

Characteristics of main research directions investigated at the institute and the achievements 2010–2014

Institute	Institute of Vertebrate Biology of the CAS, v. v. i.
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We focus on advanced research in frontier areas of evolutionary ecology, biodiversity and medical zoology. These activities are of both fundamental and applied nature and significance. Here, we provide a brief summary of the main research directions and the most important achievements. The institute has no stable structure of individual research teams (i.e. composition of teams is flexible and is influenced by actual projects). Our research is distinctly based on broad international collaboration but in this report we have selected mainly the scientific results in which the Institute's share on their elaboration is clearly dominant. Comprehensive information will be given during the on-site visit of the Commission, and further details can also be found at <http://www.ivb.cz/publikace.html> and <http://www.ivb.cz/biennial-report.html>.

Evolutionary ecology

This research focuses on understanding the ecological and evolutionary basis of interactions between and within species. It explicitly considers evolutionary effects of competition, co-evolution and the role of parasites. The investigations are performed on both laboratory and natural populations using the most advanced methods and approaches of molecular genetics and genomics, animal physiology and behavioural ecology.

Cues for increasing fitness: mating systems and sexual selection

Sexual selection is a powerful evolutionary process that results in adaptations aimed to maximize reproductive success. We studied mechanism of sexual selection in bitterling fishes, small cyprinids that lay their eggs into the gills of freshwater mussels. In a series of studies, we demonstrated that female choice and outcome of male-male competition for best mating opportunities frequently disagree. Females often reproduced with dominant (but not preferred) males because they possessed the most valuable resources and were able to exclude other males from reproduction. We found that female mate choice decisions are likely based on olfactory signals that communicate dissimilarity between partners (Reichard et al. 2012: PLoS One) and such decisions have major consequences for offspring survival (Agbali et al. 2010: Evolution). Availability of breeding resources (freshwater mussels) was important determinant of individual variability and population level recruitment (Konečná et al. 2010: Behav Ecol Sociobiol; Casalini et al. 2010: Behaviour; Liao et al. 2013: Behaviour) and identified selection gradients of particular phenotypic traits associated with the strength of sexual selection (Reichard et al. 2009: Evolution). We also studied morphological and behavioural adaptations to sperm competition (Pateman-Jones et al. 2011: Biol J Linn Soc; Spence et al. 2013: Behav Ecol; Smith and Reichard 2013: Behaviour; Smith et al. 2014: J Evol Biol).

We further explored the role of sexual selection on reproductive isolation tactic in annual killifish. We found that mate choice is an important part of reproductive output but played only a marginal role in reproductive isolation between populations, despite their long separation and distinct colour differences (Reichard and Polacik, 2010: Biol J Linn Soc). In another sympatric species pair, we found incomplete and asymmetric reproductive isolation (Polačik and Reichard 2011: PLoS One). Finally, using Endler's guppy, we revealed major effect of social environment on the expression of alternative male mating tactics (Řežucha and Reichard 2014: Anim Behav).

In socially monogamous birds, extra-pair fertilization (EPF) leads to sperm competition and may intensify the strength of sexual selection. We studied rates of EPF in both non-passerine

and passerine birds (e.g., Kreisinger et al. 2010: J Avian Biol, Promerova et al. 2011: J Avian Biol) and tested if EPF contributes to the development of carotenoid-based male feather ornamentation in a sexually dichromatic passerine, the scarlet rosefinch. We demonstrated that the colouration of ornamental breast feathers was a good predictor of male annual reproductive success only when male within-pair and extra-pair fertilization success were taken into account. EPF increased variation in male reproductive success, and we have examined the idea that carotenoid-based ornamentation signals individual quality. As a signal of quality we measured pro-inflammatory responsiveness in adult males. Surprisingly, we found that low-quality paler birds responded more strongly to phytohaemagglutinin than high-quality brighter individuals, (Vinkler et al. 2012: Anim Behav). Similar results were obtained in grey partridges (Svobodova et al. 2013: J Ornithol). We proposed a novel mechanism explaining the honesty of health signalization in animals (the so called “carotenoid-maintenance handicap”, Vinkler and Albrecht 2010: Naturwissenschaften). At the ultimate level, another trait like egg appearance serves for protection and signalling function for female quality. We could conclude that egg colouration in great reed warbler is unlikely to have a signalling function for female quality (Honza et al. 2012: J Avian Biol; Honza et al. 2011: Naturwissenschaften).

Extra-pair matings lead to post-copulatory sexual selection and sperm-sperm competition may affect the evolution of sperm phenotypes. We proposed (Lifjeld et al. 2010: PLoS One) and used (Albrecht et al. 2013: Proc R Soc Lond B; Lasemoen et al. 2013: Behav Ecol Sociobiol) between-male variation in sperm length as a novel proxy to estimate species-specific levels of sexual promiscuity in birds (for similar approach applied to murine rodents see Sandera et al. 2013: PLoS One). In addition, by comparing the occurrence of sperm abnormalities in various birds from Chernobyl area and control localities across Europe, we demonstrated for the first time a trade-off between sperm length and integrity (Hermosell et al. 2013: Biol Lett).

Host and parasite co-evolution

The relationship between the hosts and parasites is often characterized by rapid evolution of parasite adaptations to exploit the host, and counter-adaptations in the host to avoid being parasitized or to decrease the costs imposed by parasitism. To study these interesting questions two model systems were used, i.e. brood parasitism in birds and bitterling-mussel co-evolutionary relationship.

We examined both adaptations and counter-adaptations with a special respect to interaction between common cuckoo and its host. In a series of dummy experiments (Honza et al. 2010: Behaviour; Čapek et al. 2010: Condor), we explored nest defence tactics in various hosts. We demonstrated that hosts adjust not only their aggressive behaviour but also nest attendance. Our studies confirmed that egg type and nest attentiveness affect mode (Stokke et al. 2010: Acta Ornithol) and timing of egg rejection (Požgayová et al. 2011: Behav Ecol), and that the essential cues used for egg discrimination by the hosts are found at the blunt pole (Polačiková 2010: Behaviour; Polačiková et al. 2011: J Avian Biol; Požgayová et al. 2011: Behav Ecol). It was suggested that another factor affecting egg discrimination is the nest light environment but we were not able to support this statement (Honza et al. 2011: Ethology; Honza et al. 2013: Naturwissenschaften). Interestingly, we did not confirm previous assumptions that host rejection behaviour is genetically fixed (Procházka et al. 2014: Naturwissenschaften). We found that cuckoos optimize nest searching strategy in relation to the number of available nests and choose those, in which their eggs match appearance of host clutches (Honza et al. 2014: Proc. R. Soc. B; Jelínek et al. 2014: Ibis), but a lack of local adaptations of this brood parasite was revealed (Avilés et al. 2001: J Evol Biol) and some constraints on host choice were identified (Grim et al. 2011: J Anim Ecol). On the other hand, we rejected a traditional assumption that cavity nesters are unsuitable cuckoo hosts (Grim et al. 2014: Ornis Fenn). We also investigated whether host social mating system affects reproductive success of the brood parasite. We found higher cuckoo fledging success in nests of monogamous compared to polygynous males and

these results suggests that the actual level of social polygyny may considerably influence the overall reproductive success of a local cuckoo population (Trnka et al. 2012: Behav Ecol Sociobiol). We recorded that host social status plays an important role in aggression towards brood parasites and strong and repeatable aggressiveness of host females outweigh that of females serving thus as a frontline anti-parasite defence (Požgayová et al. 2013: Anim Behav; Trnka et al. 2013: Ethology).

Bitterling parasitizes freshwater mussels by laying eggs in the mussel's gills and, in turn, mussel larvae parasitize the skin and gills of the fish. We used samples from across the current range of the European bitterling to investigate possible scenarios for its colonization of Europe (Bryja et al. 2010: Mol Ecol) and found that bitterling populations vary in the history of associations with their hosts, with consequences for the state of co-evolutionary arm race (Reichard et al. 2010: Evolution). We found no cryptic lineages associated with mussel host species (Reichard et al. 2011: Mol Ecol). Potential consequences of the invasion of an Asian unionid mussel on the European bitterling populations were evaluated (Reichard et al. 2012: Biol Lett) and it was revealed that generalist strategy of mussel's parasitic larvae is the key of their invasion success (Douda et al. 2012: Biol Inv). We also investigated the effect of another non-native species, *Dreissena polymorpha*, on the bitterling reproduction (Vrtilík and Reichard 2012: Hydrobiologia), and contributed to the complete phylogeny of the bitterling subfamily (Chang et al. 2014: Mol Phyl Evol).

Role of behaviour and physiology in thermal strategies

The ectotherms cope with heterogeneity in the thermal environment using the unique combination of behavioural thermoregulation, thermal acclimation, and evolutionary adaptation. We used a non-traditional model species, the Alpine newt, to examine the generality of existing theory of evolutionary thermal biology in amphibians, which are globally the most threatened vertebrates by climate change.

Our results indicated that newts combine various behavioural and physiological components in their thermal strategy involving both behavioural and plastic mechanism probably resulting from their exposure to diverse selection pressures due to indirect development (Gvoždík 2012: Biol Lett, Hadamová and Gvoždík 2011: Physiol Biochem Zool; Kurdíková et al. 2011: PLoS One; Šamajová and Gvoždík 2010: Funct Ecol; Smolinský and Gvoždík 2012: Oecologia). Thermal adjustments affected their interactions with predators suggesting the joint influence of biotic and abiotic factors in shaping newt thermal strategies (Gvoždík et al. 2013: PLoS One; Smolinský and Gvoždík 2013: Biol J Linn Soc). Using experiments we identified new sources of variation in energy metabolism and thermal sensitivity of behaviour from individual level to species interactions (Kristín and Gvoždík 2014: J Exp Zool, Kristín and Gvoždík 2014: J Zool; Smolinský and Gvoždík 2014: J Therm Biol; Polčák and Gvoždík 2014: Anim Behav). These facts change a widely held view on ectotherms' response to heterogeneity in the thermal environment, and thus they should be incorporated into future co-adaptations models of thermal biology. Our studies clearly demonstrate the importance of studies on non-traditional model species, thereby reducing widespread taxonomic bias across biological disciplines.

A new fish model species in aging studies

African annual killifish of the genus *Nothobranchius* have a unique life history that makes them a valuable biological model across biological disciplines, with research on ageing being the most important one. In order to enable a broad use of these fish in ageing research, we conducted diverse studies aimed to improve our knowledge of the species natural history.

We mapped the distribution of *Nothobranchius* species in Mozambique and produced the first demographic and ecological analyses. We found that populations of all three studied species were more abundant than previously believed and highlighted the fact that field work must be held during the rainy season as most populations diminish at its end. We thoroughly quantified the food niche overlap using stomach content analysis (Polačik and Reichard 2010: J Fish Biol),

and stable isotope signatures (Polačik et al. 2014: Hydrobiologia). Despite their abundance, annual fishes are vulnerable to changes in the ecosystem connected with human intervention. Their diversity is still largely unexplored and we discovered a new species of killifish in central Mozambique (Reichard 2010: Zootaxa). We investigated age structure and hatching synchrony in wild populations, revealing variability in the degree of hatching date synchronization (Polačik et al. 2011: J Fish Biol). A call was initiated to reconsider the use of one species of annual killifish as a biocontrol of aquatic mosquito larvae in endemic malaria areas in Africa due to high ecological risk (Reichard et al. 2010: Paras and Vect); the initiative was successful and resulted in abandonment of the field trials.

We significantly contributed to the development of the annual killifish as a model system for research on ageing. We described phylogeny of the target species group (Dorn et al. 2011: Mol Phyl Evol) and characterized genetic structure of wild populations across their range (Bartáková et al. 2013: BMC Evol Biol). We demonstrated that they are vertebrates with the shortest generation time (Blazek et al. 2013: EvoDevo), quantified inter-sexual differences in ageing rates in the wild and in the laboratory (Reichard et al. 2014: Evol Ecol) and showed that species from different regions across gradient of aridity differ in genetically determined lifespan (Terzibasi Tozzini et al. 2013: BMC Evol Biol). Finally, we discovered unique set of alternative intra-population life-history strategies related to unpredictable environmental conditions (Polačik et al. 2014: J Evol Biol).

Wildlife immunology issues

Many researchers interested recently in ecological and evolutionary immunology have focused their studies on free-living animals. In respect of some misunderstandings in the concept of “immunocompetence”, we attempted to unify the terminology in the field with classical immunology (Vinkler et al. 2011: J Avian Biol). PHA skin-dwelling test is one of the widely used techniques for cell-mediated immunity assessment in ecology. We assumed, that the magnitude of the PHA swelling response is an indication of current stress or disease, rather than evidence for level of investment into immune defence in this species (Vinkler and Albrecht 2011: Folia Zool). In rosefinches we also demonstrated an importance of detailed haematological examination for correct health assessment (Vinkler et al. 2010: J Ornithol). Using the zebra finch we provided the first evidence of the importance of erythroagglutination in PHA reaction (Vinkler et al. 2010: Funct Ecol). However, the skin-swelling test using PHA reliably mirrored the individual general proinflammatory potential.

Major histocompatibility complex (MHC) has become a model system for studies of natural selection, host-parasite interactions and mate choice. We analysed the evolutionary mechanisms influencing genetic variability in MHC over a wide spectrum of vertebrate species (e.g. Čížková et al. 2011: Heredity). We found that MHC variation does not copy phylogeographic structure in bank voles and that positive (probably pathogen-mediated) selection is more important than historical effects for the spatial genetic structure of MHC (Malé et al. 2012: Biol J Linn Soc). In a wild population of zebrafish from Bangladesh, (Smith et al. 2011: Evol Ecol Res) no evidence for heterozygote advantage was found, while combined parasitological and genetic results suggested that MHC variability is maintained by pathogen-mediated selection fluctuating in time and space; and similar results were found in another species with simple MHC, the Grey Partridge (Promerová et al. 2013: Plos ONE).

In the context of mate choice, MHC genes are considered as either “good genes” (where all females in a population prefer the same male with a specific MHC genotype) or as “complementary genes” (where the female chooses a male according to her own genotype). In the scarlet rosefinch, we described MHC Class I (Promerová et al. 2012: Mol Ecol Res) and found some support for the “good-genes” model, as social males of high MHC-heterozygosity were cheated on by their females less frequently than less MHC-heterozygosity males (Promerová et al. 2011: J. Avian Biol). On the contrary, in bitterling fish the mate choice

experiments support the hypothesis of MHC as complementary genes (Agbali et al. 2010: Evolution; Reichard et al. 2012: PLoS One).

Besides MHC, the crucial receptors of innate immunity called Toll-like receptors have been intensively studied for the first time in wide spectrum of free-living birds and mammals (e.g. Fornůšková et al. 2014: Ecol Evol; Vinkler et al. 2015: Genetica). In contrast to previous expectations, it was found that they can be very variable and that the parasite-induced selection is the most important evolutionary mechanisms affecting this variability (Bainová et al. 2014: Develop Comp Immunol; Vinkler et al. 2014: Gen Sel Evol; Fornůšková et al. 2013: BMC Evol Biol).

Biodiversity

We focus on all three basic levels of biodiversity, i.e. the genetic, species, and community structure and its variation using both molecular and ecological approaches. Particular attention was paid to species diversity of vertebrates in tropic and sub-tropic regions where scientific knowledge is still insufficient or even lacking. These investigations were performed in diverse geographic areas, and in collaboration with local researchers.

Hybrid zones and speciation

Hybrid zones are viewed as natural laboratories where to study evolutionary processes leading eventually to speciation. We have adopted the house mouse hybrid zone (HMHZ) as our model system. The zone between *Mus musculus musculus* and *M. m. domesticus* is over 2500 km long but only 10-20 km wide. Due to long term research we keep now the richest resources for analyses of the mouse hybrid zone: 4900 individual samples from 440 localities covering area of 7250 km². Bioinformatic approach based on the analysis of 1401 SNPs spaced regularly along whole mouse genome (Wang et al. 2011: Mol Ecol) revealed that selection at incompatible loci extends to 5% at autosomal and 23% at X-linked markers (Janoušek et al. 2012: Mol Ecol). However, a targeted selection of X-linked markers demonstrated that even two loci in tight linkage may experience different selection, recombine away from their parental genome and introgress in different ways (Macholán et al. 2011). Any rigorous analysis of hybrid zone estimates the effects of stochastic factors that can mask the selection signal; in a randomization study we showed that only sampling in central parts of the zone and in 2-dimensions will provide well supported estimates (Dufková et al. 2011: Evolution).

We focused on traits that presumably affect fitness of individuals and can be linked to mechanisms underlying selection against hybrids. Phenotype data were related to a hybrid index which characterizes the level of individual hybridity. We found that males from central parts of the HZ had less motile sperm and lower sperm number in comparison to parental counterparts (Albrechtová et al. 2014: PLoS One). In addition, sperm number was shown to be associated with an extensive Y chromosome introgression (Ďureje et al. 2012: Folia Zool). Males with *M. m. domesticus* background bearing the Y chromosome from *M. m. musculus* displayed significantly higher numbers of sperm than the males with native Y chromosome (Albrechtová et al. 2012: Proc R Soc Lond B). Laboratory-based experiments were used to infer genetic basis of X chromosome linked hybrid male sterility (Bhattacharyya et al. 2014: PLoS Genet) and haplotype dependent proliferative advantage of mtDNA in conplastic mice (Burgstaller et al. 2014: Cell Rep). These results suggest that post-mating isolation barriers prevail in the HMHZ. Nevertheless, we also detected a signal for pre-mating isolation that might potentially lead to speciation. Using an originally constructed probability model we provided evidence for the divergence of olfactory cues and reinforcement of their signalling/perception power in recognition of mates of the same subspecies (Vošlajerová Bímová et al. 2011 Mol Ecol). Differences in dispersal and exploration between the two mouse subspecies add another facet to behavioural divergence than may affect HMHZ dynamics (Hiadlovská et al. 2012: Biol J Linn Soc).

One of long-term holding dogma used to explain narrowness of the HMMZ claims that hybrid mice suffer higher level of parasitism due to breaking co-evolutionary relationships between host and parasites. Analysis of 10 helminth species (N>29,500) in 689 mice from 107 localities revealed actually the opposite pattern. We detected significant decrease both in terms of parasite spectrum and individual helminth abundance in the central part of HZ (Baird et al. 2012: Evolution). The prevalence of individual pathogens (e.g. *Helicobacter*) was similar in hybrid and parental populations (Wasimuddin et al. 2012a: Mol Biochem Parasitol). Recent data suggested even more interesting scenario in parasite-host interactions. We detected genetic footprints of past co-evolution between mouse subspecies and diverse helminth and microbial parasites (Wasimuddin et al. 2012b: Appl Env Microbiol; Kváč et al. 2013: Int J Parasitol; Göy de Bellocq et al. 2015: J Virol). These findings strongly suggest that hybrid zones of hosts may host nested hybrid zones of their co-diverged parasites.

Our contribution to the house mouse biology was appreciated by Cambridge University Press that published a compendium on evolutionary processes within the genus *Mus* edited by two fellows of the Institute (Macholán et al. 2012: Cambridge Univ Press).

Genetic structure of amphibians, mammals and birds in Europe

Genetic data from the whole distribution range of taxa provide important information on the history of particular populations, such as colonization routes or location of glacial refugia. Investigations of great crested newts (*Triturus*) revealed a species complex in Central European populations, influenced by both historical and recent hybridization and introgression in areas where parapatric populations and species meet (Mikulíček et al 2012: Folia Zool). We studied (Hulva et al. 2010: Mol Ecol) highly diversified bats of the *Pipistrellus pipistrellus* group and showed that overall pattern of diversification includes a mosaic of phylogenetically basal, relatively small and mostly allopatric demes in the Mediterranean Basin, as well as two sympatric sibling species in the large continental part of the range. Using the European ground squirrel as a model species, for the first time we provided genetic evidence of the so-called "refugia of continental climate" during interglacial periods of Pleistocene and described the colonization patterns of this steppe specialist (Říčanová et al. 2013: Mol Ecol).

We contributed to the exploration of the origins of laboratory mice (Yang et al. 2011: Nature Genet). Given that laboratory strains are still dominant models in biomedical studies, our work allowed immediate targeted planning experiments that use association studies for mapping genes negatively influencing human health. In the Institute, a number of new laboratory mouse strains were developed and are kept contributing to conservation of natural genetic resources of this biological model of prime importance.

We used genetic approaches to study various vertebrate groups (European chub – Seifertová et al. 2012: J Biogeography, small passerines – Procházka et al. 2011: J Avian Biol; Neto et al. 2012: PLoS One; common shrew – Horn et al. 2012: Evolution; greater mouse-eared bat - Bryja et al. 2010: Acta Chiropterol) to answer challenging questions related to their intra-specific phylogeographic history. Our studies exemplified the power of phylogeography in revealing hidden genetic diversity, delineating manageable units for conservation and resolving taxonomic questions.

The gibel carp is a diploid-triploid complex exhibiting dual reproduction modes (gynogenesis and sexual reproduction) in mixed populations. Our research (Halačka et al. 2010: J Fish Biol) confirmed a lower proportion of club cells at spawning in diploid males and triploid females than in diploid females. The difference in MHC diversity between gynogenetic triploids and sexual diploids is congruent with the hypothesis of sexually-mediated selection and fulfil a prerequisite of the Red Queen hypothesis (Šimková et al. 2013: BMC Evol Biol). We showed that the most common MHC genotypes of gynogenetic triploids are the target of parasite selection, and investigated the basic biochemical profile of two forms of gibel carp (Vetešník et al. 2013: Fish Physiol Biochem) that could explain the changes in the reproduction mode.

Uncovering the biodiversity of vertebrates in Africa and the Middle East

Biological diversity in Africa and adjacent regions of the Middle East is still underestimated. Many currently accepted taxonomic concepts depend mainly on decades-old work. Numerous activity of the Institute focused on understanding processes forming the diversity of various vertebrates inhabiting the regions. Various genetic markers were employed and combined with classical and geometric morphometrics to resolve taxonomical and evolutionary questions.

Within amphibians and reptiles, a revision of the type material of montane tree frogs from East Africa resulted in several nomenclatorial changes (Gvoždík et al. 2014: Zootaxa). Molecular phylogenetics provided insights into biogeographical histories of African torrent frogs (Petroedetidae) and revealed several candidate undescribed species (Barej et al. 2014: Mol Phylogenet Evol). A phylogeographical analysis of Central African reed frogs supported the hypothesis of a single dispersal event from the continent to oceanic islands (Bell et al. 2015: J Biogeography). Herpetological survey in the western Democratic Republic of the Congo was conducted (Nagy et al. 2013: Herpetol Notes) and new data on an almost unknown colubrid snake from the Congo Basin was revealed (Nagy et al. 2014: Zootaxa).

Bryja et al. (2010: Mol. Ecol.) examined rodents of the *Praomys daltoni* complex, which are typical inhabitants of the Sudanian savannah ecosystem in western Africa, and represent a suitable model for testing effects of the Quaternary climatic oscillations on recent genetic variation patterns. Phylogeographic analyses across the distribution range of the complex revealed several well defined clades that do not support division into the two currently recognised species, and highlighted evolutionary processes in Sudanian savannah in Plio-Pleistocene. Additional genetic data of the speciose group Praomyini provided significant new information on distribution and taxonomy of these rodents. Comparison of the individual species distribution patterns allowed identification of cryptic centres of biodiversity where habitat protection is urgently required (e.g. Bryja et al. 2012: Folia Zool; Bryja et al. 2014: J Biogeogr). The analysis of most comprehensive genetic dataset of African taxa from the genus *Mus* (represented by African subgenus *Nannomys*) in sub-Saharan Africa revealed unexpectedly high unknown species diversity with up to dozen of undescribed species, which is very surprising in a genus that contains the best known mammal species, the house mouse (Bryja et al. 2014: BMC Evol Biol). Some species were found in the field after very long time, as exemplified by the Ethiopian endemic mammal genus *Muriculus*, which was unambiguously transferred to genus *Mus* based on the first genetic analysis of an individual captured after more than 70 years (Meheretu et al. 2015: Mammalia).

Various taxa of African bats were investigated using morphological and molecular genetic traits (Vallo et al. 2011: Acta Chiropterol; Vallo et al. 2013: Zool Scripta). We revisited (Koubínová et al. 2010: Acta Chiropterol; Koubínová et al. 2013: Front Zool) taxonomy of bats in western Africa and discovered several new cryptic species with the use of cytogenetic and molecular analyses. These studies had direct implications for conservation of the species.

In collaboration with Selçuk University in Konya, we summarized and reviewed available data on karyotypes of the mammals from Turkey and neighbouring regions in respect of their implications to taxonomy (Zima et Al. 2013: Acta Theriol; Arslan and Zima 2014: Folia Zool). Special attention was paid to exploring the pattern of chromosomal evolution in blind mole-rats in the Middle East (Arslan et al. 2011: Mamm Biol.; Arslan and Zima 2015: Zool Stud; Arslan and Zima 2015: Mamm Biol).

Disease ecology

We explore interactions between pathogens (viruses, bacteria, fungi) or parasites (protists, helminths) and their human or non-human hosts. Outbreaks of infectious diseases are affected by changes in the ecology of the host and the pathogen, or in the environment. Therefore, understanding the incidence, prevalence, or timing of diseases requires studying the ecology of

these interactions. Parasitic organisms influence the abundance and dynamics of wild vertebrate populations, and in some cases lead to host extinction. The majority of diseases of humans are zoonotic, meaning that they are transmitted from animals to humans.

Transmission of pathogens in natural ecosystems

Reviews on mosquito-borne viruses were compiled in international collaboration covering topics of zoonotic mosquito-borne flaviviruses (Weissenböck et al. 2010: Vet Microbiol), epidemiology of West Nile in Europe (Calistri et al. 2010: Open Virology J) and arboviruses pathogenic for domestic and wild animals (Hubálek et al. 2014: Adv Virus Res). Zoonoses present a significant health problem to Europe and a comprehensive monograph on microbial zoonoses and saprozooses was published (Hubálek and Rudolf 2011: Springer). In research of the mosquito-borne diseases we participated in the EDEN FP6 Programme EDENext and EuroWestNile FP7 Programmes and VBORNET.

We reported for the first time the dangerous lineage 2 of West Nile virus in the Czech Republic (Rudolf et al. 2014: Eurosurveillance) and detected a novel lineage in mosquitoes (Pachler et al. 2014: Emerg Infect Dis). Six viral isolates were obtained from female mosquitoes collected in Southern Moravia (Hubálek et al. 2010: J Med Entomol). Interestingly, some mosquito species with a largely southern Eurasian distribution were repeatedly found (Hubálek et al. 2014: J Med Entomol; Pachler et al. 2014: Emerg Infect Dis) which possibly moved into the region as a result of climate change. Detailed studies on Usutu flavivirus (Hubálek et al. 2014: Transbound Emerg Dis), which is endemic to Africa, and West Nile flavivirus (Hubálek et al. 2013: Vector-Borne Zoonotic Dis) theoretically indicate the means by which new vertebrate pathogens emerge. Mosquitoes in Europe may also transmit additional, non-viral human diseases such as dirofilariasis (Rudolf et al. 2014: Parasitol Res) and malaria.

Tick-borne viruses (tiboviruses) are characterized by their specific mode of biological transmission to endotherm vertebrates via competent haematophagous ticks. Ixodid ticks as the vectors of many emerging pathogens represent a significant health risk for vertebrates, including humans (Venclíková et al. 2014: Ticks and Tick-borne Dis; Venclíková et al. 2014: Acta Parasitol). In a seminal review on tick-borne viruses in Europe (Hubálek and Rudolf 2012: Parasitol Res) we summarized background information on 25 tiboviruses that have been detected in Europe. The review also highlighted the potential emergence of a number of neglected tiboviruses in Europe. We tested (Rudolf et al. 2010: J Basic Microbiol) effects of salivary gland extract from fed *I. ricinus*, the competent vector of Lyme borreliosis in Europe, on the growth of *Borrelia burgdorferi*. This result confirmed the substantial role of salivary glands in the pathogen transmission mechanism to vertebrate host.

The studies related to the rodent-borne diseases included investigations of small mammals from a rural landscape for the presence of hantavirus Tula (Heroldová et al. 2012: Vector Borne Zoonot Dis). The antigen prevalence in common voles *Microtus arvalis*, the main reservoir of the hantavirus, was 10% and increased with growing population density. A new staphylococcal species *Staphylococcus microti* was isolated from viscera of common voles with generalised *Brucella microti* infection (Nováková et al. 2010: Int J Syst Evol Microbiol).

White-nose syndrome in European bats

White-nose syndrome is an emerging infectious disease of hibernating bats associated with a fungal skin infection that has caused drastic die-offs in North America. The fungus, *Pseudogymnoascus destructans*, grows at cold temperatures and the drop in body temperature of bats during hibernation proves ideal for the pathogen growth.

Our research (Pikula et al. 2012: J Wild Dis; Zukal et al. 2014: PLoS One) confirmed that at least eleven bat species in Europe suffer the full range of skin tissue damage diagnostic of white-nose syndrome in North America. We used a newly developed, non-lethal method (Turner et al. 2014: J Wild Dis) to detect white-nose syndrome lesions. The fungus is a generalist, and can potentially affect all hibernating bats (Zukal et al. 2014: PLoS One). We immortalized cells

from bat issues to find reasons of different survival in immunological experiments (He et al. 2014: PLoS One).

Surprisingly, the infected bat species in Europe do not suffer mortality attributable to the infection on a scale similar to that across the Atlantic (Martínková et al. 2010: PLoS One) as also evidenced by the long-term data about numbers of hibernating bats. The contrast between the disease impact in North America and Europe, coupled with the fact that animals on both continents exhibit diagnostic features of white-nose syndrome histopathology, provides hope for bat conservation and recovery in North America.

New epidemiological challenges in Africa

Bats have in recent years gained an immense attention as hosts of viruses of potentially significant concern on human health. Due to required expertise in natural and evolutionary history of bats in otherwise medical-oriented research, we participated in several collaborative studies in Africa. The research showed the presence of SARS-related coronaviruses in European bats and inferred relationships within coronaviruses including a discovery of an influenza-related coronavirus from African bats (Drexler et al. 2010: J Virol). Further findings confirmed the importance of bats as ancestral hosts of paramyxoviruses and hepadnaviruses, which include major human diseases such as measles, distemper or mumps (Drexler et al. 2012: Nature Comm), and hepatitis B (Drexler et al. 2013: PNAS), respectively. In another study, we provided evidence of bat origin of the novel MERS coronavirus (Annan et al. 2013: Emerg Inf Dis). In addition to discoveries of origins and diversity of viruses, we also explored factors affecting viral richness in bats such as size and shape of geographic range and body weight (Maganga et al. 2014: PLoS One). We also discovered new arena- and hantaviruses in Ethiopian endemic rodents (Meheretu et al. 2012: Emerg Inf Dis). Even if we currently do not know their impact on human health, similar viruses in western African cause serious haemorrhagic fevers in human.

All great apes are considered as endangered or critically endangered species, and infectious diseases are listed as amongst their most important threats. We assessed parasite community composition (Petrášová et al. 2010: Int J Primatol; Petrželková et al. 2010: Am J Primatol) and molecular diversity of *Blastocystis* in sympatric native and introduced primates in Tanzania (Petrášová et al. 2011: Int J Parasitol). We studied also other pathogens in ape populations showing that several hookworms are shared by humans and apes co-habiting same habitat (Hasegawa et al. 2014: PLoS Neglect Trop Dis). We recorded the presence and molecular diversity of opportunistic microsporidia, *Giardia* and *Cryptosporidium*, in western lowland gorillas in Central African Republic and in mountain gorillas in Rwanda (Sak et al., 2013, 2014: PLoS One).

Our research focused on commensal entodiniomorphid ciliates that inhabit the colons of African great apes. We studied occurrence of two entodiniomorphids in wild and captive chimpanzee populations (Pomajbíková et al. 2010: Am J Phys Anthropol; Pomajbíková et al. 2012: J Eukaryot Microbiol). We evaluated molecular diversity (Vallo et al. 2012: Am J Phys Anthropol) and the basic metabolic activity of *Troglodytella abassarti* that actively participates in the digestion and storage of polysaccharides (Profousová et al. 2011: Folia Microbiol).

We also studied avian host-parasite associations in western Africa, Central America and South America with emphasis to both ectoparasites and endoparasites blood parasites (e.g. Literák et al. 2013: Parasitol Int; Čapek et al. 2014: Ticks Tick-borne Dis).

Applied ecology and conservation biology

We aim to provide research outputs that could find useful applications in nature protection and related environmental issues. Such studies were focused on introduced and invasive species and their ecological interactions in newly occupied areas and habitats. We were attempting to search

for new approaches in conservation biology, particularly in the field of conservation genetics. In this area we participated in international collaboration managed within projects of the EU (e.g. 6th FP project MODELKEY; 7th FP project ConGRESS).

Population genetics and stable isotopes: important tools for species conservation

Genetic approaches were implemented in endangered species surveys or population studies. We mapped the origin and colonization routes of introduced non-indigenous ungulates (sika deer – Barančková et al. 2012: Ecol Res; Alpine chamois – Demontis et al. 2011: Mamm. Biol; Martínková et al. 2012: Folia Zool; Zemanová et al. 2015: Conserv Genet). In marsh frogs and European ground squirrels, microsatellites provided evidence of local inbreeding, strong anthropogenic barriers to gene flow and identified populations requiring conservation management (Mikulíček and Pišut 2012: Eur J Wildl Res; Říčanová et al. 2011: Conserv Genet; Cosic et al. 2013: Conserv Genet). A similar pattern was observed in fragmented populations of the black grouse, where type of mating system was related to genetic diversity (Svobodová et al. 2011: J Ornithol). A study by Šťovíček et al. (2011: Conserv Genet Resources) showed that released captive-bred mallards were successfully integrating into breeding wild populations through hybridization. These results, based on combination of both neutral and adaptive markers, suggested that release of captive-bred individuals may threaten the genetic integrity of wild populations (Čížková et al 2012: Biol Conserv).

Stable isotopes often provide valuable indirect information where conventional approaches fail. In combination with other methods they have become an extremely useful toolbox for many ecological studies. We used this method to infer resource acquisition and trophic position in Afro-tropical birds (Procházka et al. 2010: Ostrich) and to trace breeding and wintering origin of long-distance migrants (Procházka et al. 2013: J Avian Biol; Procházka et al. 2014: Acta Ornithol).

Fish indicate freshwater pollution

Despite significant improvements in water quality over the last two decades, pollution remains a problem in a number of European river basins. Within a project aimed to modelling the impact of key pollutants on freshwater biodiversity (MODELKEY), we examined fish and macrozoobenthos communities within the River Elbe (Jurajda et al. 2010: Czech J. Anim Sci). A long-term study performed in 1992-2010 by Valová et al. (J Env Sci Health 2010) assessed trace metal contamination of fish in the River Morava (Danube basin). The findings indicated that concentrations did not continuously increase downstream, but peaks appeared immediately behind the pollution point-sources. In this respect, the River Morava did not appear a significant source of trace metals for the River Danube. We also examined the potential relationship between physiological condition of a model fish (chub) and exposure to chemical pollution (Wenger et al. 2010: Environ Toxicol Chem).

Non-native gobiid fishes – an emerging model in invasion biology

Since the 1990s, a number of gobiid species have spread beyond their original Ponto-Caspian range and successfully invaded various river systems in Europe and North America. Invasion success is often connected with phenotypic plasticity and competitiveness of the invasive species. The aim of our research (Borcherding et al. 2011: J Appl Ichthyol; Janáč et al. 2012: Fund Appl Limnology; Janáč et al. 2013a, b, Ecol Freshw Fish; J. Fish Biol.; Roche et al. 2013: Knowl Manage Aqua Ecol) was to follow the spread of invasive gobiid species in Europe, and to evaluate their pattern of spreading, ecological demands and impact on native biota. We found that invasion success of bighead goby *Neogobius kessleri* and round goby *N. melanostomus* populations was related to better somatic condition and faster growth as a result of significantly higher prey availability in the area of introduction compared to their native range.

Introduced species are often advantaged by escapement from the effects of natural predators and parasites, and parasites may play a key role in determining the success of an invasion. The continuous expansion of Ponto-Caspian gobies to various river systems (Borcherding et al.

2011: J Appl Ichthyol) provides unique system to study host-parasite associations in different stages of invasion. Our parasitological examinations have indicated a reduced parasite infection load in introduced *N. melanostomus*, which might also contributed to higher invasiveness in this species (Ondračková et al. 2010: Ecol Res). A high susceptibility to local parasites, however, has prevented the *N. kessleri* from taking advantage of parasite loss during the introduction process. In the interconnected river systems in Europe, quite recent goby populations showed decreased genetic diversity and parasite numbers (Ondračková et al. 2012: Parasitology). However, acquisition of local parasites and repeated introductions led to comparable genetic and parasite diversity between goby populations from the native and non-native range of the same river (Francová et al. 2011: J. Appl Ichthyol). Reduction in parasite species richness in the new range was also documented for another fish species expanding from marine/brackish waters to the environment with low salinity (Ondračková et al. 2012: J Appl Ichthyol). Co-introduction of non-native parasites with the host was observed in several other fish species. All co-introduced parasite species were strictly specific to their original host; therefore did not represent a threat for local fish fauna (Ondračková et al. 2011: J. Helminthol; Ondračková et al. 2012: Helminthologia).

Carnivores, herbivores, and birds - protection and management

The study of top predator ecology in time and space is a challenging issue for understanding species-specific adaptations to highly dynamic ecosystems and has number consequences for prey abundance and distribution.

Our studies focused on habitat utilization of different carnivore species in intensively used agricultural landscapes (Červinka et al. 2013: J Nat Conserv; Svobodová et al. 2011: Eur J Wildl Res; Šálek et al. 2014: Eur J Wildl Res; Šálek et al. 2014: Mamm Biol), or in urban ecosystems (Červinka et al. 2014: Urban Ecosys). Movements of mammalian nest predators in an agricultural landscape were investigated using scent stations (Šálek et al. 2010: Land and Urban Plan). We also studied distribution and habitat preferences of endangered carnivore species such as the invasively spreading golden jackal (Šálek et al. 2014: J Wildl Res) or the rapidly declining steppe polecat (Šálek et al. 2013: Acta Theriol). Spatial activity of highly endangered owl predators in Central European farmland was assessed (Šálek and Lövy 2012: Bird Conserv Internat). We studied links between breeding biology, anti-predator strategies and nest predation in birds (Padyšáková et al. 2010: Wild Res; Hořák et al. 2011: Ostrich; Javůrková et al. 2012: PLoS One; Mikula et al. 2014: Acta Ornithol; Sedláček et al. 2014: Trop Cons Sci). The white stork-vole model system was used to evaluate the predator-prey dynamics across large spatial and temporal scales (Hušek et al. 2013: Pop Ecol). Population dynamics of rodents in immission-polluted areas of northern Bohemia was studied in regard of long-term trends in pest abundance or trophic resources for carnivores (Cornulier et al. 2013: Science; Hušek et al. 2013: Pop Ecol).

Another project partly supported by the EU funds was exploring the large carnivores in north-eastern parts of the country. The status of three large carnivore species, Eurasian lynx, grey wolf and brown bear was monitored, and a variety of field and laboratory methods including field monitoring, camera-trapping, GPS telemetry monitoring, and non-invasive genetic sampling was used. The existence of all three species is threatened by habitat fragmentation and illegal hunting, in the case of wolf also by hybridisation with dogs. Our results may contribute greatly to successful conservation strategies and management.

Studies of the diet of species of conservation interest could help to understand their current status and inter-relationships in habitats. We provided the first analyses of the trophic habits of European beaver (Krojerová-Prokešová et al. 2010: J Zool), and other studies were performed on the diet of native ungulates (Krojerová-Prokešová et al. 2010: Wildl Biol; Barančková et al. 2010: Eur J Wildl Res).

Legal protection has been shown to benefit birds in Western Europe, but not yet in Eastern Europe. After 1990, trends in protected species in the Czech Republic improved more than in unprotected species and it indicates that national legislation was successfully implied. A combination of 'broad and shallow' and 'narrow and deep' protection to few species might be most efficient for securing healthy bird populations for the future (Koleček et al. 2014: Biol Conserv). To develop effective conservation measures, it is crucial that we understand how ecological and life-history traits relate to risk of species extinction. In the Czech Republic, slow life history increases the extinction risk in habitat specialists, but reduces such risk in habitat generalists (Koleček et al. 2014: Anim Conserv).