between distance to the apiary and fruit set was also evaluated using a quasibinomial GLM. GLMs were implemented in the R Statistical Environment (R Core Team 2013).

3. Factors influencing the reproductive success (plant size and surrounding vegetation) of lizard orchid species

In the third case study the dissertation shows the examination of three taxa: *Himantoglossum adriaticum, Himantoglossum calcaratum* subsp. *jankae* and *H. calcaratum* subsp. *calcaratum*. In total 12 study sites located in Hungary, in Croatia and in Bosnia and Herzegovina (Fig. 1), were investigated in two years (2013 and 2014).

1. Table: **Study sites with site characteristics and sample size**

Taxon	Country	Locality	n 2013	n 2014	Geocoordinates
H. adriaticum	Hungary	Keszthely	34	53	N 46.794°, E 17.277°
H. adriaticum	Hungary	Kőszeg	37	171	N 47.375°, E 16.526°
H. adriaticum	Hungary	Nagytevel	41	81	N 47.264°, E 17.598°
H. adriaticum	Hungary	Sümeg	47	179	from N 46.972°, E 17. 356° to N 46.945°, E 17.373°
H. adriaticum	Croatia	Letaj I.	14	64	N 45.255°, E 14.121°
H. adriaticum	Croatia	Letaj II.	12	20	N 45.255°, E 14.132°
H. adriaticum	Croatia	Paz	7	19	N 45.277°, E 14.104°
H. adriaticum	Croatia	Učka	100	84	N 45.317°, E 14.175°
H. calc. subsp. *jankae*	Hungary	Érd	9	15	N 47.349°, E 18.940°
H. calc. subsp. *jankae*	Hungary	Gyulafirátót	34	42	N 47.175.°, E 17.934°
H. calc. subsp. *jankae*	Hungary	Szava	101	130	N 45.882°, E 18.194°
H. calc. subsp. *calcaratum*	Bosnia and Herzegovina	Sutjeska	67	142	from N 43.496°, E 18.736° to N 43.308°, E 18.656°

During the first survey, flowering specimens were recorded in the studied populations, and flowering and environmental characteristics were assessed. About one month after the end of the flowering period, each populations were visited again and total number of flowers and of fruits was counted for each individual and established reproductive success. Shoots which were damaged during the flowering period were excluded from the analyses. A total of 1502 plants were examined in two years.

The author examined the effect of the following variables on reproductive success:

- plant height and inflorescence length (in cm)
- the number of flowers per inflorescence
- the number of opened flowers per inflorescence, that characterized timing of flowering of each specimen
- NND: median distance (in m) of 5 nearest *Himantoglossum* individuals
- the protrusion (in cm) of inflorescence from the surrounding vegetation

- the tree, shrub and herb cover (in %) around each *Himantoglossum* individual (in a plot of 1 m × 1 m)
- the number of co-flowering, rewarding plant species within radii of and 2.5 m around lizard orchids

For the analyzes, flowering plant species occurring around orchids were grouped in several ways, according to published sources: plant families, genera (Király 2009), flower color (Arnold *et al.* 2010), and flower shape (Vamosi *et al.* 2014). Based on the results of our preliminary studies, categorization according to flower shape was used in the analyzes. Based on the flower shape was categorized rewarding plants in two groups, as “restrictive” or “unrestrictive” (Vamosi *et al.* 2014) to determine they possessed a morphological barrier that prevents some pollinators from accessing their floral rewards.

We analyzed factors influencing reproductive success of *Himantoglossum* orchids using Generalized Linear Mixed Model with binomial errors (binomial GLMM). We first made a comparison between taxa, but we also performed an analysis of the total records for reliable results. The binomial GLMM was implemented using the lme4 package in R Statistical Environment (Bates *et al.* 2015, R Core Team 2017).

3. RESULTS AND DISCUSSION

1. Data to the management of the Adriatic lizard orchid populations in Hungary

In the study period (2013–2017) the number of flowering stems varied between 34 (Keszthely, 2013) and 179 (Sümeg, 2014) per year per sites. The inflorescences were in the largest number in Kőszeg and Sümeg, furthermore Keszthely and Nagytevel showed a similar tendency in the number of flowering individuals.

Among the traits, the height of the shoots and the length of the inflorescences were not independent from each other, accordingly they changed in the same way: they differed significantly between places and years too, and the interaction of years and places was also significant. The relationship between plant heights and number of flowers is weaker. In the case of the number of flowers, only the effect of the places and the years were significant, their interaction was not. Inflorescence lengths differed significantly in all four places. The smallest variability was shown by the number of flowers. So, the number of the flowers in the inflorescences is a stable trait, but reproductive success is influenced by the location and the environmental factors of the given year.

The number of fruits and fruit set differed significantly between places and years too. There were lower (typically around 20% annual averages) fruit set in Keszthely and Sümeg, and higher in Kőszeg and Nagytevel (around 30–60% annual averages). On the basis of the Corine surface cover, the Keszthely and Sümeg populations occur in forested areas or in forested and scrubland mosaic, while the population of Kőszeg and Nagytevel grow in a complex of meadow and cultivated areas which, among other things, can affect the reproductive succes.

2. Honeybee (*Apis mellifera*) mediated increased reproductive success of a rare deceptive orchid

The importance of apiaries in the pollination of Lizard Orchids is shown by the average reproductive success of the H. adriaticum population at Nagytevel in 2013 was significantly higher than in other population studied by us (with three exceptions: Nagytevel 2010, Keszthely 2004 and Sümeg 2003). The author confirmed the role of honeybees in outstanding reproductive success: that that individual reproductive success in the population at Nagytevel decreased significantly with increasing distance from the apiary.

3. Factors influencing the reproductive success (plant size and surrounding vegetation) of lizard orchid species

Three predictors had the largest effects according to the analysis of the total records: length of the inflorescence (0.426), tree cover (-0.508) and scrub cover (-0.367). Median distance of 5 nearest Himantoglossum individuals and relative flowering were both negatively related to fruit set, albeit with lower effect sizes (-0.137 and -0.170, respectively). Fruit set was significantly positively related to the number of unrestrictive and restrictive flowers (effect sizes 0.291 and 0.184, respectively).

Comparison between taxa also confirmed the positive effect of inflorescence length and the negative effect of tree and shrub cover (in case of *H. calcaratum* subsp. *calcaratum* only the tree cover had a significant effect). However, differences also were observed. In the case of *H. adriaticum*, the number of surrounding unrestrictive flowers (i.e. rewarding flowers, which attract generalist pollinators) had a positive effect on fruit set, while the fruit set of *H. calcaratum* subsp. *jankae* and *H. calcaratum* subsp. *calcaratum,* was significantly positively related to the number of surrounding restrictive flowers (i.e. rewarding flowers, which attract specialist pollinators). In the case of Adriatic and Janka Lizard Orchid, the year had a significant effect on fruit set.

In total, we recorded 170 co-flowering rewarding species (in 137 species group) in the plots around individual *Himantoglossum* species, with an average of 6.7 ± 3.6 species per plot, although there were differecies among the study taxa.

In preliminary studies, we found that all of the color ranges (except in one case) perceived by the bees had a positive effect on reproductive success. Furthermore our preliminary results suggest that the presence of several nectar producing families are positively influence to the fruit set, however their statistical support was low. That is, the studies did not confirm that a particular color or taxa had significant role of the reproduction success of *Himantoglossum* species.

In conclusion, the more flowering plants occur around the individuals of *Himantoglossum* species, results the higher fruit set, which reflects the importance of diverse habitats.

3. NEW SCIENTIFIC RESULTS

1. During the study period (2013–2017) of the four Hungarian populations of *Himantoglossum adriaticum*, the number of flowering individuals was determined, which differed between years and locations. The number of inflorescences did not increase above 100 stems even under ideal weather conditions, in Keszthely and Nagytevel while the number of flowering individuals in Kőszeg and Sümeg approached to 200 flowering stems.
2. During the five years of the study the reproductive success varied between 9.2 and 61.7% in Hungary. The realised 61.7% fructification rate in Nagytevel is the highest ever published population-level average reproductive success of *H. adriaticum* showing that a *Himantoglossum* population could reach the average fructification level of rewarding orchids.
3. In 2013, honeybees contributed to the high reproductive success of the Nagytevel population: the reproductive success of individual flowers in this population was negatively related to their distance from the beehives.
4. The average reproductive success of the studied Lizard orchid populations was independent from the size of the population.
5. Plant height, inflorescence length, number of flowers are significantly related to each other, of which inflorescence length showed the greatest variability and the number of flowers the smallest.
6. The author of the dissertation identified the factors influencing the reproductive success. Fruit set was significantly related to
   a. the inflorescence length (positively, based on overall records and per species study as well)
   b. tree cover and scrub cover (negatively, based on overall records and per species study as well)
   c. median distance of 5 nearest *Himantoglossum* individuals (negatively, based on overall records)
   d. relative flowering (negatively, based on overall records) and
   e. the number of unrestrictive and restrictive flowers (positively, based on overall records).
7. The author did not find any species acting as a „magnetic species”. Heterospecific density and richness increased reproductive success.

4. CONCLUSIONS AND SUGGESTIONS

Based on the results of the dissertation, the flowering and reproductive success in the Hungarian populations of the Adriatic Lizard Orchids was greatly influenced by habitat conditions of the localities.

In summary, the plant traits that in general increase their visibility and attractiveness (inflorescence size; shrub and woody vegetation) had a significantly effect in the reproductive success of *Himantoglossum* species, confirming the findings of other studies concerning deceptive orchids (Kindlmann & Jersakova, 2006; Jersakova *et al.*, 2016). That is habitat maintenance, mowing and shrub clearing are necessary for the orchid species, for example, on roadside verges (Fekete *et al.* 2017), where they can flower in high numbers (Bódis *et al.* 2019). The traditional regime of mowing increase recruitment however can reduce seed production (Sletvold *et al.* 2010), therefore, in the long-therm, the application of extensive land use methods is expedient. This is partially realized in the Hungarian populations.

In semi natural habitats, surrounded by nectar-producing species, rare, deceptive orchids may also achieve higher reproductive success. The species richness of the semi-natural habitats is also essential for the survival of bee communities (Brown & Paxton 2009), therefore maintaining extensive farming has a global nature conservation interest.

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6. SELECTED PUBLICATIONS

Articles on the topic of the dissertation in english in professional journals with impact factors

	Specialization / position of the journal	**IF**
Bódis, J., **Biró, É.,** Nagy, T., Takács, A., Sramkó, G., Bateman, R. M., Gilián, L., Illyés, Z., Tökölyi, J., Lukács, B. A., Csábi, M. Molnár, V. A. (2019): Biological flora of Central Europe *Himantoglossum adriaticum* H. Baumann. Perspectives in Plant Ecology, Evolution and Systematics, 40: 1-17 https://doi.org/10.1016/j.ppees.2019.125461	Ecology 63/168 **Q2** Plant Science 68/234 **Q2**	**2,54**
Fekete, R., Nagy, T., Bódis, J., **Biró, É.,** Löki, V., Süveges, K., Takács, A., Tökölyi, J., Molnár, V. A. (2017): Roadside verges as habitats for endangered lizard-orchids (*Himantoglossum* spp.): Ecological traps or refuges? Science of the Total Environment, 607–608: 1001–1008. https://doi.org/10.1016/j.scitotenv.2017.07.037	Environmental Science 27/241 **Q1**	**4,61**
Biró É., Bódis J., Nagy T., Tökölyi J., Molnár V. A. (2015): Honeybee (*Apis mellifera*) mediated increased reproductive success of a rare deceptive orchid. Applied Ecology and Environmental Research 13: 181-192. https://doi.org/10.15666/aeer/1301_181192	Ecology, Evolution, Behavior and Systematics **Q4**	**0,5**

Other not closely related to the topic of the dissertation in english in professional journals with impact factors

Bódis, J., **Biró, É.,** Nagy, T., Takács, A., Molnár, V. A., Lukács, B. A. (2018): Habitat preferences of the rare lizard orchid. Tuxenia, 38: 329–345. https://doi.org/10.14471/2018.38.020	Plant Science, Ecology, Nature and Landscape Conservation **Q3**	**1,267**
Gilián, L. D., Bódis, J., Eszéki, E., Illyés, Z., **Biró, É.,** Nagy, J. Gy. (2018): Germination traits of Adriatic lizard orchid (*Himantoglossum adriaticum*) in Hungary. Applied Ecology and Environmental Research, 16(2): 1155–1171.	Ecology, Evolution, Behavior and Systematics **Q4**	**0,689**

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Biró É., Bódis J. (2015): Sallangvirág (*Himantoglossum*) fajok virágzás-fenológiája és elterjedési mintázata. Kitaibelia 20 (1): 157–167. ISSN 2064-4507 (Online), ISSN 1219-9672 (Print)

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Biró É., Bódis J. (2017): Adatok a hazai adriai sallangvirág állományok természetvédelmi kezeléséhez. – In: Mizsei E., Szepesváry Cs. (szerk.): XI. Magyar Természetvédelmi Biológiai Konferencia „Sikerek és tanulságok a természetvédelemben". Absztrakt-kötet. Eszterházy Károly Egyetem Eger; Magyar Biológiai Társaság, MTA Ökológiai Kutatóközpont, p. 47. (poszter)

Fekete R., Nagy T., **Biró É.,** Bódis J., Takács A., Tökölyi J., Molnár V. A. (2016): Útszegélyek, mint orchidea élőhelyek. – A XI. Aktuális Flóra- és Vegetációkutatások a Kárpát-medencében nemzetközi konferencia, 2016. 02. 12-14. Budapest. Előadások és poszterek összefoglalói pp. 41-42.

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Biró, É., Bódis, J., Nagy, T., Takács, A., Tökölyi, J., Molnár, V. A. (2015): Reproductive success of *Himantoglossum* species. In: Sven Wagner (ed.): *International Conference on temperate Orchids. Research and Conservation. TORC'15 programme and abstracts.* Samos Island, Greece, 13-19. April 2015, Sails-for-Science Foundation, Greece p. 75. (előadás)

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Biró É., Bódis J., Nagy T., Takács A., Tökölyi J., Molnár V. A. (2014): Mely növényi jellegek és környezeti tényezők befolyásolják a sallangvirágok szaporodási sikerét? Diószegi Szeminárium, Debreceni Egyetem TTK Növénytani Tanszék 2014.02.20. (előadás)

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