

OBSERVATIONS ON A POPULATION OF *OPHRYS SPHEGODES* MILL. IN ROMANIA

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Abstract: The aim of this paper was to analyze some aspects of the population biology of *Ophrys sphegodes* near the locality of Tinaud, Bihor region. This population was first mentioned, and documented with herbarium specimens, by Dr Gh. Coldea in 1968, but not studied since. This is the only description of *Ophrys sphegodes* known so far from Romania. Our study aimed to estimate population size and density, the analysis of individual orchids association, the correlation of the morphological parts of the plants, the percentage of plants visited or pollinated by the co-evolved species *Andrena nigroaenea* and the associations with other plant species. The results were analysed for two distinct groups of the population distributed on separate surfaces with different ecological factors and anthropological influences. This allowed us to study also the effects of different management methods on the population. It was shown that there are clear differences between the two groups as concerning their number and density as well as flower morphology and the efficiency of pollination. This differentiation is caused by both ecological and anthropogenic factors. This study should be further developed with the goal of implementing protection measures for this rare and evolutionarily most interesting terrestrial orchid.

Keywords: co-evolution, conservation, flower visitation rate, human impact, *Ophrys sphegodes*, pollination, population biology

Introduction

The Mediterranean orchid genus *Ophrys* has one of the most fascinating pollination strategies in flowering plants. It offers no reward whatsoever, but lures its male insect pollinators, mainly bees (*Andrenidae*, *Colletidae*, *Megachilidae* and *Apidae*) and in a few cases beetles (*Elateridae* and *Scarabaeidae*), by mimicking the visual, olfactory and tactile cues of the females [1]. Thus attracted, the male tries to copulate with the flower and thereby removes the pollinia and by the next visit assures the pollination of the orchid. The relationship between flower and pollinator is highly specific, each *Ophrys* species having only one pollinator [2].

The main distribution area of the genus *Ophrys* is in the Mediterranean region, but a few species do extend further north. In Romania six species have been reported: *O. sphegodes*, *O. insectifera*, *O. apifera*, *O. oestriifera* spp. *cornuta*, *O. fuciflora*¹ and *O. fusca*¹ [3], although the last two species have not been found in recent years and there is no herbarium material to sustain these old records. The area of *O. sphegodes* in Romania is restricted to the western borders of the country, in the Plopiș Mountains, near the village of Tinaud, Bihor region, where it was first described by Coldea in 1968 [4]. As is the case of most orchid species in Romania, there have not been any detailed studies of *O. sphegodes* since its discovery, neither on its pollinator, the mining bee *Andrena nigroaenea*, nor on any aspects of the demography and ecology of the species.

The present paper concentrates on relatively simple aspects of population size and spatial structure, pollination success, correlation of morphological characters and aspects of

¹ The description of these species in Romania is very old and has not been verified; also new molecular data have shown that *O. fusca* and *O. fuciflora* actually comprise two groups of several species.

conservation, regarding the orchid *O. sphegodes*. It should not be seen as a complete study, but as a first step for future studies.

Methods

The study site is located in the Bihor region, in the western part of Romania, near the village of Tinăud, and covers an area of approximately 0.25 km² on a S-SW-facing hillside. The natural vegetation belongs to the association *Festuco rupicolae-Danthonietum* Csürös *et al.* 1961 [5], but large portions are planted with *Pinus sylvestris* and other species; in the lower part are extensively used orchards. Besides this, due to lack of management (no mowing or grazing) some parts are strongly covered by *Pinus sylvestris*, *Ligustrum vulgare*, *Rosa* sp. and *Crataegus monogyna* shrubs. Additionally, two main areas can be distinguished within the study site, with different ecological conditions and usage. Being submitted to different treatments, these two separate areas were used to compare the effects of anthropogenic factors on the *Ophrys* population. Additionally the visitation rate by the pollinator *Andrena nigroaenea* and the overall distribution of the individual plants was determined, also on the basis of the two areas. To this we add some correlation data on important morphological characters in order to also evaluate their variability, such as: basal leaf length and width, plant height, number of flowers and labellum length and width. Correlation was calculated by linear regression, using a standard calculation programme. Data were collected over the two years 2007 and 2008, during April to May.

Results and Discussions

The study site near the village of Tinăud, Bihor region, with an approximate overall size of 0.25 km², was divided, using natural landmarks, into two separate areas. The main difference between the two areas lay in the humidity gradient, steepness, vegetation structure and composition and management. The first area, area A (Fig. 1a), had a southern orientation, with a steep slope and low humidity levels. The vegetation was strongly overgrown by shrubs and had a cover of approximately 70–80%, with several patches of bare soil, where solitary bees often nested. From the appearance of the vegetation it could be concluded that the grass had not been cut for at least two to three years. The second area, area B (Fig. 1b), with a south-western orientation, was strongly influenced by human activity, being partly used as an orchard and mown every year between the beginning and middle of July. The humidity was also significantly higher and the slope less steep than in area A.



Fig. 1: a. Area A, with low anthropogenic influence; b. Area B, used as orchard and grazing ground for horses, regularly mown.

During our studies we estimated the size of the population of *O. sphegodes* to be 1500–2000 individuals, with a density of 0.67 individuals/m². At the same time it must be taken into consideration that not all plants develop flowers every year, and some can remain dormant for

several years [6]. The distribution of individuals was not even, but different in the two areas, area A having 80% of the flowering individuals. Also most of the individual plants (75.51%) grew isolated, 22.45% occurred as small groups and only 2.05% in medium-sized groups. Vegetative reproduction seems to be low in this species [7], which would explain at least a part of the observed distribution pattern.

We also tried to determine the plant species that often accompanied the individuals of *O. sphegodes*. Twenty-four species were identified, but only a few were constant associates of this orchid, the most frequent being *Inula ensifolia*, *Prunella vulgaris* and *Filipendula vulgaris*. To these we should add as important neighboring plants *Ligustrum vulgare* and *Pinus sylvestris*, because it is on these plants that the male pollinators mark their flight routes and patrol in search of females.

Pollinator visits were quantified by numbering plants with developing fruits, with removed pollinia and with deposited pollen massulae. The results were rather surprising, because the visits were much higher than expected and also it was easy to see a difference between the two study areas (Fig. 2). In area A the visitation rate was almost double that of area B, even if one takes into account the different numbers of individuals. Although these data suggest that taller plants are far better visited than smaller plants, similar results being obtained by Paulus [2], this author showed that in *O. speculum* smaller plants had higher reproductive success, this being correlated to the foraging behaviour of male pollinators. So our data might be explained also by the hypothesis that co-evolved pollinator flying route and height influences the height of pollinated orchid species (unpublished data).

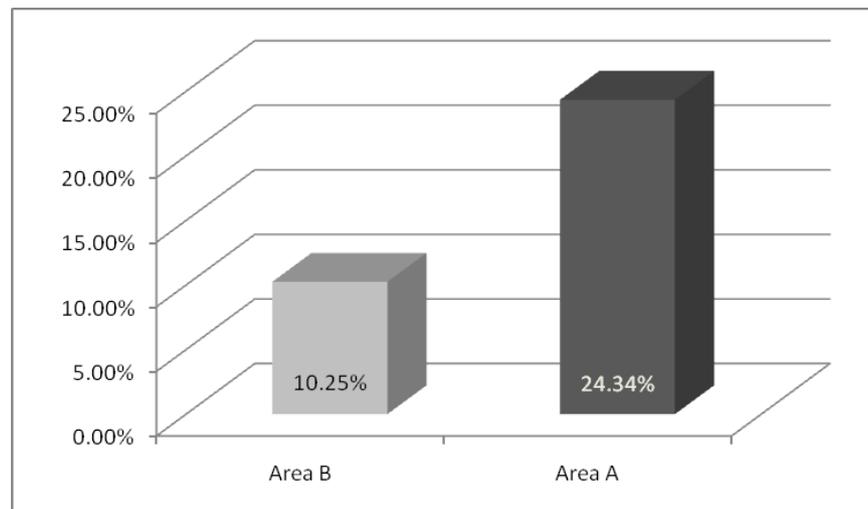
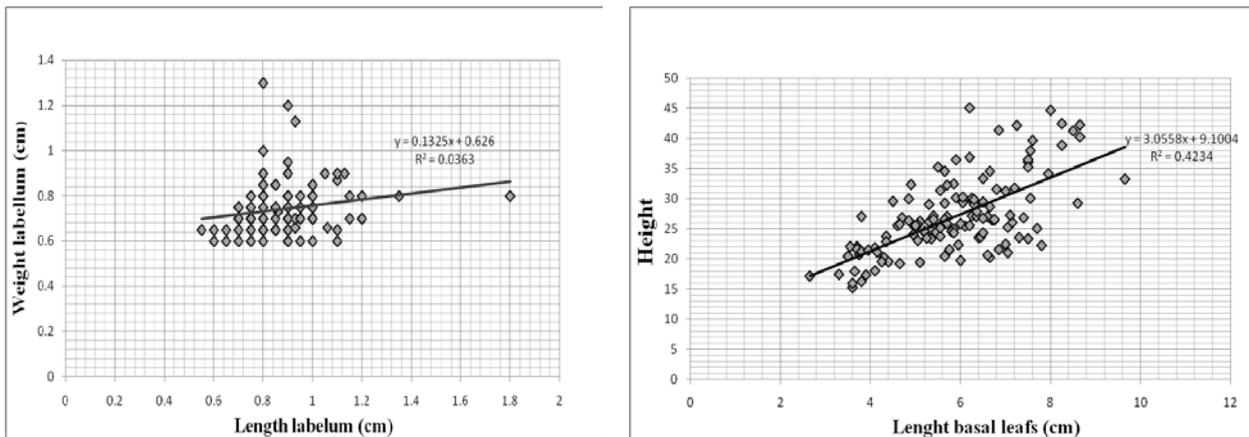


Fig. 2: Visitation rate by pollinators to *O. sphegodes* individuals, showing different values between area A and B.

For the correlation analyses we measured several important characteristics, such as length and width of basal leaves, height, number of flowers, length and width of labellum. The results were very variable, some gave relatively strong correlation, others not and yet others changed their relations after analysing a higher number of individuals. The strongest correlations were between: length of leaves and height (Fig. 3), width of basal leaves and number of flowers, number of flowers and height, middle correlations were between length of basal leaves and flower number, weight of basal leaves and height, and low correlation resulted between the labellum width and length (Fig. 4).



Figs. 3-4: Correlations between length of basal leaf and flower height ($R^2 = 0.4234$, $r = 0.65$) and between length and width of the labellum ($R^2 = 0.0363$, $r = 0.19$).

The most unexpected correlation was between the length and width of the labellum; this is because the labellum is that petal of the orchids that imitates the female of the pollinator, and the right dimensions are crucial for getting the males in the position to remove the pollinia (the viscidia sticking to the head of the pollinator) (Fig. 5). Simple correlations will not be enough to explain this phenomenon, but further studies should provide answers to this question. High correlations between leaf characters and height are not surprising, as they are indicators of the individual's development. One of the important characteristics of the genus *Ophrys* is the very high variability of the individual flowers, not only between individuals but also within the inflorescence. This makes determinations sometimes extremely difficult. In the case of the present study, population morphological variation was, as expected, very high, some flowers having some remarkable traits, such as red petals and prominent labellum protuberances. This high variability is an adaptation to the learning abilities of the pollinating bees, which after visiting one flower often learn to avoid it in future [2]. So for flowers to increase their pollination rate, variability is a strategy to ensure that pollinia are transported from one flower to the next, while making sure that the pollinators do not revisit the same flower, so avoiding self-fertilization.



Fig. 5: Pollinia of *O. sphegodes* under inverted microscope Olympus CK-2 (left 40X; right 200X), the sticky viscidia adhering to the tip of the scalpel.

Although it must be taken into consideration that in *O. sphegodes* olfactory cues are more important than visual ones, due to the mating system of the pollinator, but these also show similar patterns of variability [8, 9].

The population studied at Tinăud is remarkable not only because it is the only one discovered in Romania, but also because of its size and uneven distribution at the site, which allowed us to make a comparison between the effects of different management types on this rare and endangered species. So in area B, where the influence of human activity is high – regular early mowing, grazing, use of pesticides, etc. – the population has fewer individuals and the visitation rate by the pollinator is also very low. On the other hand in area A, where human impact is low, the population is very well developed and visitation rates are remarkably high for orchids. Of course ecological factors are very important for the development of the populations but it is clear that human impact is also a major factor that influences the survival of this species.

Being the only population of *O. sphegodes* discovered in Romania, its protection should be a high priority, even if at a European level it is not considered a particularly endangered species. Management actions in this case should not be very expensive; because we believe that simple actions could contribute to the conservation of this population. One such action, that has proved effective for other orchids as well [10], is to fix a later mowing time, after the fruits are mature and the seeds have dispersed. An appropriate time would be between mid-July and early August. At this time grazing could also be permitted; this would help keep shrubs from covering the orchid's habitat. In area A, additional clearing of some portions, of shrubs (especially *Pinus sylvestris*), would also be advisable. Of course these are just suggestions, and for their application further studies and appropriate funding are necessary, but at least constant monitoring of the population will be carried out.

Conclusions

1. The Romanian population of *O. sphegodes* could thus be reconfirmed, and an estimate made of its size and density of individuals. Also the population is quite large, and it shows differences in distribution according to human activity and ecological factors, confirming our expectations.
2. The pollinator visitation rate to the flowers was very high, also differences could be determined between the two study sites, which leads us to believe that the distribution of the pollinator is affected by certain factors (like insecticides use or the unavailability of patrol sites for the males in area B). This should be a subject for further studies.
3. Correlation analysis of important morphological characters gave interesting results, such as the low correlation between labellum width and length, which indicates an independent development of these two characters.
4. For the conservation of this population of *O. sphegodes* some management actions have been proposed: regular mowing after ripening of the fruits, partial removal of shrubs, especially *Pinus sylvestris*, which has been planted here, and continuous monitoring of the population.

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OBSERVAȚII ASUPRA UNEI POPULAȚII DE *OPHRYS SPHEGODES* MILL. DIN ROMANIA

(Rezumat)

În lucrarea de față se prezintă studiul unei populații de *Ophrys sphegodes* din comuna Tinăud (județul Bihor), acesta fiind primul studiu populațional efectuat pe această specie la noi în țară. În acest studiu s-a estimat mărimea și densitatea populației, s-a determinat procentul de plante vizitate de polenizatorul *Andrena nigroaenea* (Hymenoptera), corelația între unele caracterele morfologice importante ale plantelor și s-au stabilit cei mai importanți factori care pun în pericol această populație.

În analiza rezultatelor s-a ținut cont de repartiția diferențiată a indivizilor de *O. sphegodes* pe două suprafețe A și B, care se deosebesc atât în ceea ce privește factorii ecologici, cât și din punct de vedere al influenței antropice și s-au comparat indivizii acestor două zone. Aceste diferențe între suprafețe sunt reflectate de numărul, densitatea și gradul general de dezvoltare a plantelor. Numărul total de indivizi estimați pe suprafața de studiu (0,25 km²) a fost între 1500 și 2000. 20% din populație se găsește pe suprafața B, cu influența antropică mai ridicată, pe când pe suprafața A, cu influența antropică redusă, se găsesc 80% din indivizii populației. Densitatea totală a fost de 0,69 indivizi/m². Modul de asociere a indivizilor de *O. sphegodes* a fost preponderent sub formă izolată, mai rar sub formă de grupuri sau pâlcuri mici.

O importanță deosebită pentru biologia speciei o are gradul de vizitare/de polenizare a florilor de către himenopterul specific *Andrena nigroaenea*. Cu toate că, specia polenizatoare nu a putut fi colectată, numărul mare de flori polenizate indică prezența ei în zonă. Gradul de vizitare/de polenizare a florilor este diferit pe cele două suprafețe A și B, florile de pe versantul sudic (B) sunt mai frecvent vizitate (peste 24%), decât cele din livadă (peste 10%). Un rol în polenizarea florilor de *O. sphegodes* îl are și distribuția tufelor de *Ligustrum*, respectiv a pinului, pe care masculii polenizatori le folosesc ca puncte de marcare a rutelor de zbor. Orhideele situate în apropierea acestora au șanse mai mari să se afle și la intersecția rutelor de zbor ale polenizatorului. Pentru a susține aceste afirmații trebuie efectuate însă și experimente comportamentale.

Dintre cele 16 corelații efectuate, cea mai semnificativă a fost cea între numărul de flori și lățimea frunzelor bazale ($r = 0,68$). În urma comparării datelor dintre cei doi ani de studiu a rezultat modificarea unor corelații pe baza creșterii numărului de date (mai mare în 2008), unele caractere devenind astfel medii sau ne semnificative (lungime/lățime label).

Din datele obținute reiese, că factorul antropic influențează puternic populația de *O. sphegodes*. Această influență se observă atât în cazul numărului diferențiat de indivizi pe cele două suprafețe, cât și în gradul de dezvoltare a indivizilor de *Ophrys*. Data timpurie de cosire în livadă și extinderea plantației de pini pun în pericol populația analizată, pe când o lipsă totală de întreținere ar duce la împădurirea lor treptată, ceea ce ar determina dispariția habitatului speciei. O metodă optimă de management a zonei ar fi cosirea anuală a pajiștii în luna iulie, o îndepărtare parțială a unor arbuști și pășunatul în perioada în care orhideele se găsesc în faza de tubercul.