UNIVERSITY OF PÉCS

Biological Doctoral School

Long-term dynamics of *Himantoglossum adriaticum* populations: demography and life history characteristics

PhD Thesis

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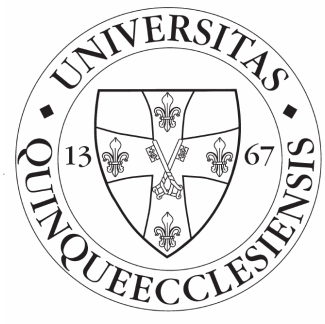
PhD



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INTRODUCTION AND AIMS

Because of their attractive and bizarre flowers, their complex and unusual life-cycle and their doubtful annual appearance, the terrestrial orchids have attracted considerable attention, not only by the naturalists and scientists but also by the 'man in the street'. In Europe orchids are often perceived as the flag-ship of the nature conservation movement.

Orchids are considered one of the most successful groups of flowering plants. The number of species is the highest among plant families and they occur in numerous habitats except for the areas, where they are unable to live, such as deserts and arctic regions where the soil is constantly frozen. Their world-wide dispersion confirm the extreme plasticity that the great species richness imply.

During the last decades, most orchid species in Europe have suffered of significant decline, most likely as a result of land use changes, habitat deterioration and fragmentation. Some of them adapted to the habitats maintained by human land use and now their survival depends on the existence of the extensive agricultural practices.

The endangered status of the orchids was the main reason why the knowledge of biology of orchids became important besides the knowledge of their distribution. Long term ecological research have provided the essential information for determining factors controlling and regulating the population structure and its dynamics in space and time. Long term studies have generally been focused on rare and conspicuous species, predominantly orchids. Publications dealing with the population ecology of terrestrial orchids have appeared since 1980 in Europe, the United States and Canada.

The investigation of population demography of different species is important from many viewpoints. First, it is widely accepted in contemporary phytocoenology that further advances in understanding how plant communities function will not be possible without the quantitative analysis of single species behaviour and the measure of population parameters. Second, the effective protection of species requires predictive models of population behaviour which are built on the basis of a detailed knowledge.

In Hungary, orchids have been quite frequently studied and are well-known, but most of the knowledge is reduced only to their presence data and general flowering characteristics. However, hardly anything is known about the biology of Hungarian orchids, even that of strictly protected species. The long term protection of plant species is impossible without a comprehensive and thorough knowledge of their biology.

The Adriatic Lizard orchid (*Himantoglossum adriaticum* Baumann) is a Natura 2000, CITES species and strictly protected plant in Hungary. Its hypothetical value is HUF 100 000.

The dissertation deals with some important aspects of the population biology of *Himantoglossum adriaticum*. Our aims were the followings:

1. To study the morphometrical characteristics. *Himantoglossum adriaticum* was described in 1978 by Baumann, which means it has been acknowledged as an independent new species for a relatively short time. It is important to study the morphometrical characteristics, which are used to separate related species, and the variability at species level, too (in the Hungarian populations).

2. To carry out life-cycle studies:

- What are the emerging and the growth features in certain life-stages (e.g. seedling and adult stages) characterised by and what factors are they influenced by?
- How does the reproductive behaviour of the species change with time and what factors is it affected by?
- How can the structure of the investigated populations be characterised?
- What temporal pattern do the stage transformations exhibit?
- What is the long term dynamics of the population like (mortality, survival)?

3. Is it possible to gain any information on the fitness of population based on simple count data, which reflect only one aspect of the reproduction?

2. MATERIALS AND METHODS

Study species

Until the 1980-es all the *Himantoglossum* specimens found in Hungary had been determined as *H. hircinum* (L.) Sprengel. H. Baumann found a Lizard orchid population on the Istrian-penninsula in 1973 and declared that plants differed from the *H. hircinum* and named the new species *H. adriaticum* Baumann in 1978. There is no strong goat odour in the case of *H. adriaticum*, and there are narrower leaves in the rosette, the inflorescence is looser and has fewer flowers, the helmet is smaller and closed, the spur is shorter, the colour of the flower is reddish-brown and the labellum is less twisted, but the cut is deep.

According to Baumann, *H. hircinum* does not live in Hungary, but this new species occurs in West-Hungary (Sopron, Kőszeg, Keszthely Hills, West Bakony), and *H. caprinum* (M.-Bieb.) Sprengel lives in the other part of the country. Taxonomists agree with Baumann about the occurrence of *H. adriaticum* and *H. caprinum* in Hungary. The new species occurs in the central and northern parts of Italy, Croatia, Albania, Slovenia, in the south and the west of Austria, in the west of Hungary and Slovakia. Transdanubia (West Hungary) is the territory where the eastern margin of the distribution of *H. adriaticum* and the western boundary of the distribution of *H. caprinum* overlap (besides South Albania).

Study site, sampling and data analyses

The morphometrical characteristics (size of the spur and the outer perianth) were measured in four populations of *H. adriaticum*. There were two sampling sites in Keszthely Hills (Pilikán-Szoroshad and Rezi) and two on the Sümeg-Tapolca Ridge (along the road to Sümeg and along the road to Nyirád). To compare the species, these characteristics were measured in the case of *H. caprinum* too. Sampling sites were in the Villányi Hills (Tenkes and Fekete Hill) and in the Gerecse Hills (Nagy-Teke).

The locality, where the majority of *H. adriaticum* studies were carried out is situated in the western part of Hungary, in the Keszthely Hills, at the boundary of the village Gyenesdiás and Keszthely town. This is the oldest known *H. adriaticum* population, situated on both sides of the Pilikán-Szoroshad minor road, in dolomite grassland, on the edge of the forest. In the study area calciphilous oak woodland, shrub woodland and pine plantations form a vegetation mosaic with the dolomite grassland. Habitats are disturbed, the vegetation is degraded (due to

mainly human activities). *H. adriaticum* grows in the grassland near the road and on the edge of the expanding shrubs and trees.

Some investigations were performed in this population:

- the flowering individuals were searched and counted along a 1 km long section of the road in every June (1992-2008),
- permanent plots were set to study the recruitment and plant individuals were recorded (1999-2009),
- observations of individual plants were made in order to follow the fate of the 1993-1996 cohorts (till 2007), censuses were made annually.

Leaf Growth Rate (LGR) and Relative Leaf Growth Rate (RLGR) were calculated so as to describe the growth pattern of the plants. Number of leaves, maximum leaf area and maximum value of Leaf Area Duration (LAD) (at the end of vegetation period) were used to characterize the condition of the plants. We assumed that the different basal rosette sizes meant different growth patterns, so the plants were divided into 3 groups on the basis of the number of basal leaves (≥4 leaves, 3 leaves, 2 leaves), and these groups were analysed separately.

Several statistical analyses were used to evaluate the data: test of normality, single factor ANOVA, linear regression, multiple linear regression, chi-square test for independence, non-parametric tests: Mann-Whitney and Kruskal-Wallis H test.

R 2.9.0. software package was used to analyse multiple regression. Other statistical analyses were carried out using the software package SPSS 13.1.

Variables describing weather conditions (mean temperature per month, number of frost days per month, precipitation per month) were measured by the Hungarian Meteorology Service at Keszthely (W: 46°44'52" E:17°14'35", height above sea-level: 112.0 m), 6 km far from the study site.

3. NEW RESULTS

3.1. Morphometrical characteristics of *Himantoglossum adriaticum*

1. The length of the spur is a better and a less overlapping characteristic than the size of the outer perianth among the distinctive marks between *H. adriaticum* and *H. caprinum*.

There were no significant differences among the size of the spur in the studied populations. The sizes did not differ from the values known from literature..In the case of the outer perianth there was a significant difference between the populations having the lowest and the highest mean. The characteristic size of the outer perianth was smaller (7.5-9 mm) than we had known (8-11 mm).

The size of the spur and the outer perianth are influenced by the weather, they grew larger when the amount of the precipitation was larger, but remained in the characteristic range.

2. The other morphometrical traits measured (height of the stem, length of the inflorescence, number of the flowers) varied considerably between years. Among them the number of flowers proved to be the least variable during the years.

3. The morphometrical characteristics of the individuals in the population examined were the following: the height of plant is 40-60 cm; the 15-25 cm long, loose inflorescences contain 25-35 flowers; the spurs are 2-3.5 mm, the outer perianths are 7-10 mm long. The rosettes consists of 2-5 leaves. The largest leaves are 7.5-17.5 cm long and 1.5-4.5 cm wide, 3.5-4 times longer than wide (in sunny habitats).

3.2. Life-cycle of the studied *Himantoglossum adriaticum* population

3.2.1. Germination and emergence of the recruitment

1. Seedlings emerged in large numbers in the 3rd year after the adult plants had flowered, that is the seeds need to spend two years in the soil before they are able to develop their first green leaf.

2. The emergence of the seedlings was continuous during the vegetation period, their survival depended on the environmental factors. When the external conditions were suitable they develop to two-leaves rosette plant, when unfavourable, they died and disappeared. Presumably, survival is the critical stage, rather than emergence.

3. The phenology of *H. adriaticum* is similar to other orchid species distributed in the Mediterranean region. The leaves of the larger plants appear after the late-august – early-september rainfall, usually in September. The weather is suitable for the recruitment, when the autumn is wet and cool, seedlings follow the appearances of adult plants with a small lag, but the emergence of the seedlings is continuous during the vegetation period. Cold winters are unsuitable for seedlings.

4. In the emergence of the seedlings, besides the meteorological factors, an important role is played by the current year's status of the parent plant (vegetative, reproductive, dormant) too. The seedlings move together with the parent plant: when the parent plant is dormant, the seedlings also remain in the soil. The status of the parent plant in the previous years do not affect the number of the emerged seedlings.

3.2.2. Growth of the individuals

5. The seedlings (94%) have one leaf mostly, when they emerge first, the width of the leaf is 0.1-1 cm. They can grow till spring, reaching their maximum leaf area in springtime apart from those individuals which are damaged and are unable to compensate the damage. These seedlings reach their largest size during the autumn or winter.

6. There was an intensive growth period after the autumn appearance in all individuals. The intensive growth lasted till November, then the growth of individuals in different size classes were different: large plants showed a stagnation or only a slight growth till the end of March in all three years. During this time the assimilation area often got reduced because of the damage caused by frosts and herbivores. The leaf growth of large plants was very intensive from the end of March till the arrival of the warm period in May, i.e. the end of the growing

period of orchids. Large plants followed the following growth pattern: intensive growth period in September-October and in April, stagnating or slight growth in winter.

In the case of the medium and small size plants the stagnation period is not typical, the growth was characterized by an almost constant rate, no considerable differences could be observed between the autumn and spring or the winter phases.

7. Hardly more than 10% of individuals increased their leaf number year-by-year. The importance of the decrease or increase of the leaf number is that it determines whether the plant remains in its size category or goes over to the larger or the smaller size categories. For *H. adriaticum* the most interesting status is that of the 4-leaf plant, which is the starting point of the large size in this species.

3.3. Dynamics of flowering and fruiting

1. Regarding the temporal fluctuation of the number of flowering plants in the Keszthely Hills (Pilikán-Szoroshad), the investigated population seems to be stable, because during the long-term study period (17 years) the years richest in inflorescence appeared accidentally.

2. The number of flowers was the most stable, while the number of fruits was the most variable reproductive trait. In those years, when there were a lot of flowering individuals, they grew higher and developed more flowers than in the years when there were only a few flowering individuals.

At the population level there is significant correlation between the number of flowering plants (and therefore the number of flowers) and the number of fruits (seedpods), but the value of the correlation coefficient is very low showing a weak relationship. There was no correlation between the average seedpod number per individual and the number of flowering plants, the height of the stem, the length of the inflorescence, but there was a negative correlation with the average number of flowers.

3. There were more than 4000 seeds per seedpod, on average.

4. It was found that the temperature and the rainfall of the current and of the previous years were important weather variables influencing the number of flowering individuals, the height of the stem and the average number of flowers. A positive correlation was confirmed between the above mentioned reproductive traits and the temperature and the rainfall in the previous year and a negative correlation between the traits and the temperature in spring of the current year.

5. Good fruit-producing years, which can ensure the long-term survival of the population under suitable environmental conditions, do not coincide with the ’good orchid years’, that is years of great number of inflorescences.

6. Five Hymenoptera species were proved as pollinators of *H. adriaticum*: *Osmia caerulescens* (L.), *Lasioglossum (Evylaeus) morio* (F.), *Lasioglossum (Evylaeus) lucidulum* (Schck.), *Megachile melanopyga* Costa, *Colletes similis* (Schck.). They visit a wide range of flowers, none of them being an orchid-specialist.

7. The fertility is influenced by the fact whether or not there is nectar secretion in the flowers. Some of our results suggested that the flowers of the *H. adriaticum* population investigated are deceptive, and this influences the pollination too.

There was a negative correlation between the mean number of seedpods and the mean number of flowers, so in those years when the mean number of flowers per inflorescence were higher the mean number of seedpods per inflorescence were lower.

Fertility rate was 16.7% (17 years' average) (cf.: the deceptive orchids' flowers are characterized by a fertility rate under 20%).

8. In the case of *H. adriaticum* the critical size for flowering seemed to be 50 cm^2, which was usually reached in the four-leaf stage of the rosettes. Above the critical size the probability of flowering rises with the increase of leaf number.

9. The leaf number and the leaf area of reproductive plants had already been larger for two years before the flowering took place, than those of the plants which remained vegetative. It means despite the twin-tuber life form, there is a possibility to accumulate carbohydrates, probably in a way that from a larger tuber a larger plant develops, which again can produce even larger tubers. On this basis, the strategy of resource utilisation of *H. adriaticum* does not differ from that of other perennials, which are able to accumulate carbohydrates even for many years before the flowering.

10. The cost of reproduction is usually 2 leaves, but we did not distinguish the cost of flowering and fruiting.

3.4. Demography

During the life history studies (1997–2005) we proved the followings:

1. A large part of the population (53.5–76.9% yearly) consists of vegetative plants. The majority of them have one or two leaves.

The proportion of the flowering plants varied between 4.1% and 34%, the proportion of the dormant ones varied between 1.6 and 12.3%. The difference between the minimum and maximum values was eightfold both in the case of flowering and dormant status.

2. In the case of adult plants dormant period lasted from 1 to 6 years, and only one third of them was one year long. The adult plants spent in dormant status 7-57% of the period investigated. The dormant status was two times as frequent in the vegetative adults as in the reproductive ones.

Dormancy of recruitment was detected, too. It was the rarest in the smallest life-stage category. Dormancy is more characteristic of the recruitment than the adults, but its duration was restricted only for one year in the case of recruitment.

3. The recruitment was much more dependent on the small-scale environmental effects than adults were. The mortality rate of the seedlings was size-dependent, it decreased with increasing size of the seedlings.

4. Annual mortality rate of adults varied between 5.7 and 20.6%.

5. The survivorship curve of the population was Deveey II. type, which means a constant mortality rate and has been described as a characteristic feature of other terrestrial orchids,

too. There is a 2.5 times difference between the lowest and highest yearly mortality rate, which does not mean large fluctuation.

6. *H. adriaticum* is a long-lived orchid, the average life span is 8 years and the tenth of them live at least 15 years. Half-life span is 5.5 years.

Year to year transitions were determined based on the 1997-2005: period:

7. The most frequent transition was the vegetative-vegetative transition (41.2%) in the case of the adult plants. The rate of the reproductive-vegetative (14.2%) and vegetative-reproductive (13.8%) transitions were much lower than the vegetative-vegetative one. The reproductive-reproductive transition was 7.9%.

8. The reproductive status occurred in 2% after the dormant status, in 62% after the vegetative status and in 36% after reproductive status, respectively. Nearly one third of the flowering plants will flower in the next year again, more than half of them will be vegetative and 10% of them will dye. Only 3% will be dormant.

9. Before the vegetative status two thirds of the adults were vegetative, 24% were reproductive and 6% were dormant. After the vegetative status the most likely stage will the vegetative status (in 63%) again, one fifth of them will be reproductive, 6% will be dormant and 10% will die.

10. The previous status in the case of the dormancy is in 46% the dormant status, in 46% the vegetative status and only in 8% the reproductive status. After the dormant status the most likely is the dormant status (in 52%) again, 44% of them will be vegetative, and only 4% will be reproductive.

11. Before they die, 72% of adults spend their last year in vegetative status and 28% in reproductive status.

12. On the basis of the stage-transition probability the stasis and retrogression proved to be the most important features in the stage structure of the population. [Stasis means survival from one year to the next in the same stage class, while retrogression means plants decreasing in size during the year or reverting from stage (e.g. flowering status to a vegetative one or becoming dormant)].

4. SUMMARY

New results are summarised as the followings:

- Morphometrical characteristics of different Hungarian populations of *H. adriaticum* were determined;
- The processes of the emergence of recruitment and the growth of individuals were determined, as well as their relationships with environmental (meteorological and biotical) factors;
- New results were established in connection with the dynamics of flowering and fruiting, with special regard to the pollination biology of the species;

- The life cycle diagram of *H. adriaticum* based on the different stages were constructed and parameterized.

5. PRACTICAL APPLICATIONS OF THE RESULTS

The key to the successful protection of the species is its detailed knowledge. There are 10 orchids among the Natura 2000 species, the ones of community importance (Natura 2000 marker species), four of which occur in Hungary: *Cypripedium calceolus, Liparis loeselii, Himantoglossum caprinum* and *H. adriaticum*. The action plans of conservation of *Cypripedium* and *Liparis* were ready, but that of the two *Himantoglossum* species have not been developed yet.

Our growth studies, the investigations of the flowering and fruiting dynamics, as well as the description of the life-cycle characteristics contribute to the revealing the biology of these long-lived, rare, terrestrial orchid species. Our results make possible the development of a conservation strategy for the *H. adriaticum*.

1. The necessity of the monitoring, being carried out currently, is confirmed by our results. Although the total number exemplars in the population and flowering frequency cannot be estimated if only flowering individuals are used for monitoring, due to the long time period it enables to detect tendencies and temporal variations in population size.

2. Considering the fact that the fitness of the population depends on the recruitment, and for them survival rather than emergence is the critical status, it is suggested that the monitoring should be extended to the recruitment as well (e.g. with permanent plots).

3. It will be useful to clarify the uncertainty of the fecundity (pollination, nectar production) to help the protection of the species.

4. According to our results, nowadays the best habitats for *H. adriaticum* are influenced by human activity. The disappearance of the extensive land-use (rarely mowed orchards, uncultivated vineyards, grass covered shoulder of the roads) can cause destruction of the actual and potential habitats of the species. Known habitats need nature conservation management.

Proposals concerning the investigated habitats:

- The main problem is the shrub encroachment in the case of the Pilikán population, so the removal of the shrub overgrowth would be desirable.
- The populations which live along the shoulders of the roads (Keszthely-Várvölgy, Sümeg-Tapolca, Sümeg-Nyirád line) suffer a lot of damage because their habitat is mowned in the flowering or fruiting time. We should more efficiently encourage the official in charge to order mowing at a more orchid-friendly time.
- The present mowing technology is harmful to the plants, because the cutting is done too low and it includes both sides of the dikes. We propose less close cutting in late July.

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