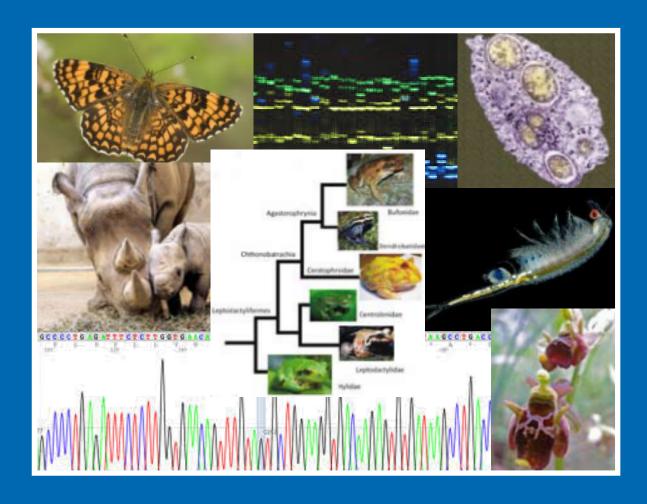
# "When phylogeny and geography meet conservation"



Debrecen 2014 August 29-30

Debreceni Egyetem Élettudományi Épület F.015-ös terem









#### **PROGRAM OF THE SYMPOSIUM**

#### 08 29 Friday

#### Morning session I

8.30-9.30	Registration
9.30-9.40	Ákos Pintér Dean of the Faculty of Science and Technology
9.40-9.50	Zoltán Barta Head of the Department of Evolutionary Zoology
9.50-10.25	Michael Bruford: Phylogeography of Large African Mammals: are we near to a continent-wide paradigm?

10.25-11.20 Coffee break

#### Morning session II

- 11.20-11.55 Rudi Verovnik: Asellus aquaticus model organism for study of phylogeography, ecological speciation and parallel evolution.
- 11.55-12.15 Krisztián Szabó: Natal dispersal of eastern imperial eagle (Aquila heliaca) in the Carpathian Basin.
- 12.15-12.35 Zsolt Végvári: Temporal patterns in diversity, population size and voltinism in Noctuid moths (Noctuidae).
- 12.35-12.55 Gábor Sramkó: A preliminary look into the genetic diversity of Carpatho-Pannonian steppic species poor westernmost outpost of the Eurasian steppes?

13.00-14.00 Lunch break

#### Afternoon session I

- 14.00-14.35 Lajos Rózsa: Shifting bushmeat consumption and the rise of Armillifer grandis (Crustacea: Pentastomida) infections in the Democratic Republic of Congo.
- 14.35-14.55 Judit Vörös: Conservation genetics of the olm (Proteus anguinus) in Croatia.
- 14.55-15.15 Péter Takács: Population genetic patterns of a vulnerable, stagnophilic fish species (Umbra krameri Walbaum. 1792) in Hungary.
- 15.15-16.20 Coffee break

#### Afternoon session II

- 16.20-16.55 Emese Meglécz: Microsatellite evolution at a genome scale and implications on genetic marker development.
- 16.55-17.15 Edina Nemesházi: Individual identification of white-tailed eagles, based on microsatellite loci.
- 17.15-17.35 András Kosztolányi: Breeding behaviour in a biparental beetle.
- 17.35-17.55 Rita Rácz: Microsatellite markers in the survey of Lethrus apterus

#### 08 30 Saturday

#### Morning session I

- 9.00-9.35 Niklas Wahlberg: The wings of butterflies: active movements through time and space.
- 9.35-9.55 János P. Tóth: How improvement of scientific methods could change our thinking on the same question? A historical over-
- 9.55-10.15 Judit Bereczki: The pattern of Wolbachia infestation in the social parasitic Maculinea butterflies (Lepidoptera: Lycaenidae)
- 10.15-11.00 Coffee break

#### Morning session II

- 11.00-11.35 Gabriel Neve: Identification of Conservation Units: cases studies in Lepidoptera and Crustacea.
- 11.35-11.55 Katalin Pecsenye: Conservation units of Parnassius mnemosyne in the Carpathian Basin.
- 11.55-12.15 Péter Takács 2: Genetic heterogeneity reveals cryptic taxonomic diversity of stream-dwelling gudgeons (Teleostei, Cyprinidae) in the middle Danubian hydrosystem (Hungary)
- 12.15-14.00 Lunch break

#### Afternoon session I

14.00-14.35 Thomas Schmitt: Known centres and new borders: The biogeography of the odonates of Eurasia.

14.35-14.55 Edit Juhász: Contrasting climatic responsiveness of wing and genital characters in the Heath Fritillary (Melitaea athalia, Lepidoptera: Nymphalidae).

14.55-15.15 András Tartally: The re-discovered Maculinea rebeli: Host ant usage, parasitoid and initial food plant around the type locality (Lepidoptera, Lycaenidae).

15.15-16.00 Coffee break

#### Afternoon session II

16.00-16.20 László Ronkay: The Himalayan winter Noctuidae fauna and its biogeographic importance.

16.20-16.55 Zoltán Varga: Outline of Faunal History of Southeastern Central Europe: a Synthesis of Palaeoecology and Phylogeography.

16.55-17.10 Zoltan Varga is 75 years old: more than 50 years in evolutionary biology

#### PARTICIPANTS OF THE SYMPOSIUM

#### **INVITED SPEAKERS**

Bruford, Michael: Cardiff School of Biosciences, Cardiff, UK

e-mail: BrufordMW@cardiff.ac.uk

Meglécz, Emese: Aix-Marseille Université, Marseille, France

e-mail: emese.meglecz@imbe.fr

Nève, Gabriel: Aix-Marseille Université, Marseille, France

e-mail: gabriel.neve@imbe.fr

Rózsa, Lajos: Ecology Research Group,

Hungarian Academy of Sciences, Hungary

e-mail: lajos.rozsa@gmail.com

Schmitt, Thomas: Senckenberg Deutsches Entomologisches Institut,

Müncheberg, Germany

 $e\hbox{-}mail: Thomas. Schmitt@senckenberg.de$ 

Varga, Zoltán: University of Debrecen, Debrecen, Hungary

e-mail: varga.zoltan@science.unideb.hu

Verovnik, Rudi: University of Ljubljana, Ljubljana, Slovenia

e-mail:Rudi.Verovnik@bf.uni-lj.si

Wahlberg, Niklas: University of Turku, Turku, Finland

e-mail: niklas.wahlberg@utu.fi>

#### Debrecen, 2014 August 29-30

**PARTICIPANTS** 

Bereczki, Judit: University of Debrecen, Debrecen, Hungary

e-mail: bereczki.judit@gmail.com

Juhász, Edit: University of Debrecen, Debrecen, Hungary

e-mail: juhasz.edit@science.unideb.hu

Kosztolányi, András: MTA-DE Lendület Behavioural Ecology Research Group

University of Debrecen, Debrecen, Hungary e-mail: andras.kosztolanyi@gmail.com

Nemesházi, Edina: Szent István University, Budapest, Hungary

e-mail: enemeshazi@gmail.com

Pecsenye, Katalin: University of Debrecen, Debrecen, Hungary

e-mail: katalin.pecsenye@science.unideb.hu

Rácz, Rita: MTA-DE Lendület Behavioural Ecology Research Group

University of Debrecen, Debrecen, Hungary

e-mail: ritaracz89@gmail.com

Rákosy, László Babes-Bolyai University, Cluj, Romania

e-mail: laszlorakosy@hasdeu.ubbcluj.ro

Ronkay, László: Hungarian Natural History Museum,

Budapest, Hungary e-mail: ronkay@nhmus.hu

Sramkó, Gábor: Ecology Research Group,

Hungarian Academy of Sciences, Hungary e-mail: sramko.gabor@science.unideb.hu

Szabó, Krisztián: Szent István University, Budapest, Hungary

e-mail: kr.szabo@gmail.com

Tartally, András: University of Debrecen, Debrecen, Hungary

 $e\hbox{-}mail: tartally.and ras@science.unideb.hu\\$ 

Takács, Péter: Balaton Limnological Institute, Tihany, Hungary

e-mail: takacs.peter@okologia.mta.hu

Tóth, János Pál: Research Institute for Viticulture and Oenology,

Tokaj (RIVOT), Hungary

e-mail: acutiformis@yahoo.com

Újvárosi, Lujza: Babes-Bolyai University, Cluj, Romania

e-mail: lujza1@yahoo.co.uk

Végvári, Zsolt: Hortobágy National Park, University of Debrecen,

Debrecen, Hungary

e-mail: zsolt.vegvari@gmail.com

Vörös, Judit: Hungarian Natural History Museum,

Budapest, Hungary e-mail: jvoros@nhmus.hu

#### **ABSTRACTS**

#### INVITED SPEAKERS

## Phylogeography of Large African Mammals: are we near to a continent-wide paradigm?

#### Michael Bruford

This talk will examine current knowledge of pan-African patterns of genetic diversity in large mammals. It will compare and contrast what we know and identify knowledge gaps for rainforest, savannah and generalist species. Two examples will be provided to illustrate these issues: that of the black rhinoceros, for which the recent history of this species is (depressingly) well-known and which is a savannah/desert specialist and that of the rainforest-dependent okapi, for which knowledge is almost absent and which was only photographed in the wild for the first time in 2008. The link between these studies and conservation action will be explicitly made, and future research priorities will be identified.

## Microsatellite evolution at a genome scale and implications on genetic marker development.

Emese Meglécz, Gabriel Nève, Ed Biffin and Michael G. Gardner

Microsatellites are ubiquitous in Eukaryotic genomes. A more complete understanding of their origin and spread can be gained from a comparison of their distribution within a phylogenetic context. Although information for model species is accumulating rapidly, it is insufficient due to a lack of species diversity, thus intragroup variation is necessarily ignored. We have studied random sequences of 154 Eukaryote species to test whether there was a phylogenetic pattern in microsatellite abundance. A very strong heterogeneity of microsatellite abundance was observed within most taxonomic groups and the age of the taxon seems to be a primary factor in degrading the phylogenetic pattern.

Among the many hypotheses to explain the origin and spread of microsatellites within genomes, association to transposable elements has been frequently evoked, but no systematic genome wide study involving a large number of diverse species has been done. We have used the same dataset as for the abundance study to investigate the presence of microsatellites in potentially repetitive sequences to examine this hypothesis. The results are discussed in function of their influence on microsatellite marker development.

## Identification of Conservation Units: cases studies in Lepidoptera and Crustacea

#### Gabriel Nève

In conservation biology, one of the key points is to assess the scale at which conservation measures are to be taken. In this respect tools in population genetics may help define what populations are. Linderiella massaliensis (Crustacea, Branchiopoda) is endemic to the Provence area (South France) where it is found only in temporary water bodies on calcarious rocks. Only nine such pools are known. Sequencing of COI of series of individuals coming from all pools allowed us to identify on one hand a case where the species has been artificially introduced; on the other hand introduction could be ruled out when a new population was found about 60 km north of all the other populations, as this population showed several unique haplotypes.

Coenonympha oedippus is a threatened butterfly in Europe. A population genetics study on populations in southwest France showed that populations in this area are weakly differentiated from each other; on the other hand population in the plains of Dordogne are different from the ones from the foothills

of the Pyrenees, about 200 km to the south. This is probably due to different selection pressures on the 6PGD locus.

These case studies show that the identification of population genetics processes may be important in the identification of the characteristics of individual populations, in order to guide conservation action: preservation of existing populations, re-introductions and in some cases identification of previous introductions.

## Shifting bushmeat consumption and the rise of Armillifer grandis (Crustacea: Pentastomida) infections in the Democratic Republic of Congo

Mihály Sulyok, Lajos Rózsa, Imre Bodó, Dennis Tappe, and Richard Hardi

Livestock production in Congo Basin countries is limited, thus people strongly rely on bushmeat as a source of animal protein. As populations of large-bodied birds and mammals are increasingly overexploited people shift towards the consumption of reptiles and snakes in particular. African snakes also act as definitive hosts of two pentastomid species (Armillifer armillatus and A. grandis) that utilize mammal intermediate hosts. Thus A. grandis -- formerly scarcely reported from humans -- became an emerging pathogen in the area. Apparently, while most infections are symptomless and remain undetected, the rare ocular manifestations of the disease are highly conspicuous and thus reliably indicate ongoing epidemiological changes.

LR's research was supported by the EU and Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence' Program.

## Known centres and new borders: The biogeography of the odonates of Eurasia

#### Thomas Schmitt and Markus Heiser

To enhance our knowledge on the biogeographic structuring of Eurasia, we selected odonates as a study group to unravel the location and extension of faunal regions and elements all over this region and to better understand the transition between the Palearctic and the Oriental realm in East Asia. Furthermore, we searched for biogeographic differences between the strongly flying dragonflies and the more sedentary damselflies. For our analysis, we elaborated regionalised species lists for all 1,765 Eurasian odonate species for a total of 63 pre-defined biogeographic regions. A more refined analysis was performed for the western Palearctic, split into 97 regions harbouring 162 species. Cluster and principal components analyses were performed to extract faunal regions and faunal elements. In general, we distinguished three major biogeographic regions: (1) Europe, North Africa, North Asia; (2) India, Indochina, South China; (3) North China, Korea, Japan. Notable substructures and independent faunal elements were detected in each of these groups, especially in Southeast Asia representing the diversity hotspot of odonates. Some biogeographic differences exist between dragonflies and damselflies; thus, western Palearctic Anisoptera influence the species composition in Northeast Asia more than Zygoptera; damselflies have more island elements than dragonflies; and they are more strongly isolated by the Strait of Gibraltar, but do not show a more fine-grained structure of their faunal elements. Consequently, Southeast Asia apparently was the most suitable part of Eurasia for odonate survival during cold stages. The influence of these elements into East Asia is more intense than often thought so that the northern border of the Oriental realm has to be located more to the North for odonates than mostly assumed. Even an independent realm in the region of China, Japan, Korea and the Far East of Russia might be discussed. The biogeographic structure within the western Palearctic supports many of the classical assumptions for this region, but also might justify extra-Mediterranean refugia. The differences in the active flight capacity between dragonflies and damselflies might be responsible for the observed biogeographic differences between these two groups.

#### Outline of Faunal History of Southeastern Central Europe: a Synthesis of Palaeoecology and Phylogeography

#### Zoltán Varga

#### Synopsis

General zonality and faunal types

- (i) deviation from the general zonality in Carpathian basin
- (ii) diversity of faunal types in the Carpathian basin

The 'palimpsest' of the faunal picture – the layers

- (i) the soil fauna with relict endemisms (Lumbricidae) oldest
- the fauna of springs and running waters (mostly small) (Trichoptera, Plecoptera, groups of Diptera; Gastropoda)
- terrestrial fauna: the faunal cycles of Quaternary the paleoecological setting phylogeography of
  - a) Mediterranean species the 'paradigmatic' core areas
  - b) continental the extra-Mediterranean core areas
  - c) oreal/oreotundral species periglacial and montane core areas
  - d) eremic species autochtone speciation in the Pannonian region]

Conclusions and perspectives

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### Asellus aquaticus - model organism for study of phylogeography, ecological speciation and parallel evolution

Rudi Verovnik, Marjeta Konec, Simona Prevorčnik

The water louse Asellus aquaticus is a widespread, euryoecious species, mostly uniform in morphology throughout its large European range. Within this contiguous range some populations invaded subterranean environments and developed specific morphological traits termed troglomorphoses (eye and

pigment loss, elongation of appendages etc.). We studied phylogeographic patterns of the species at continental scale and more specifically in regions where repeated cave invasions have occurred (Dinaric Karst - Slovenia, Dobrodgea - Romania) using mtDNA and nuclear gene markers. At regional scale microsatellites were also used. Phylogeographic analysis suggested that the first dispersion of the species proceeded possibly during late Miocene from the western part of the Pannonian basin. Several areas colonized from here have served as secondary refugia which correspond in part with classical Mediterranean refugia. At regional scale at least three separate cave invasions were confirmed with putative source populations still present on isolated karst poljes outside caves. Comparative analysis of Romanian and Slovenian pairs of ecomorphs has shown that several morphological traits underwent the same evolutionary change – parallel evolution, while others were regionally specific indicating local adaptations as the cave systems compared have a completely different ecology apart from darkness. Microsatellite data analysis suggests that all cave populations are distinct species with evidently no gene admixture with ancestral surface populations. Due to sharp difference in ecology between surface and subterranean environment ecological speciation may have an important role in the process of speciation in A. aquaticus.

#### The wings of butterflies: active movements through time and space

#### Niklas Wahlberg

Recent advances in using molecular data, calibrated with fossil evidence, to estimate the times of divergence of lineages in almost any group of organisms has brought fresh understanding to where various organisms have originated and how they got to where they are today. I will review the state of knowledge in butterflies, a group that has long been speculated to have been influenced by the break up of Gondwana. Our work on the family Nymphalidae shows that most lineages are too young for vicariance to have played a major role in explaining the current distributions of species. In contrast, it is very clear that

butterflies have actively dispersed, although it is apparent that successful colonizations of new areas happens rarely enough that clear phylogenetic patterns are retained.

#### **PARTICIPANTS**

## The pattern of Wolbachia infestation in the social parasitic Maculinea butterflies (Lepidoptera: Lycaenidae)

Judit Bereczki, Rita Rácz, Zoltán Varga and János P. Tóth

Wolbachia is a common group of bacteria found in arthropods and nematodes. This intracellular bacteria genus has attracted considerable interest since the past decade primarily because of their vast abundance, fascinating effects on hosts, which range from reproductive manipulation to mutualism and their significance in the evolution of their hosts. Here we present the prevalence and infestation pattern of these bacteria in Maculinea butterflies in the Carpathian Basin. Besides, we evaluate the possible relation between the presence of Wolbachia and the reduced genetic diversity of the target species.

The research was supported by the OTKA (K109223) and János Bolyai Scholarship of the Hungarian Academy of Sciences.

## Contrasting climatic responsiveness of wing and genital characters in the Heath Fritillary (Melitaea athalia, Lepidoptera: Nymphalidae)

Edit Juhász, Zsolt Végvári, János Pál Tóth, Katalin Pecsenye and Zoltán Varga

Nowadays it is of particular interest how different organisms react to climatic changes and weather conditions on extended temporal scales. Because of this

importance, we aim to investigate the effects of climatic patterns on the wing size and genital characteristics in a butterfly species which is considered a threatened species in Western Europe but not yet endangered in the eastern part of the continent.

The main object of this investigation is to reveal climate-induced changes in several morphometric measures in Aggtelek Karst area. We found that climatic responsiveness of lepidopteran wing characters is prone to be modified by climatic patterns Wing size was predicted by spring temperatures during larval development indicating that environmental conditions experienced by caterpillars are carried over to adult stage. In contrast to wing morphology, we found that processus posterior size and aedeagus length showed no clear temporal trends and were not related to any of the measured climatic predictors over the study period.

Our study highlights the importance of climatic responsiveness in various metrics of wing morphology of a lepidopteran decreasing on European scale, which might be of key importance in adapting to accelerated climatic trends.

#### Breeding behaviour in a biparental beetle

András Kosztolányi & Zoltán Barta

The beetle Lethrus apterus (Coleoptera: Geotrupidae) is a biparental species with Eastern European and Anatolian distribution. According to the literature the sexes are specialized to different parental roles: the male defends the nest burrow from intruders and pulls cut leaves into the nest underground, whereas the female forms balls from the leaves in the brood chambers where the eggs were laid previously. The balls serve as food for the hatched offspring. Recent observations, however, suggest that this role specialization of sexes changed recently in this species of conservation concern. In new project we aim to investigate whether parental role division is different between populations and whether it may be attributed to the differences in the social and abiotic environment and/or genetic divergence.

## Individual identification of white-tailed eagles, based on microsatellite loci

Edina Nemesházi, Krisztián Szabó, Zoltán Horváth and Szilvia Kövér

White-tailed eagle (Haliaeetus albicilla [LINNAEUS, 1758]) populations in Europe suffered a drastic decrease in the early 20th century, but as a result of conservation measures, nowadays the populations are successfully recovering. Recently, several investigations payed attention on the philogeography and population genetics of the species, but we do not know much about the species at individual level. To investigate territorial dynamics, mate fidelity or natal dipsersal, individual identification is indispensable. We elaborated a suitable molecular method for this goal (15 microsatellite loci, originally published for different species), based on both non-invasive and invasive sampling. Using this method, in 2013, we successfully started a long-term study of territorial dynamics and mate fidelity around the Boronka protected area, a high quality habitat with an outstanding population density (11 breeding pairs in about 1200 ha).

## Conservation units of Parnassius mnemosyne in the Carpathian Basin

Katalin Pecsenye, Noémi Szolnoki, Judit Bereczki, Edit Juhász and Zoltán Varga

The present geographic distribution of genetic variation in e.g. Lepidoptera species basically depends on two sets of processes: (i) the past history of the given species; (ii) the current distribution of its suitable habitats. The history of the European populations of Parnassius mnemosyne (Linnaeus, 1758) has been surveyed by using mtDNA sequences (Gratton et al 2008). The Pannonian/Carpathian region has been shown to harbour two sets of haplotypes all

belonging to the Eastern ancestral lineage: EN and EE. The aim of the present study was to analyse enzyme polymorphism in the P. mnemosyne populations of the Carpathian Basin to reveal the contemporary pattern of their genetic differentiation.

The results of Bayesian clustering analyses supported the presence of two genetic lineages/clusters in the Carpathian basin. One cluster was typical for the Northern and Transdanubian ranges of Hungary and it seems to be identical to lineage EN described by Gratton et al. (2008). Another cluster was characteristic for populations of the Bereg region and Transylvania and it probably corresponds to lineage EE in Gratton et al. (2008). The genetic composition of the populations of the Körös region, however, remained unexplained.

#### Microsatellite markers in the survey of Lethrus apterus

Rita Rácz, Judit Bereczki, Gábor Sramkó, András Kosztolányi and Zoltán Barta

Lethrus apterus is a member of the family Geotrupidae and this beetle is well-known for its biparental care. Until recently this species was common in Hungary, however, the agricultural activities changed its preferred habitats in the last century. Therefore, L. apterus can be found only in fragmented areas. This locally increased density of individuals may augment the frequency of extrapair copulations. Until now, there were no microsatellite markers available for any species in the genus Lethrus, thus we developed 15 microsatellite loci for L. apterus in order to investigate its reproductive biology and the genetic structure of Hungarian populations. Here we present some preliminary results of the population genetic survey.

#### The Himalayan winter Noctuidae fauna and its biogeographic importance

#### László Ronkay

The Himalayan winter moth fauna is an assemblage of generic/species complexes of rather heterogeneous phylogenetic origin, their main zoogeographic characteristics are, however, often show common features in their distribution patterns and the routes of dispersion. These common trends are easily detectable when studying the wide sense Himalayan (more precisely Himalayan-Sino-Pacific) genera of the winter Noctuidae. The taxonomic and zoogeographical analysis of these groups is based on the thorough study of ca 450 species of the Noctuidae from practically all countries of the Himalayan-Sino-Pacific

The analysis revealed the autochtonous Himalayan origin of these winter noctuid genera, the processes of allopatric and sympatric speciation during their spreading along the main routes of dispersion which produced the great diversity of species and subspecies (including numerous endemic taxa in different parts of this vast area). The core areas and the secondary centres of speciation and/or dispersion of the discussed groups outline rather precisely these major routes (the "direct Himalayan", "great S-type", "Himalayan combined", "small S-type", "Himalayan forceps", "Manchurian-Pacific" and "northern temperate" routes), indicating also important bionomic properties of the given groups (e.g. the potential of spreading, ecological plasticity) as well as the supposed time of their genesis and the location of the missing links.

The genesis and expansion of this large faunal type has a great importance in the population of practically the entire northern temperate zone, especially the monsoonic forest habitats and the temperate arboreal biomes. Spreading along the two sides of the Tibetan Plateau, the representatives of the monsoonic arboreal fauna conquered the temperate zone forests in both continents. They crossed the Beringian to spread across the extensive woodlands of North America and they reached Mexico via the Rocky Mountains. The western branch and the descendents of the Pacific temperate forest fauna spread

along the taiga belt and the Eurasian mountain ranges as far to the west as the Atlantic Ocean. The behaviour and the environmental needs of these species far away from their homeland have undergone significant changes, and this flexibility has played an important role throughout the distribution process: except for the tropical regions of the northern hemisphere, they could gain ground almost everywhere. Their dominance is obvious even in Europe, at a great distance from the place of their origin.

#### A preliminary look into the genetic diversity of Carpatho-Pannonian steppic species—poor westernmost outpost of the Eurasian steppes?

#### Gábor Sramkó

The steppes of the Carpatho-Pannonian region are considered to be the westernmost representatives of the Eurasian, large steppes. As western outposts, the steppic plants and animals are considered to be steppic relicts, and somehow as a consequence - a decreased genetic variability of such populations is implied, although there are very few studies showing such pattern. Three partly ongoing phylogeographic studies, the case of Adonis volgensis (Ranunculaceae), Pulsatilla patens (Ranunculaceae) and Sicista subtilis trizona (Dipodidae), are shown, where we have found different patterns of genetic variability from the above assumption. In fact, the case of P. patens explicitly shows high-levels of genetic variability in the Carpatho-Pannonian region, which - together with the phylogeographic pattern - implies northeastward colonisation of the western part of the Eurasian steppes from the Carpathian Basin. Our other examples also highlight the possible importance of our geographic region as source of genetic variability of steppic organisms, thus arguing for a much higher conservation value of this westernmost outpost of steppes.

The author expresses his gratitude to his co-authors for contributing to the data on which this presentation is based. This study was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'.

## Natal dispersal of eastern imperial eagle (Aquila heliaca) in the Carpathian Basin

#### Krisztián Szabó

After a severe bottleneck and habitat loss in the second half of the 20th century, population census of eastern imperial eagles in the Carpathian Basin began to grow, along with an expansion in the breeding area. Recently the population seems to be far from reaching its maximal size, and still only a fraction of suitable habitats are occupied by the species. Studied on the individual level, dispersal movements of eagles can provide detailed information about the patterns of population dynamics in this expanding population (e.g. whether individual movements are affected by sex, environmental factors, territory quality and structure). Here we report a study on natal dispersal patterns (i.e. the movement of an individual from its birth site to its first breeding site) of eastern imperial eagles in the Carpathian Basin, carried out by comparing multilocus DNA-profiles of breeding birds (2011-2013) with previously ringed (2001-2011) nestling eagles' profiles.

## Genetic heterogeneity reveals cryptic taxonomic diversity of stream-dwelling gudgeons (Teleostei, Cyprinidae) in the middle Danubian hydrosystem (Hungary)

Péter Takács, Péter Bihari, Erős Tibor, András Specziár, Ildikó Szivák, Péter Bíró and Eszter Csoma

With the forefront of genetic analyses the taxonomy of many European fish taxa have been reevaluated in the last decade, including the stream dwelling Gobio genus. Recent genetic analyses proved the existence of two morphologically very similar species from the Carpathian region. The Carpathian gudgeon (Gobio carpathicus) was identified from the eastern, while the Danube gudgeon (Gobio obtusirostris) from the western (Transdanubian) region of the country. However, these studies examined on limited number of samples which originated from the verge of the region. Therefore, we still lack detailed information about the species identity, phlyogenetic relationships and genetic diversity of the Gobio species inhabiting the inner area of the Carpathian Basin. In this study 241 specimens from 27 sites were sampled and analyzed with two molecular methods, sequencing the mtDNA Control Region and Amplified Fragment Length Polymorphism. Results suggest an unambiguous east-west separation of the studied Gobio stocks. However, the haplotype of the Danube gudgeon could be identified only in the north-western region of Hungary, whereas the haplotype of the Carpathian gudgeon occured only at the northernmost sampling site, in the eastern part of the country. In the central and southern part of the Transdanubian region previously unknown haplotypes were found, and another different haplotype group appeared also at the eastern sites. These results indicate the presence of cryptic gudgeon lineages in the Carpathian Basin, Hungary.

## Population genetic patterns of an endemic fish species (Umbra krameri Walbaum. 1792) in Hungary

Péter Takács, Tibor Erős, András Specziár, Zoltán Vitál, Péter Sály, Péter Bíró and Eszter Csoma

The aim of this work to show the recent distribution pattern of this protected, vulnerable fish species in Hungary, and to reveal its population genetic structure within and among regions. As the result of our field surveys muddminnow noted from more than 40 sampling sites from Hungary. For genetic studies 9 microsatellite markers were used and we analysed fin clips of 390 specimens were collected from 33 locations across 8 regions. Results show high level of differentiation among regions, and various levels of separation detected within regions. In some cases recent population bottlenck was revealed.

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## The re-discovered Maculinea rebeli: Host ant usage, parasitoid and initial food plant around the type locality (Lepidoptera, Lycaenidae)

András Tartally and Anton Koschuh

The taxonomy of the myrmecophilous Maculinea alcon group (Lepidoptera: Lycaenidae) is highly debated. Maculinea 'rebeli' has generally been considered to be the xerophilous form of Ma. alcon (Ma. alcon X hereafter) with Gentiana cruciata as initial food plant. However, the type locality and all other known sites of Ma. rebeli are found above the coniferous zone, and are well separated from the lower regions where Ma. alcon X sites are found. Furthermore, no food plant, host ant and parasitoid data for the nominotypic Ma. rebeli have yet been published. Two populations were discovered, both parasitized exclusively My. sulcinodis host ant around Gentianella rhaetica host plant (confirmed by oviposition). The results highlight the differentiation of Maculinea rebeli from Ma. alcon X, from both conservation biological and ecological points of view. Thus, it should be concluded that Ma. rebeli does not simply represent an individual

form of Ma. alcon but it can be considered as at least an ecological form adapted to high mountain conditions both in its initial food plant and host ant species.

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## How improvement of scientific methods could change our thinking on the same question? A historical overview.

János P. Tóth, Judit Bereczki and Zoltán Varga

Scientific methods have improved a lot in the last few decades. Nowadays, phylogeny and geometric morphometrics have become some of the most important tools for modern taxonomy providing better understanding on the evolutionary or biogeographical history of the taxa. Thus, it results in a clearer and more robust picture on species delimitations. Effective conservation of a species is only possible if sufficient amount of information is available for the nature conservation authorities which usually make decisions on habitat management or reintroduction plans. The story of Melitaea ornata kovacsi Varga, 1967 is a good example of how improvements of scientific methods and more and more knowledge on species biology could influence our thinking on the same question.

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## Temporal patterns in diversity, population size and voltinism in Noctuid moths (Noctuidae)

Zsolt Végvári, Edit Juhász, János Pál Tóth, Zoltán Barta, Sándor Boldogh, Sándor Szabó and Zoltán Varga

Using a long-term dataset of 476 Noctuid moth species captured by a standardized light trap in Aggtelek Karst area spanning 20 years, we analysed temporal patterns in various biodiversity measures, population size and the degree of voltinism. Although biodiversity metrics and population dynamics showed

contrasting temporal patterns, the degree of voltinism exhibited only minor changes, implying that the length of our study period is too short to detect climate-driven changes in the number of annual generations.

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#### Conservation genetics of the olm (Proteus anguinus) in Croatia

Judit Vörös and Dusan Jelić

The olm (Proteus anguinus) is the only cave-dwelling vertebrate in Europe, inhabiting the karst cave systems of the Dinaric Alps. Due to its adaptation to underground habitats studying even the distribution of the species is extremely difficult. We developed a sampling method based on environmental DNA which helps to detect the presence of the animal from the cave systems. Using 454 pyrosequencing we developed several species-specific microsatellite markers to characterize two Proteus populations in Croatia, and to provide a useful tool for conservation genetic studies of this extremely vulnerable creature.