# DRY GRASSLAND LAND USE TREATMENT REGIME EXPLAINS THE OCCURRENCE OF THE GREEN WINGED ORCHID, *ANACAMPTIS MORIO* (L.) R. M. BATEMAN, PRIDGEON & M. W. CHASE IN THE GORIČKO NATURE PARK, NE SLOVENIA

# REŽIM UPRAVLJANJA S SUHIMI TRAVIŠČI DOLOČA POJAVNOST NAVADNE KUKAVICE, *Anacamptis Morio* (l.) r. M. Bateman, pridgeon & M. W. Chase v Krajinskem Parku goričko, sv slovenija

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#### ABSTRACT

Dry grassland land use treatment regime explains the occurrence of the Green winged orchid, *Anacamptis morio* (L.) R. M. Bateman, Pridgeon & M. W. Chase in the Goričko Nature Park, NE Slovenia

The green winged orchid (Anacamptis morio) is a common, widespread species in Slovenia, distributed in areas up to altitudes of 1000 m. The population, located inside the borders of Goričko Nature Park, is one of the richest in the country, even though the appropriate habitats have deteriorated considerably. Species frequency, density and maximum density were studied, according to the following: the land use treatment regime, species richness and diversity and selected landscape parameters of these 80 dry, Hypocherido-Festucetum rupicolae Steinbuch 1980 and also Ranunculo bulbosi-Arrhenatheretum elatioris Ellmauer and Mucina et al. 1993, semi-natural grassland fragments. The species was present on 46 fragments with a total of 3812 specimens. Species density and maximum density are in significant positive relation with the elevation of the dry grassland fragments. The maximum density of the species in significant, negative relation with the species richness of the dry grassland fragments and also with the species diversity of the grassland fragments. Both species richness and diversity are closely related to the land use treatment regime of the particular grassland fragments. The orchid prefers grasslands that have been regularly mowed, but is less frequent on those with higher species diversity rates. These include fragments at different succession stages with containing woody and herbaceous plant species that have generally higher diversity index values. Half of the population located in NE Goričko

# IZVLEČEK

Režim upravljanja s suhimi travišči določa pojavnost navadne kukavice, *Anacamptis morio* (L.) R. M. Bateman, Pridgeon & M. W. Chase v Krajinskem parku Goričko, SV Slovenija

Navadna kukavica (Anacamptis morio) je v Sloveniji pogosta, splošno razširjena vrsta do nadmorskih višin približno 1000 m.n.m. Populacija, ki se nahaja v Krajinskem parku Goričko je ena največjih v smislu števila osebkov kljub dejstvu, da se število fragmentov ekstenzivnih, suhih in pol suhih travnikov naglo zmanjšuje. Spremljali smo frekvenco vrste (število cvetočih osebkov) ter njeno povprečno in maksimalno gostoto v povezavi z režimom rabe tal na fragmentih suhih travišč, vrstno pestrostjo suhih travišč in izbranimi krajinskimi parametri. Na območju raziskave smo zabeležili 80 fragmentov suhih in pol suhih travišč, ki pripadajo asociacijama Hypocherido-Festucetum rupicolae Steinbuch 1980 in Ranunculo bulbosi-Arrhenatheretum elatioris Ellmauer in Mucina et al. 1993. Vrsta je bila prisotna na 46 fragmentih s skupno 3812 cvetočimi primerki. Povprečna in maksimalna gostota vrste sta v značilni, pozitivni zvezi z nadmorsko višino fragmentov suhih travišč. Maksimalna gostota vrste je v značilni negativni zvezi s številom vrst na obravnavanih fragmentih suhih travišč kot tudi z vrstno pestrostjo (vrednosti indeksa Shannon- Weaver) fragmentov suhih travišč. Število vrst na fragmentih suhih travišč kot tudi vrstna pestrost sta v tesni povezavi z režimom rabe tal posameznih fragmentov travišč. Navadna kukavica preferira redno košena travišča, številčno pa je manj pogosta na fragmentih z višjimi vrednostmi indeksa vrstne pestrosti. Taki fragmenti travišč so v zgodnjih fazah sukcesije z zelna-

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was found on ruderalized (disturbed) dry grassland fragments (road verges, graveyards, house front yards). Since dry grassland fragments are in a serious decline, alternative habitats such as road verges could support the local population of the green winged orchid in future. The land use treatment regime of the dry grassland fragments and the altitude do significantly explain today's occurrence and the spatial distribution of *Anacamptis morio* in NE Goričko Nature Park. Conservation efforts should be focused to preserve the remaining grassland fragments through proper management.

Keywords: Traditional agricultural landscape, Festuco-Brometea and Molinio-Arrhenatheretea semi-natural grasslands, Anacamptis morio, land use treatment regime, landscape parameters, Goričko Nature Park, NE Slovenia timi trajnicami in enoletnicami ali pa v kasnih stadijih sukcesije z drevesno grmovnimi vrstami in imajo v splošnem višje vrednosti indeksa vrstne pestrosti Shannon-Weaver. Ugotovili smo, da se polovica populacije vrste (23 fragmentov, 1908 cvetočih primerkov) na območju raziskave pojavlja na motenih (ruderaliziranih) fragmentih suhih travišč. Taki habitati so dvorišča hiš, pokopališča, pasovi travišč v neposredni bližini cest. Glede na dejstvo, da je število redno košenih fragmentov suhih travišč na območju raziskave v naglem upadanju bi lahko prav ruderalizirana (motena) travišča predstavljala pomemben refugij vrste na območju raziskave v prihodnje. Režim rabe tal in nadmorska višina statistično značilno pojasnjujeta današnji vzorec razširjenosti (število in gostoto primerkov) navadne kukavice v Krajinskem parku Goričko. Trajnostno varovanje navadne kukavice bi moralo biti usmerjeno v zaščito in redno vzdrževanje (košnja) pol naravnih fragmentov suhih travišč, preprečevanje zaraščanje še obstoječih fragmentov.

Ključne besede: Tradicionalna kmetijske krajina, pol naravna travišča iz razredov Festuco-Brometea in Molinio-Arrhenatheretea, Anacamptis morio, režim rabe tal, krajinski parametri, Krajinski park Goričko, SV Slovenija

#### **1 INTRODUCTION**

The alteration of landscapes throughout the world results in fragmentation of natural and semi-natural vegetation, imposing a great threat on numerous plant and animal species (KIVINIEMI & ERIKSSON 2002). In fragmented landscapes, plant species persistence depends on functional connectivity in terms of pollen flow to maintain genetic diversity within populations, and seed dispersal to re-colonize habitat patches following local extinction (RICO et al. 2011). Habitat fragmentation is predicted to lead to an area-related reduction in population size and a decreasing colonization rate due to isolation (KIVINIEMI 2008). Traditionally managed, unfertilized semi-natural grasslands, such as pastures and meadows, are known to support a rich flora and are often characterized by high species diversity (KULL & ZOBEL 1991; NORDER-HAUG et al. 2000). Goričko Nature Park is located in the extreme NE of Slovenia (Figure 1), and was established to protect the well-preserved traditional, central-European agricultural landscape. The mosaic structure of the landscape and the variety of secondary habitats in Goričko were caused by the simultaneous effect of factors both natural and anthropogenic, geopolitical, economic and social (KALIGARIČ et al. 2008). The landscape structure in Goričko is a typical result of "frozen processes" (FORMAN 1995) that appeared or influenced the landscape in the past. Small patches and their diversity reveal the "typical" traditional agri-

cultural landscape, fragmented at the end of the 19<sup>th</sup> and at the beginning of the 20<sup>th</sup> century. In the traditional agricultural landscape of Goričko, there were basically four kinds of intense land-use: agricultural fields, grassland, pasture and vineyards. During the past century, traditional land use was drastically changed. The area used for pasture decreased, mainly because of land abandonment and forest progression. The area used for hay-making was largely transformed into fields or, more often, abandoned and overgrown. Dry, semi-natural grasslands, remnants of the traditional agricultural landscape are today threatened because of quick overgrowing with woody species of the zonal, "Querco-Carpinetum" type.

Although it was previously included in the genus Orchis (synonym Orchis morio L.), phylogenetic studies based on DNA sequences have shown that the green winged orchid and related species are more closely related to the pyramidal orchid (Anacamptis pyramidalis (L.) Rich.) than to 'true' species of Orchis (BATEMAN et al. 1997, PRIDGEON et al. 1997). Anacamptis morio is a widespread Euromediterranean species occurring from southern Norway to Iran (FAY & RANKOU 2010). Although it has been reported to be monocarpic (CHATTERS 1994), in a demographic study WELLS et al. (1998) refuted this, with some individuals being demonstrated to flower for up to 17 years! The species is pollinated by deceit, in that the spur contains no nectar to reward the insect (FAY & RANKOU 2010). Pollinators include bumble bees, honey bees and solitary bees (SUMMERHAYES 1976). Across its distribution range, it has a broad ecological range and occurs in various types of grassland and prefers neutral or calcareous soils (FAY & RANKOU 2010). The green winged orchid (Anacamptis morio) is widespread in the Goričko Nature Park, especially in its NE part. Under suitable conditions, populations of some thousands of flowering plants can still be found in NE Goričko. Green winged orchid (Anacamptis morio) is found in selected dry grassland-fragments under differing land use treatment (management) regimes. The species appears on semi-natural, dry and semi-dry grassland fragments, road verges and also on ruderalized (disturbed) grassland fragments. These are the only remaining fragments of larger areas, fragmented at the beginning of the 19th century; we were keen to understand the driver that causes/explains today's spatial distribution of the species in a selected area.

The aim of this study was to reveal the effect of management or abandonment and also of selected

landscape parameters (dry grassland fragment area, inclination, aspect, altitude and distance to human settlements/houses) on the spatial distribution of the green winged orchid (*Anacamptis morio*) in the selected research area. This particular species was chosen in the study since it serves as a flagship species for conservation (Böhnert et al. 1986), the same as other grassland orchid species. Orchids in general are well suited to be indicators of the health and biodiversity of the European semi-natural grasslands but also other habitats. We addressed the following specific questions;

How does land use treatment regime (management or abandonment) affect orchid frequency and density on dry grassland fragments?

Is there any relation between the species richness and species diversity of these studied grassland fragments and the green winged orchid frequency and density?

How do selected landscape parameters- dry grassland area, inclination, aspect, altitude and distance to human settlement (houses) affect orchid frequency and density on dry grassland fragments?

# 2 METHODS

#### Study area and field methods

The study area (centered at 46°50'-46°52' N, 16°15'-16°52' E) is located in NE Goričko Nature Park, NE Slovenia, and covers 9.26 km<sup>2</sup> (Fig. 1B and 1C). The area of Goričko Nature Park has a central-European climate with a relatively dry winter. Average annual rainfall is between 750 mm and 820 mm. The driest months are January and February, while most rainfall occurs in July and August. Mean annual temperature is between 9°C and 10°C (ARSO 2014). It is a mosaic of



Figure 1. Geographic position of the research area in Europe (A), in Slovenia (B) and the spatial distribution of the 80 dry grassland fragments.

arable land, fields, forest patches, grassland orchards, vineyards and dry grasslands on non calcareous sandstones. The geologic substrate in NE Goričko is made up mainly of Tertiary sediments (between 1.6 and 66 million years old) and forms a soft hilly landscape with relatively small inclination differences (ČINČ JUHANT & PLANIŠEK 2002). The soils are acidic, with a rich surface network of fresh water. The area includes the land associated with the settlements of Budinci and Dolenci, and is bounded on all sides by larger fragments of black locust (Robinia pseudoacacia), deciduous, beech (Vicio oroboidi-Fagetum) (and mixed) forests and oak hornbeam forests (Erithronio-Carpinion) on acid soils (BAKAN 2006). The dry grasslands belong to the Festuco-Brometea and Molinio-Arrhenatheretea class, with the dominant associations being Hypochoerido-Festucetum rupicolae Steinbuch 1980 and Ranunculo bulbosi-Arrhenatheretum elatioris Ellmauer and Mucina et al. 1993. Dry and semi-dry grasslands in Goričko are not as rich with species as similar grasslands elsewhere in Slovenia, owing to the low pH value, so the soil is quite acid (BAKAN 2006).

All the grassland fragments having the floristic composition of dry grasslands (which also includes also earlier or middle successional stages after abandonment) found in the area after a thorough scanning of the field in 2013/2014, were systematically mapped, sampled and analyzed. The total number of all plant species per grassland fragment (species richness) was sampled. In In addition, the total sampling of randomly dispersed square meter plots (10 per fragment) was done to reveal each plant species frequency per fragment in order to calculate the species diversity (Shannon-Weaver diversity index) of these dry grassland fragments.

The total number of green winged orchid specimens (frequency) per fragment was counted on all dry meadow fragments. Green winged orchid density per dry grassland fragment was calculated (number of specimens per grassland fragment/fragment area). The maximum density of the species per grassland fragment was obtained using a square meter plot. The plot was randomly displaced around the area of each grassland fragment in areas where a higher density of the species was detected, ten times. All the orchid specimens within the plot were counted. The highest value (out of ten) represents the actual maximum density of the species per dry grassland fragment.

#### Data analysis

In the first step, grassland fragments were grouped according to their similarity in total number of species present per grassland fragment (species richness), using TWINSPAN analysis (HILL 1979), in order to establish varied land use treatments in terms of management type or abandonment stage. The output was also validated in the field through observation (expert knowledge) and additional interviews with farmers. As a measure of grassland (alpha) diversity, Shannon's Index (H) was calculated for all fragments, using the equation by Shannon & Weaver (1963). The spatial geometry (dry grassland fragment area, inclination, aspect, altitude and the distance to human settlements, houses) of the grassland fragments was obtained using ArcGIS 9.3 Spatial analyst tools (ESRI 2010) by vectorizing the plots, which were first drawn on printed orthophoto images (pixel size= 0.5 m) (GURS 2010). Spearman's Rank correlation coefficient for the nonnormally distributed data was used to identify and test the strength of the relationship between the green winged orchid frequency and density and the selected landscape parameters, also species richness and diversity of the dry grassland fragments. The nonparametric, Kruskal-Wallis one-way analysis of variance by ranks, which does not assume a normal distribution of the data was used in order to determine whether the orchid's frequency and density vary significantly between differing land use treatment regime categories (SPSS Inc. 2006). The Mann-Whitney test (MANN & WHITNEY 1947), a nonparametric test of the null hypothesis that two populations are the same against an alternative hypothesis, was used in order to find statistically significant differences between the groups (in our case land use treatment regimens) (SPSS Inc. 2006).

#### **3 RESULTS**

## Land use treatment regime in relation to the species richness and diversity of the dry meadow fragments

After a thorough scanning of the field, 80 semi-natural grassland fragments having the floristic composition of dry grasslands (which includes some earlier or middle successional stages after abandonment) were found. In total, 180 plant taxa were recorded, (45 of which were habitat specialists), being characteristic species of the classes *Festuco-Brometea* (*Mesobromion*) and *Molinio-Arrhenatheretea*. The orchid flora is restricted to five grassland species: Anacamptis morio, Neotinea *ustulata*, Neotinea tridentata, Gymnadenia conopsea and Spiranthes spiralis. The Spiranthes spiralis is the abundant one in the area. The total number of species per fragment varies from 30 to 61, with an average of 42.5 species per fragment (Figure 2A). In the terms of their species richness and appearance, the grassland fragments are quite similar, but in general we can distinguish between still managed (N=55) and abandoned fragments (N=25). Fragments with the highest species richness are those in successional stages and not the regularly mowed ones. Twinspan analysis on the basis of all the species present (species richness) and recorded per grassland fragment revealed 4 categories that could clearly be explained with land use treatment regime, as recorded in the field by observation and interviews with the farmers; these are as follows: (1) regularly mowed, typical, dry grassland fragments (N=21), (2) abandoned fragments already overgrown with woody perennials (N=14), (3) ruderalized (disturbed) fragments (N=34) and (4) abandoned fragments in early succession stages without woody species (N=11). The fragments, typically mowed twice a year (late May and late August) belong to the first category. Hay is removed and used for cattle. Those fragments are species rich with a maximum of 40-50 dry grassland vascular plant species per grassland fragment. Abandoned fragments in the late successional stages are fragments overgrown with tree or shrub species up to 1.5 m in height. Typically, these are no longer mown. Ruderalized (disturbed) fragment are located in close proximity to houses or roads. They are disturbed in the sense of traffic (constant trampling) or other human activities (mulching etc.). The grasslands in this category are present in graveyards, playgrounds, house front yards and road verges. They are mown more than 3 times a year, and the hay is usually not removed. Abandoned fragments in early succession stages without woody species are those that are not regularly mowed (maybe once a year), with tree or shrub species present, with their canopies lower than 0.5 m. These abandoned grasslands are species richest.

The mean number of all plant species present (species richness) on regularly mowed fragments of dry grassland is 41.19, and 42.35 on abandoned fragments already overgrown with woody perennials (Figure 2A). The mean number of species on ruderalized (disturbed) fragments is 39.38 (the lowest) and 54.81 on abandoned fragments in early succession stages without woody species (the highest). There are statistically significant differences in plant species richness (total number of plant species per grassland fragment) between the four established land use treatment categories (Kruskal-Wallis sum test,  $\chi^2 = 25$ . 395; df = 3; p = 0.000). The Mann-Whitney test proved that there are statistically significant differences in plant species richness between category 1 and 4, 2 and 4 and also 3 and 4 (p < 0.05).

The total number of habitat specialists varies from 7 to 24, with an average of 14.7 habitat specialists per fragment. The mean number of specialist (typical) species present on regularly mowed fragments of dry grasslands is 16.57, and 10.21 on abandoned fragments already overgrown with woody perennials (the lowest). The mean number of typical species on ruderalized (disturbed) fragments is 14.5, and 18.0 on abandoned fragments in early succession stages without woody species (the highest). There are statistically significant differences in typical plant species (dry grassland habitat specialists) richness (total number of typical species per grassland fragment) between different land use categories (Kruskal-Wallis sum test,  $\chi^2$ = 28.860; df= 3; p = 0.000). The Mann-Whitney test proved that there are statistically significant differences in species diversity index values between category 1 and 2, 2 and 3 and also 2 and 4 (p < 0.05).

The species diversity (Shannon-Weaver index value) of the grassland fragments was calculated sepa-

rately for each land use category. The mean species diversity index value (Shannon's Index values) on regularly mowed, typical fragments of dry grasslands is 3.34, and 3.29 on abandoned fragments already overgrown with woody perennials. The mean diversity index value on ruderalized (disturbed) fragments is 3.21 (the lowest), and on abandoned fragments in early succession stages without woody species it is 3.47 (the highest). In general; there are statistically significant differences in plant species diversity rates between different land use categories (Kruskal-Wallis sum test,  $\chi^2$ = 10.773; df = 3; p = 0.013). The Mann-Whitney test proved that there are statistically significant differences in species diversity index values between category 1 and 3 and 4 (p <0.05).

### Dry grassland area, slope inclination, aspect, altitude and the distance to human settlements (houses).

We are dealing with small fragments of similar area (on average 3200  $m^2$ ). The total fragment area covers 25.56 ha. The size of the smallest fragment is  $280 \text{ m}^2$ ; the largest is 11200 m<sup>2</sup>. On average, the largest fragments are those regularly mowed (mean 3660 m<sup>2</sup>), while the smallest fragments are those overgrown with woody perennials (mean 2780 m<sup>2</sup>). Ruderalized fragments (mean 3106 m<sup>2</sup>) and fragments in early succession (mean 3100 m<sup>2</sup>) are almost uniform in area. The slope inclination of all 80 dry meadow fragments varies from flat ground up to 15.42°. On average, the abandoned fragments already overgrown with woody perennials are located on slopes with a mean inclination rate of 8.30°. Ruderalized (disturbed) fragments are located on slopes with the lowest inclination rates mean 6.47°. Dry meadow fragments located on the steepest slopes are abandoned fragments in early succession stages without woody species (mean 8.46°). Fragments in different succession stages are generally located on steeper slopes, while regularly mowed and disturbed fragments occur on slopes with lower inclination rates. The dry meadow fragments are almost equally distributed on slopes with all aspect rates: 0-90° (N=23). 91-180° (N=20). 181-270° (N=21) and 271-360° (N=16). Regularly mowed, typical fragments of dry grassland are oriented towards the SE (18.7 % of their total area), 16.7% to the NE, 16% exactly to the S and 13.1% to the NW. Abandoned fragments already overgrown with woody perennials are oriented towards the NE (32.2% of their total area), 27.6 % of their area is oriented to the SE and 16.7 % to the NW. Ruderalized fragments are generally oriented towards the

SSE (155.52°), but 25% of their area is oriented to the NE, 20.7 % to the E and 13.5 to the NW. Almost exactly oriented towards the south one can find abandoned fragments in early succession stages without woody species (179.75°). 23.3% of their total area is oriented to the SW, 19.8 % to the S and 16.7 to the E. The rolling hills of the research area have an altitudinal range between 220m - 350m. The differences in mean altitudes between the 4 land use treatment regimens are low: regularly mowed, typical fragments of dry grassland (mean altitude 291.61 m), abandoned fragments already overgrown with woody perennials (290.12 m), ruderalized (disturbed) fragments (301.95 m) and abandoned fragments in early succession stages without woody species (286.79 m). In general, the managed fragments are located on higher ground than the abandoned ones. On average, the managed grasslands that are regularly mowed are located closest to buildings (mean 79.28 m). Abandoned fragments already overgrown with woody perennials are located on average 107.23 m from the nearest building and ruderalized fragments, 92.35 m. The most distant from the human settlements are the abandoned fragments in early succession stages without woody species (mean 169.99 m). In general, grassland that is still managed is located closer to buildings, while abandoned (overgrown) grasslands are the most distant.

# Green winged orchid (Anacamptis morio) frequency

Green winged orchid (Anacamptis morio) was present on 46 (out of 80) dry meadow fragments with, a total of 3812 flowering specimens recorded. According to the different land use treatment regimes, the orchid was present on (1) Regularly mowed, typical fragments of dry meadows (N=12), 1550 specimens, (2) abandoned fragments already overgrown with woody perennials (N=4), 35 specimens, (3) ruderalized (disturbed) fragments (N=23), 1908 specimens and (4) abandoned fragments in early succession stages without woody species (N=7), 319 specimens. On average, the highest frequencies of the species are found on regularly mowed, typical fragments of dry meadows (mean 129.2 specimens per grassland fragment), Figure 2B. On average, only 8.75 specimens per grassland fragment are found on abandoned fragments already overgrown with woody perennials and 82.92 on ruderalized (disturbed) fragments. 45.57 specimens are found on abandoned fragments in early succession stages without woody species. There are no statistically significant differences in the total number of orchid specimens (species frequency) between the four analyzed land use treatment regimens (Kruskal-Wallis sum test,  $\chi$ 2=3.328; df= 3; p= 0.349). Spearman's correlation co-



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Figure 2: Total number of plant species per grassland fragment (A), (B) green winged orchid (Anacamptis morio) frequency (number of flowering specimens per dry grassland fragment), (C) green winged orchid density (number of flowering specimens / grassland fragment area) and (D) maximum density of the species (number of flowering specimens / *1m2 plot) according to the land use* treatment regime of the analyzed dry grassland fragments. (1) Regularly mowed, typical fragments of dry grassland, (2) abandoned fragments already overgrown with woody perennials, (3) ruderalized (disturbed) fragments and (4) abandoned fragments in early succession stages without woody species.

efficient was used in order to identify and test the strength of the relationship between the green winged orchid frequency, species richness and diversity of the dry meadow fragments and the selected landscape parameters (Table 1).

Table 1: Relations between green winged orchid frequency, species richness and diversity of the dry meadow fragments and the selected landscape parameters.

	Spearman's coefficient ( <i>o</i> )	p-value
Fragment area (m2)	-0.193	0.200
Altitude (m)	0.240	0.108
Aspect (°)	0.257	0.084
Inclination (°)	-0.193	0.198
Distance to human settlements (m)	-0.155	0.303
Total number of species	-0.193	0.200
Number of habitat specialists	0.011	0.940
Shannon-Weaver index values	-0.194	0.194

The frequency of the species is not significantly in correlation with any of the selected landscape parameters, nor with the species richness or species diversity of the analyzed grassland fragments.

#### Green winged orchid (Anacamptis morio) density

The species reaches the highest densities on regularly mowed, typical fragments of dry meadows (mean 0.48 specimens per m<sup>2</sup>), Figure 2C. The lowest densities were reported on abandoned fragments already overgrown with woody perennials (0.003 specimens per m<sup>2</sup>). On average, 0.042 specimens per m<sup>2</sup> are found on ruderalized (disturbed) fragments and 0.015 specimens per m<sup>2</sup> on abandoned fragments in early succession stages without woody species.

There are no statistically significant differences in the density of the green winged orchid (number of specimens/fragment area) between the four analyzed land use treatment regimens (Kruskal-Wallis sum test,  $\chi 2=2.671$ ; df =3; p= 0.445). Relations between the green winged orchid density, species richness and diversity of the dry meadow fragments and the selected landscape parameters are given in Table 2. Table 2: Relations between green winged orchid density, species richness and diversity of the dry meadow fragments and the selected landscape parameters.

	Spearman's coefficient (ρ)	p-value
Altitude (m)	0.348	0.018*
Aspect (°)	0.236	0.077
Inclination (°)	-0.284	0.056
Distance to human settlements (m)	-0.211	0.158
Total number of species	-0.195	0.195
Number of habitat specialists	0.076	0.617
Shannon-Weaver index values	-0.164	0.275

The density of the green winged orchid shows a statistically significant, positive relation with the altitude of the dry grassland fragments (Table 2).

#### Green winged orchid (*Anacamptis morio*) maximum density

Again, the species reaches the highest maximum densities on regularly mowed, typical fragments of dry meadows (mean 8.42 specimens per m<sup>2</sup>), Figure 2D. The lowest maximum densities were reported on abandoned fragments already overgrown with woody perennials (1.75 specimens per m<sup>2</sup>). On average, 6.09 specimens per m<sup>2</sup> are found on ruderalized (disturbed) fragments and 2.71 specimens per m<sup>2</sup> on abandoned fragments in early succession stages without woody species. There are no statistically significant differences in the maximum density of the green winged orchid (maximal number of specimens per square meter plot) between the four analyzed land use treatment regimens (Kruskal-Wallis sum test,  $\chi 2=5.620$ ; df =3; p= 0.132). Relations between the green winged orchid maximum density, species richness and diversity of the dry meadow fragments and the selected landscape parameters are given in Table 3.

Table 3: Relations between green winged orchid maximum density, species richness and diversity of the dry meadow fragments and the selected landscape parameters.

	Spearman's coefficient (ρ)	p-value
Fragment area (m <sup>2</sup> )	0.143	0.343
Altitude (m)	0.314	0.034*
Aspect (°)	0.197	0.189
Inclination (°)	-0.285	0.055
Distance to human settlements (m)	-0.206	0.169
Total number of species	-0.327	0.026*
Number of habitat specialists	-0.087	0.565
Shannon-Weaver index values	-0.314	0.034*

The maximum density of the green winged orchid shows a statistically significant, positive relation with the altitude of the dry grassland fragments. The maximum density of the species shows a significant, negative relation with the species richness of the dry grassland fragments and also with the species diversity of the grassland fragments (Table 3). The relation between the maximum density of the green winged orchid and the inclination of the dry grassland fragments is also partially evident.

# **4** CONCLUSIONS

Over the past century, grasslands and other semi-natural plant communities in southern and temperate Europe have suffered dramatic decline in their area, owing to land use changes (LUOTO et al. 2003). One of the consequences of the rationalization of agriculture in Europe over the last half-century is the loss and fragmentation of traditionally managed habitats, such as seminatural grasslands (COUSINS 2001, KIVINIEMI & ERIKS-SON 2002). Many researchers have concluded that habitat fragmentation poses an important additional threat to biodiversity (HANSKI 2005), but others claim that fragmentation per se is generally of secondary importance (FAHRIG 2003). One reason why the effect of connectivity (or fragmentation itself) might not be significant is the slow response of populations to environmental changes (HANSKI 2005). The agricultural landscape of NE Goričko is one of the most traditional landscapes in the region. The landscape is still dominated by smallscale fields and grasslands, but overgrowing processes are now dramatically changing their appearance. These dry and semi dry, semi-natural grassland fragments are remnants of once bigger (and connected) areas, and their isolation could prove to be really important in the sense of future conservation efforts.

Anacamptis morio (Orchidaceae), typical of species distributions centered in the Mediterranean, has experienced drastic population extinctions within the last century in Central Europe (JERSAKOVA et al. 2002, JA-CQUEMYN et al. 2005, KULL & HUTCHINGS 2006). In Central Europe, it is now restricted to isolated grassland habitats and serves as a flagship species for conservation (BÖHNERT et al. 1986). No direct, statistically significant differences were found between the four land use treatment regime groups in the orchid's frequency or density (according to results obtained using the Kruskal-Walls sum test). On the other hand, it is quite obvious that both frequency and density of the species decline along with abandonment, succession. Both frequency and density values were significantly higher on managed (regularly mowed and disturbed) fragments than on abandoned (overgrown) ones. The density of the species (number of specimens/fragment area) was found to be in a significant, positive relation with the altitude of the dry grassland fragments. As already mentioned, the managed fragments are located on higher ground than the abandoned ones. The orchid reaches higher frequencies (and densities) on regularly managed, mown fragments of dry grassland and ruderalized fragments (road verges etc.). Both categories can be found in close proximity to human settlements (human infrastructure in general). Historically, settlements (and roads) were built on a higher ground, on the tops of hills and ridges, while the valleys were wetland. The maximum densities (maximum number of orchid specimens per square meter plot) were also found to be positively correlated with the altitude. Population size has been shown to be a good predictor of plant fitness and population viability, (LEIMU et al. 2006) and maximum species density (in our case the maximum number of orchid specimens per square meter plot on each dry meadow fragment) can be a measure of proper habitat conditions on a micro scale. The maximum species density is in significant negative correlation with the total number of species per dry grassland fragment. As already mentioned, the fragments richest in species are those in succession and not the regularly managed ones. Long-lived perennials (such as Anacamptis morio) are expected to have a higher probability of showing time-delayed extinction, compared with short-lived species (MORRIS et al. 2008). Although the species is long-lived, it disappears from such habitats sooner or later. Again, the maximum density of the species is strongly related to the management regime. The maximum density of the species is in significant negative correlation with the species diversity (Shannon-Weaver diversity index rates) of these dry grassland fragments. The fragments with the highest diversity rates are those in succession, and not the regularly mowed ones. Half of the local population of the green winged orchid was found on ruderalized, disturbed habitats (23 grassland fragments, 1908 specimens). Those fragments are regularly mowed but often not at the proper time. They are often disturbed by constant trampling. Anacamptis morio produces its leaves in early October (occasionally even in September). The green winged orchid starts flowering relatively early, in the first half of April. It quickly produces seed and stays dormant until the following spring. The leaves remain green and functional throughout the winter and spring, and die down in mid-June after the plants have flowered (WELLS et al. 1998). According to the life cycle (strategy) of the species, human disturbance (regular

mowing and trampling) in the case of road verges and other disturbed dry grassland fragments does not represent a serious threat to the local green winged orchid populations. It seems that these alternative habitats could become important refugia for the orchid in the future, since the regularly mowed dry grasslands are disappearing quickly, owing to abandonment or conversion into arable land. The species is often found in the close vicinity of graveyards, playgrounds, churches etc. The land use treatment regime (management or abandonment) and the altitude (probably also related to the spatial distribution of the settlements and roads across the research area) are the important parameters that can explain the recent spatial distribution and abundance of the green winged orchid (density and also frequency) within the research area of NE Goričko Nature Park. The slow response of the green winged orchid population to habitat loss and fragmentation has important implications for conservation. Since ruderalized (disturbed) dry meadow fragments seem to be an important alternative habitat for the green winged orchid (and also for other dry grassland specialists), it is important to develop a proper strategy for their management in the future. On the other hand, it is of great importance to maintain appropriate management (mowing at least twice a year) of the remaining regularly mowed dry grassland fragments in the area. We can conclude that management (regular mowing at the right time, and preserving the remaining fragments in the current trophic conditions), is the only real factor that affects- indeed, determines the recent spatial distribution of the green winged orchid on fragmented dry grassland in Goričko Nature Park and that will continue to do so in the future.

# **5 POVZETEK**

V prispevku obravnavamo vpliv režima rabe tal ter izbranih krajinskih parametrov (površina fragmentov suhih travišč, nadmorska višina, ekspozicija, naklon in oddaljenost od naselij) na frekvenco (število cvetočih primerkov) pojavljanja navadne kukavica (*Anacamptis morio*), gostoto in maksimalno gostoto pojavljanja vrste na fragmentih suhih travišč v SV delu Krajinskega parka Goričko. Prav tako nas je zanimalo ali obstajajo povezave med številom prisotnih rastlinskih vrst na traviščih, številom tipičnih vrst (habitatni specialisti) ter vrstno pestrostjo fragmentov suhih travišč (izraženo z vrednostmi indeksa Shannon-Weaver) in frekvenco vrste *Anacamptis morio* ter gostoto pojavljanja vrste na fragmentih suhih travišč. Na območju raziskave v SV delu Krajinskega parka Goričko smo locirali 80 fragmentov suhih, pol–naravnih travišč iz asociacij *Hypocherido-Festucetum rupicolae* Steinbuch 1980 in *Ranunculo bulbosi-Arrhenatheretum elatioris* Ellmauer in Mucina & al. 1993 (Slika 1). Potrdili smo prisotnost vrste *Anacamptis morio* na 46 fragmentih s skupno 3812 cvetočimi primerki. Glede režima rabe tal smo travišča razdelil v štiri kategorije: (1) fragmenti redno košenih travišč (N=12) s skupno 1550 cvetočimi primerki navadne kukavice, (2) fragmenti v fazi sukcesije z drevesno grmovnimi vrstami (N=4) s skupno 35 cvetočimi primerki, (3) motena (ruderalizirana) travišča (N=23) s skupno 1908 cvetočimi primerki in (4) opuščeni fragmenti suhih travišč v zgodnjih fazah sukcesije z zelnatimi trajnicami in enoletnicami (N=7) s skupno 319 cvetočimi primerki navadne kukavice. Dokazali smo statistično značilne razlike med štirimi kategorijami travišč glede števila prisotnih rastlinskih vrst (test Kruskal-Wallis, χ2= 25. 395; df = 3; p = 0.000). Rezultat testa Mann-Whitney dokazuje razlike v številu prisotnih vrst med kategorijami 1 in 4, 2 in 4 ter 3 in 4 (p < 0.05). V povprečju dosega vrsta Anacamptis morio največje frekvence (število cvetočih primerkov) na redno košenih (povprečje znaša 129.2 primerka na fragment travišča) in ruderaliziranih fragmentih, najnižje pa na fragmentih v fazah sukcesije (kategoriji 2 in 4), Slika 2B. Med štirimi kategorijami travišč glede režima rabe tal ni statistično značilnih razlik v frekvenci pojavljanja navadne kukavice (Kruskal-Wallis test,  $\chi$ 2=3.328; df= 3; p= 0.349). Vrsta dosega največje gostote (število vseh primerkov / površina fragmenta suhega travišča) na redno košenih traviščih (povprečje znaša 0.48 primerka na m<sup>2</sup>), najmanjše pa na opuščenih fragmentih v fazi sukcesije z drevesno grmovnimi vrstami (povprečje znaša 0.003 primerka na m<sup>2</sup>), Slika 2C. Med štirimi kategorijami travišč glede režima rabe tal ni statistično značilnih razlik v gostoti pojavljanja navadne kukavice (Kruskal-Wallis test,  $\chi 2=2.671$ ; df =3; p= 0.445). Gostota pojavljanja vrste je v statistično značilni, pozitivni zvezi z nadmorsko višino fragmentov suhih travišč (ρ=0.348, p=0.018). Zanimala nas je maksimalna gostota vrste. Odziv populacije na mikro relief, razlike v pedologiji ter razpoložljivosti hranil in prisotnosti mikoriznega simbionta se kažejo kot strnjene gruče primerkov. Maksimalno gostoto navadne kukavice smo merili s pomočjo kvadrata površine 1m<sup>2</sup> Postopek smo na vsakem fragmentu travišča ponovili deset krat. Največje število primerkov v kvadratu je predstavljalo največjo gostoto vrste na posameznem fragmentu suhega travišča. Vrsta dosega največje maksimalne gostote na redno košenih fragmentih suhih travišč (povprečje znaša 8.42 primerka na m<sup>2</sup>), najmanjše pa na fragmentih v fazi sukcesije z drevesno grmovnimi vrstami (v povprečju 1.75 primerka na m<sup>2</sup>), Slika 2D. Maksimalna gostota pojavljanja vrste je v statistično značilni, pozitivni zvezi z nadmorsko višino fragmentov suhih travišč (p=0.314, p=0.034). Maksimalna gostota pojavljanja vrste je v statistično značilni, negativni zvezi s številom vrst na fragmentih suhih travišč (p=-0.327, p=0.026) in tudi v statistično značilni, negativni zvezi z vrstno pestrostjo fragmentov suhih travišč ( $\rho$ = -0.314, p=0.034). Ugotavljamo, da frekvenca (število primerkov) kot tudi gostota navadne kukavice (povprečna in maksimalna) upadata na fragmentih v fazi zaraščanja (sukcesije). Dokazali smo statistično značilno, pozitivno zvezo med povprečno gostoto (in tudi maksimalno gostoto) vrste na fragmentih travišč

in povprečno nadmorsko višino fragmentov travišč. V povprečju se redno košeni fragmenti nahajajo na višjih nadmorskih višinah kot tisti v fazah sukcesije. Navadna kukavica dosega višje gostote populacij (in frekvence) na redno košenih in motenih fragmentih (dvorišča, pasovi travišč ob cestah, pokopališča, travišča v neposredni bližini sakralnih objektov). Taki fragmenti se nahajajo v bližini naselij, hiš, infrastrukture nasploh. Na obravnavanem območju se razložena naselja nahajajo na temenih hrbtov, gričev. V dolinah pa prevladujejo higromezofilni travniki. Tudi maksimalna gostota vrste je v statistično značilni pozitivni zvezi z nadmorsko višino fragmentov travišč. Maksimalna gostota vrste je v našem primeru merilo viabilnosti populacij, ugodnega stanja habitata na mikro nivoju. Vrstno najbogatejša so namreč travišča v fazah sukcesije in ne redno košena travišča (Slika 2A). Dolgožive trajnice, kot je navadna kukavica izginjajo iz habitatov z zakasnitvijo po pojavu motnje (faze zaraščanja) v primerjavi z enoletnicami, ki iz habitata, združbe izginejo relativno hitro (MORRIS et al. 2008). Tudi maksimalna gostota vrste je tesno povezana z načinom rabe tal, upravljanja s fragmenti suhih travišč. Tudi indeks vrstne pestrosti je na fragmentih v sukcesiji (vrednosti indeksa Shannon-Weaver) višji kot pa na redno košenih fragmentih travišč. Polovica populacije navadne kukavice na območju raziskave uspeva na motenih fragmentih (23 fragmentov, 1908 cvetočih primerkov). Taki fragmenti so običajno prekomerno košeni in izpostavljeni teptanju (kmetijska mehanizacija, avtomobilski promet itd.). Navadna kukavica razvija liste pritlične rozete že oktobra (lahko tudi že v septembru). Listi ostanejo funkcionalni preko zime ter odmrejo nekako do sredine junija, po cvetenju. Na Goričkem prične navadna kukavica s cvetenjem v prvi polovici aprila, posamezne primerke pa lahko opazujemo še v maju. Rastlina nato hitro razvije semena. Glede na omenjeno strategijo vrste lahko sklepamo, da motnje kot sta pogosta košnja v poletnem času ter teptanje ne predstavljajo grožnje vrsti na ruderaliziranih, motenih habitatih (v kolikor na gre za dodaten vnos hranil). Ruderalizirani (moteni) habitati bodo morda, ob sočasnem upadanju števila redno košenih fragmentov travišč, pomenili refugij, nadomestni habitat vrste v prihodnje. Strnemo lahko da je režim upravljanja s travišči (redno vzdrževanje v smislu košnje ter odvoza pokošene biomase) ter sekundarno tudi nadmorska višina fragmentov, v povezavi z oddaljenostjo od naselij ključni dejavnik, ki določa (razlaga) današnji vzorec razširjenosti vrste na SV Goričkem, gostoto in frekvenco. Povprečna površina fragmentov, naklon in ekspozicija pa statistično značilno ne pojasnjujejo frekvence in gostote pojavljanja navadne kukavice v Krajinskem parku Goričko.

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