

Babeş-Bolyai University

Faculty of Biology and Geology

Integrative Biology Doctoral School

PHD THESIS

**Population study on *Maculinea arion* (Lepidoptera: Lycaenidae)  
in Transylvania**

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## **Keywords**

grazing, habitat quality, host ants, host plant, mark-recapture population demography, mobility,

*Myrmica*, shrubs, vegetation heterogeneity

## 1. Introduction

### 1.1. The importance of population studies in case of butterflies

Recent habitat loss and degradation due to land-use changes led to the decline of both the distribution and population size of numerous butterfly species in Europe (Thomas et al. 2004; Van Swaay et al. 2010). Nine percent of the European butterflies are threatened and about one third of them have declining populations (Van Swaay et al. 2010). Conservation actions can improve the status of butterflies (e.g. Davies et al. 2005), however, any conservation effort will fail if the necessary information on the ecology of target species is not available (Thomas et al. 2009, 2011). Beside habitat use and resource use of both caterpillars and adult butterflies, demographic parameters and local population dynamics are also essential information to predict the viability of populations (Baguette and Schtickzelle 2003; Schtickzelle et al. 2005). Adult lifespan, sex ratio and asynchrony between male and female emergence can profoundly affect the reproductive success of butterflies and thus population viability (Calabrese and Fagan 2004; Calabrese et al. 2008). These are especially important in those species that do not form classical metapopulations (Baguette 2004), i.e. when occupied sites are loosely connected via dispersal, or when butterflies occupy most of the suitable habitat patches and extinction-colonization dynamics are weak, such as *Maculinea* butterflies (Nowicki et al. 2007).

Butterflies from the genus *Maculinea* Van Eecke, 1915<sup>1</sup> (synonymized with *Phengaris* Doherty, 1891) are among the most endangered butterfly species (Munguira and Martin 1999) and

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1 We follow the recommendation of Balletto et al. (2010) on the generic names *Maculinea* Van Eecke, 1915 versus *Phengaris* Doherty, 1891.

are in the European Red List (Van Swaay et al. 2010). Their populations are decreasing in many European countries due to habitat loss and fragmentation caused by agricultural intensification, land abandonment or improper habitat management (Van Swaay and Warren 1999; Van Swaay et al. 2010). The unique obligate myrmecophilous life-cycle of *Maculinea* butterflies (Thomas 1995; Thomas et al. 1998c) has also attracted much scientific interest (see Witek et al. 2014 for a review). The complex life history of these butterflies requires the presence of particular host plant and host ant species during their development (Thomas et al. 1998c). *Maculinea* butterflies are flagship species in nature conservation, because their quick response to changes in habitat quality instantly indicates habitat deterioration, and they are also regarded as umbrella species occupying habitats of high biodiversity value (Settele et al. 2005; Spitzer et al. 2009).

## **1.2. Vegetation heterogeneity and meadow management**

European semi-natural grasslands are largely the result of human activity. Traditional ways of grassland management such as low-intensity mowing and/or grazing led to high plant (Moog et al. 2002; Babai and Molnár 2014; Szépligeti et al. 2016) and invertebrate (Schmitt and Rákossy 2007) species richness, and unique habitat types which have developed over hundreds of years in response to the local climate, soil, geology and management methods (Baur et al. 1996; Cremene et al. 2005; Bullock et al. 2011). Due to recent land-use changes in Europe, large areas of semi-natural grasslands have been lost and/or fragmented and the process is still going on (see Bubová et al. 2015 for a review). On the one hand, intensification in agricultural management is a major driver of the loss of semi-natural grasslands, but on the other hand, the total abandonment of former extensive use causes also a decline of species richness in these grasslands (Stoate et al.

2009; Bullock et al. 2011; Bubová et al. 2015; Szépligeti et al. 2016). Intensive management goes hand in hand with high inputs of inorganic fertilizers and intensive cutting or grazing. In turn, land abandonment gives way to successional processes such as encroachment by shrubs and trees which gradually overgrow meadow vegetation (Bullock et al. 2011), and expropriate space and light from less competitive species (Jutila and Grace 2002).

Many species inhabiting semi-natural grasslands are highly sensitive to land-use changes, abandonment in particular. The species associated with these habitats are likely to decline after abandonment, and experience local extinctions in longer time perspective.

## **2. Aims of study**

Our focal organism is a flagship species of grassland biodiversity conservation in Europe (Settele et al. 2005). Although numerous studies have been published on the ecology and evolution of *Maculinea arion*, there is still a lack of information on its populations' demography except a few case studies (Pajari 1992; Pauler et al. 1995). Nevertheless, despite its severe declines in recent decades, including extinctions in some regions (e.g. Thomas 1980; Van Swaay et al. 2010), the consequences of succession and shrub encroachment on its populations have not been studied so far in Central and Eastern Europe (Thomas et al. 1998b, 2009).

In light of this, our aim was to study the demography of a *M. arion* population by estimating demographic parameters such as survival, abundance and sex ratio. Moreover, in our study, we investigated the impact of meadow succession on the endangered *M. arion* (Linnaeus,

1758), and especially on the availability of its crucial resources, i.e. host ants and host plant, as well as on the mobility of adult butterflies within their habitat.

### 3. Materials and methods

#### 3.1. Study site

The study was carried out on a 3 ha, northwestern exposed meadow located near Dej (47°7' N, 23°51' E, 345-380 m a.s.l., Cluj County, Romania). The meadow consisted of a drier and a wetter zone, and it was grazed by sheep and goat (Fig. 1).



**Figure 1.** a) Habitat of study species at Dej; b) Host plant *Origanum vulgare* (Photo: Osváth-Ferencz M.)



### 3.2. Field surveys

*Maculinea arion* adults were surveyed with mark-release-recapture (MRR) method in two subsequent years (2014 and 2015) (Fig. 2). Sampling effort varied slightly among the two years (see Table 1), but the sampling always followed the same protocol.

**Table 1.** Summary data of sampling

Year	Sampling occasions	Start	End	Persons	Hours/day
2014	13	28.06	28.07	1	6
2015	24	29.06	30.07	2-3	6



**Figure 2.** Marked *Maculinea arion* individual (Photo: Osváth-Ferencz M.)

In order to investigate the effect of succession on the host plant *Origanum vulgare*, *Myrmica* host ants and mobility of adult butterflies of the summer form of *M. arion*, we surveyed general vegetation features of the study site. For host plant sampling, 25-25 quadrats of 4 m × 4 m were randomly placed out in 2014 and 2015. Within these plots, we estimated different vegetation characteristics. The ant community was sampled in the same 25-25 quadrats with two complementary methods, pitfall traps and ant nest searching.

## 4. Results

### 4.1. Population demography of *Maculinea arion* in two subsequent years

The numbers of marked butterflies and proportion of recaptured individuals across the investigated seasons are shown in Table 2.

**Table 2.** Summary data of datasets

Year	Marked butterflies		Prop. of recaptured individuals		Mean (and max.) no. of days between first and last captures	
	Males	Females	Males	Females	Males	Females
2014	67	98	0.40	0.17	3.68 (7)	3.94 (11)
2015	47	62	0.47	0.39	2.86 (7)	2.42 (8)

We revealed considerable differences between male and female survival and recapture rate in both years (Table 3). The best JS models also varied among the datasets (Table 4).

**Table 3.** The best CJS models for each dataset after model selection.  $\phi$ : apparent daily survival rate,  $p$ : recapture probability. (.) denotes that the given parameter was constant, npar: number of parameters estimated by the model. 'Time\_mon' indicates that the given parameter changed monotonously with time.

Year	Sex	Model name	npar	AICc weight
2014	male	$\phi(\text{Cohort})p(\text{Age})$	4	0.675
	female	$\phi(.)p(.)$	2	0.163
2015	male	$\phi(\text{Time\_mon})p(.)$	3	0.216
	female	$\phi(\text{Cohort})p(\text{Cohort})$	4	0.217

**Table 4.** The best JS models and their estimates of population size for each dataset. N: size of the seasonal population  $\pm$ SE (95% confidence intervals: lci – lower confidence interval, uci – upper confidence interval), gross N: estimated number of all individuals that were present in the population during the whole sampling period  $\pm$ SE (95% confidence intervals: lci – lower confidence interval, uci – upper confidence interval). 'Time' is an abbreviation for 'Time\_mon' which indicates that the given parameter changed monotonously with time.

Year	Sex	Model name	npar	AICc	N±SE (lci–uci)	gross N±SE (lci–uci)
2014	♂	$\phi(\text{Time})p(\text{Time})\text{pent}(\text{Time})N(.)$	7	0.511	73.9 ± 5.71 (68.7–95.5)	113.2 ± 10.07 (93.5–132.9)
	♀	$\phi(.)p(\text{time})\text{pent}(. )N(.)$	16	0.59	335 ± 329.5 (129–1908)	439 ± 321 (-190.4–1068.3)
2015	♂	$\phi(\text{Time})p(. )\text{pent}(\text{Time})N(.)$	6	0.442	54.4 ± 4.34 (49.5–68.5)	72.2 ± 6.95 (59.8–87.1)
	♀	$\phi(\text{Time})p(\text{Time})\text{pent}(\text{Time})N(.)$	7	0.538	108 ± 19.6 (82.6–164.6)	148.5 ± 23.1 (103.2–193.8)

#### 4.2. The effects of meadow succession on the endangered butterfly *Maculinea arion* and its resources

Altogether 16 ant species from six genera were recorded on the study site, four of which belonged to the genus *Myrmica*. Most species were observed both in pitfall traps and during nest counts. The most abundant among potential host ant species was *Myrmica scabrinodis* Nylander, 1846. Furthermore, *M. scabrinodis* proved to be the most evenly distributed between all *Myrmica* species.

Bare ground cover was significantly negatively related to the diversity of ant species active on the ground, but neither to the number of ant species nor to the abundance of foraging ants. The number of *Myrmica* nests was inversely related with the turf height. Host plant cover was significantly negatively related to shrub cover and positively to turf height.

The numbers of the recorded movements between different sectors of the investigated site reached 57 in 2014 and 74 in 2015. In both years of the study the inter-sector movement probabilities were significantly higher between sectors not separated from each other by shrubs.

## 5. General conclusions

- a) We did not find significant differences between apparent daily survival of males and females, though apparent survival depended on different covariates for the two sexes.
- b) Recapture probability highly varied among the sexes and years. If there was a significant difference between sexes then male recapture probability was higher. The high variation in recapture probabilities within and between years suggests that this parameter was likely affected by many factors (such as weather, sampling intensity) that hampers the detection of any general pattern.
- c) Seasonal population size of females was usually higher than that of males. Concerning the sex ratio we found large biases and it meant a female-biased sex ratio in both years.
- d) We found strong protandry in both sampling years. Protandry often explained as a reproductive strategy of males in the competition for mating females.
- e) In case of a low sampling frequency in a small population, the occurrence of such encounter histories can be very low. Therefore our recommendation is to conduct sampling in MRR studies of *M. arion* optimally every day if weather permits, but at least every second day.
- f) We found that the vegetational changes associated with meadow succession negatively influenced the availability of the vital resources for the endangered *M. arion* butterfly. Specifically, the increasing turf height had an inverse effect on host ant abundance, whereas host plant availability was negatively affected by shrub encroachment.
- g) Shrub belts restricted the movements of *M. arion* within the investigated site. Although currently the subpopulations inhabiting different fragments of the site are not really

separated from one another, since the exchange of individuals among them is still substantial, it is likely that further expansion of shrubs will lead to their partial isolation.

- h) The *M. arion* population in Dej is the largest known in Romania and hence its preservation is of high importance. While at present the negative impacts of succession on this population that we documented in the present study do not appear to threaten its persistence, the situation is likely to deteriorate in the future, and thus management actions aimed at halting the successional processes are clearly needed.

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## List of scientific publications

### Publications

1. Osváth-Ferencz, M., Kőrösi, Á., Czekes, Zs., Rákósy, L., Nowicki, P. (2017) The effects of meadow succession on the endangered butterfly *Maculinea arion* and its resources. *Insect Conservation and Diveristy*, submitted.
2. Osváth-Ferencz, M., Bonelli, S., Nowicki, P., Peregovits, L., Rákósy, L., Sielezniew, M., Kostro-Ambroziak, A., Dziekańska, I., Kőrösi, Á. (2016) Population demography of the endangered large blue butterfly *Maculinea arion* in Europe. *Journal of Insect Conservation*, DOI: 10.1007/s10841-016-9944-0. **IF: 1.431**
3. Osváth-Ferencz, M., Czekes, Zs., Molnár, Gy., Markó, B., Vizauer, T.Cs., Rákósy, L., Nowicki, P. (2016) Adult population ecology and egg laying strategy in the 'cruciata' ecotype of the endangered butterfly *Maculinea alcon* (Lepidoptera: Lycaenidae). *Journal of Insect Conservation*, 20: 255-264. **IF: 1.431**
4. Osváth-Ferencz, M. (2016) Bérlok, háziurak és szomszédok: populációvizsgálat erdélyi módra. In *Intelligens háló 2016 – Határon túli fiatal kutatók tanulmányai*, Edutus Főiskola, Tatabánya, Hungary, pp. 135-141.
5. Loos, J., Horcea-Milcu, A.I., Kirkland, P., Hartel, T., Osváth-Ferencz, M., Fischer, J. (2015) Challenges for biodiversity monitoring using citizen science in transitioning social-ecological systems. *Journal for Nature Conservation*, 26: 45-48. **IF: 1.646**
6. Czekes, Zs., Markó, B., Nash, D., Osváth-Ferencz, M., Lázár, B., Rákósy, L. (2014) Differences in oviposition strategies between two ecotypes of the endangered myrmecophilous butterfly



*Maculinea alcon* (Lepidoptera: Lycaenidae) under unique syntopic conditions. *Insect Conservation and Diversity*, 7: 122-131. **IF: 2.174**

### **Participation in international conferences**

1. Osváth-Ferencz, M., Kőrösi, Á., Czekes, Zs., Rákósy, L., Nowicki, P.: The effects of vegetation heterogeneity on the host ants of the endangered butterfly *Maculinea arion*. *7th Central European Workshop of Myrmecology*, 21-24 April 2017, Kraków, Poland (poster)
2. Markó, B., Csata, E., Keresztes, K-K., Osváth-Ferencz, M.: Reproductive and behavioural correlates of dominance hierarchies in queens in the facultative polygynous ant *Myrmica scabrinodis*. *Euro IUSSI - Meeting of the European Sections of the International Union for the Study of Social Insects*, 8-11 August 2016, Helsinki, Finland (talk)
3. Osváth-Ferencz, M., Czekes, Zs., Onodi, H., Molnár, Gy., Markó, B., Nowicki, P., Rákósy, L., Kőrösi, Á.: From butterflies to ants: a population study of *Maculinea arion* (Lepidoptera: Lycaenidae) in Romania. *International Symposium: Future 4 Butterflies in Europe*. 31 March-2 April 2016, Wageningen, The Netherlands (talk)
4. Osváth-Ferencz, M., Czekes, Zs., Markó, B., Rákósy, L.: Ant community structure of a *Maculinea arion* (Lepidoptera: Lycaenidae) population's habitat. *IV. Central European Meeting of IUSSI*, 26-29 March 2015, Lichtenfels, Germany (poster)
5. Ferencz, M., Czekes, Zs., Nowicki, P., Molnár, Gy., Vizauer, T.Cs.: Vital signs in Transylvania: population structure of *Maculinea alcon* 'xerophila' (Lepidoptera: Lycaenidae) by mark-recapture method. *7th International Symposium, The Ecology and Conservation of Butterflies and Moths*, 4-6 April 2014, Southampton, United Kingdom (poster)

6. Loos, J., Ferencz, M., Kirkland, P.: Butterfly Monitoring Romania – two steps ahead. *7<sup>th</sup> International Symposium, The Ecology and Conservation of Butterflies and Moths*, 4-6 April 2014, Southampton, United Kingdom (poster)
7. Loos, J., Wiemers, M., Ferencz, M., Kirkland, P.: Tagfalter Monitoring in Rumänien. *Symposium für Schmetterlingsschutz und 16. UFZ-Workshop zur Populationsbiologie von Tagfaltern & Widderchen*, 6-7 March 2014, Helmholtz-Zentrum für Umweltforschung - UFZ, Leipzig KUBUS, Germany (talk)
8. Czekes, Zs., Molnár, Gy., Ferencz, M., Markó, B.: Ant communities of *Maculinea alcon* and *M. 'rebeli'* populations' habitats in Transylvania, Romania. *The 3<sup>rd</sup> Central European Section Meeting of the IUSSI*, 14-18 March 2013, Cluj-Napoca, Romania (talk)
9. Czekes, Zs., Molnár, Gy., Ferencz, M., Lázár, B., Markó, B.: Ant community structure of syntopic populations of *Maculinea alcon* and *Maculinea 'rebeli'* (Lepidoptera: Lycaenidae). *5th Congress of the European Sections of the International Union for the Study of Social Insects*, 26-30 August 2012, Montecatini Terme, Italia (poster)
10. Czekes, Zs., Ferencz, M., Lázár, B., Molnár, Gy., Markó, B.: Differences and similarities in the egg laying preference of *Phengaris alcon* and *Phengaris 'rebeli'* (Lepidoptera: Lycaenidae) in a sympatric population. *2nd Central European Meeting of the IUSSI*, 25-28 March 2011, Papenburg, Germany (poster)

### **Participation in national conferences**

1. Osváth-Ferencz, M., Onodi, H., Molnár, Gy., Czekes, Zs., Markó, B., Rákossy, L., Nowicki, P., Kőrösi, Á.: Populációvizsgálat Erdélyben: mi hír a nagyfoltú hangyaboglárkáról (Lepidoptera:

- Lycaenidae)? *2<sup>nd</sup> Lepideptorological Meeting in Hungary*, 7-10 July 2016, Szögliget, Hungary (talk)
2. Osváth-Ferencz, M., Onodi, H., Molnár, Gy., Rákósy, L., Nowicki, P., Körösi, Á.: Lepkeszámlálás Erdélyben: mi hír a nagyfoltú hangyaboglárkáról (Lepidoptera: Lycaenidae)? *17. Biology Days*, 8-9 April 2016, Cluj-Napoca, Romania (talk)
  3. Osváth-Ferencz, M., Czekes, Zs., Markó, B., Körösi, Á., Rákósy, L.: Lepkéktől a hangyákig: a *Maculinea arion* (Lepidoptera: Lycaenidae) egy romániai populációjának vizsgálata. *16. Biology Days*, 16-18 April 2015, Cluj-Napoca, Romania (talk)
  4. Ferencz, M.: Egy védett lepkefaj, a *Maculinea alcon* 'xerophila' (Lepidoptera: Lycaenidae) torockói populációjának felmérése. *XXXII. National Conference of Scientific Students' Associations*, 8-10 April 2015, Pécs, Hungary (talk)
  5. Osváth-Ferencz, M., Körösi, Á., Rákósy, L.: Egy nagyfoltú hangyaboglárka populáció (Lepidoptera: Lycaenidae) szerkezetének vizsgálata Erdélyben. *16th Congress of the Hungarian Ethological Society*, Tihany, 28-30 November 2014, Tihany, Hungary (poster)
  6. Osváth-Ferencz, M., Körösi, Á., Rákósy, L.: Szereti vagy nem szereti? Mennyire igényes a nagyfoltú hangyaboglárka dési populációja? *IX. Hungarian Conference of Conservation Biology*, 20-23 November 2014, Szeged, Hungary (poster)
  7. Osváth-Ferencz, M., Loos, J., Kirkland, P.: Merre tart Romániában a lepke-monitoring? *IX. Hungarian Conference of Conservation Biology*, 20-23 November 2014, Szeged, Hungary (poster)
  8. Ferencz, M., Molnár, Gy., Czekes, Zs., Vizauer, T.Cs., Nowicki, P.: Egy erdélyi *Maculinea alcon* 'xerophila' populáció szerkezetének vizsgálata jelölés-visszafogás módszerével. *15th*

*Congress of the Hungarian Ethological Society*, 29 November-1 December 2013, Budapest, Hungary (talk)

9. Ferencz, M., Molnár, Gy., Czekes, Zs., Vizauer, T.Cs.: Dinamica și structura unei populații de *Maculinea alcon 'xerophila'* (Lepidoptera: Lycaenidae) din Ardeal. *A doua circulară a simpozionului BIOTA: BIOdiversitate: Tradiții și Actualitate*, 8-9 November 2013, Biology and Geology Faculty, Babeș-Bolyai University, Cluj-Napoca, Romania (poster)
10. Ferencz, M.: Egy védett lepkefaj, a *Maculinea alcon 'xerophila'* (Lepidoptera: Lycaenidae) torockói populációjának felmérése. *XVI. Transylvanian Students' Scientific Conference*, 23-26 May 2013, Cluj-Napoca, Romania (talk)
11. Molnár, Gy., Ferencz, M., Czekes, Zs., Markó, B.: A hangyaközösség szerkezetének vizsgálata a szürkés hangyaboglárka (*Maculinea alcon xerophila*) szintopikus és nem szintopikus populációinak élőhelyén. *14. Biology Days*, 12-14 April 2013, Cluj-Napoca, Romania (talk)
12. Ferencz, M., Molnár, Gy., Czekes, Zs., Vizauer, T.Cs.: Egy védett lepkefaj, a *Maculinea alcon 'xerophila'* (Lepidoptera: Lycaenidae) torockói populációjának felmérése. *14. Biology Days*, 12-14 April 2013, Cluj-Napoca, Romania (talk)
13. Czekes, Zs., Ferencz, M., Molnár, Gy., Markó, B.: Eltérő peterakási stratégia a szürkés (*Maculinea alcon alcon*) és karszti hangyaboglárkánál (*Maculinea alcon xerophila*) (Lepidoptera: Lycaenidae). *14. Biology Days*, 12-14 April 2013, Cluj-Napoca, Romania (talk)
14. Molnár, Gy., Ferencz, M., Czekes, Zs., Markó, B.: Hangyagazdafajok elterjedése és a hangyaközösség szerkezete szimpatrikusan élő *Phengaris alcon* és *P. 'rebeli'* populációk élőhelyén. 9. Magyar Ökológus Kongresszus, 5-7 September 2012, Keszthely, Hungary (poster)

15. Ferencz, M., Lázár, B., Molnár, Gy., Czekes, Zs., Markó, B.: A *Maculineaalconalcon* és *Maculineaalcon 'rebeli'* (Lepidoptera: Lycaenidae) tápnövény-használata közötti különbségek szintopikus populációk esetén. 4. *Myrmecological Symposium in the Carpathian Basin*. 30 July-3 August 2012, Kisnamény, Hungary (talk)
16. Molnár, Gy., Ferencz, M., Czekes, Zs., Markó, B.: A *Maculineaalcon* és *M. 'rebeli'* hangyagazda-fajainak elterjedése és a hangyaközösség szerkezete szintopikus populációk élőhelyén. 4. *Myrmecological Symposium in the Carpathian Basin*. 30 July-3 August 2012, Kisnamény, Hungary (talk)
17. Czekes, Zs., Markó, B., Ferencz, M., Lázár, B., Molnár, Gy., Nash, D.R., Rákossy, L.: The effect of host plant characteristics and ant community structure on egg laying patterns in sympatric populations of *Maculineaalconalcon* and *M.alcon 'rebeli'*. 3. *Myrmecological Symposium in the Carpathian Basin*, 15-19 September 2011, Cluj-Napoca, Romania (talk)
18. Czekes, Zs., Ferencz, M., Lázár, B., Molnár, Gy., Markó, B.: Strategii de ovipozitare la populații simpatrice de *Phengarisalcon* și „*rebeli*”. 21. *National Symposium of Romanian Lepidopterological Society*, 16-17 April 2011, Cluj-Napoca, Romania (talk)
19. Ferencz, M., Lázár, B., Molnár, Gy., Czekes, Zs., Markó, B.: Differences in egg laying strategies of *Phengarisalcon* and *Ph. 'rebeli'* (Lepidoptera: Lycaenidae) in sympatric populations. 12. *Biology Days*, 8-10 April 2011, Cluj-Napoca, Romania (talk)

## Prizes

1. Ferencz, M.: Egy védett lepkefaj, a *Maculineaalcon 'xerophila'* (Lepidoptera: Lycaenidae) torockói populációjának felmérése. XXXII. *National Conference of Scientific Students'*

*Associations*, 8-10 April 2015, Pécs, Hungary (talk) – I. Prize

2. Ferencz, M.: Egy védett lepkefaj, a *Maculineaalcon 'xerophila'* (Lepidoptera: Lycaenidae) torockói populációjának felmérése. *XVI. Transylvanian Students' Scientific Conference*, 23-26 May 2013, Cluj-Napoca, Romania (talk) – III. Prize