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Summary of the Doctoral thesis

Adaptive evolution of Toll-like receptors in birds

Adaptivní evoluce Toll-like receptorů u ptáků

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Abstract

Toll-like receptors (TLRs) are one of the key and presumably also evolutionary most original components of animal immune system. As Pattern recognition receptors they form the first line of innate immune defence against various pathogens. The proper receptor binding of pathogenic ligands is crucial for their correct recognition and for subsequent triggering of an appropriate immune response. Because there exists a direct interaction between the receptor surface and the pathogenic ligand, host-pathogen coevolution on molecular level can be predicted. Thus, through variability of their ligands, TLRs are exposed to extensive selective pressures that may be detected on both genetic and protein levels. Surprisingly, the variability we revealed in birds is even higher than previously expected based on the reports from other vertebrates, mainly mammals. In my doctoral thesis I summarise the results of my contribution to the avian TLR research. We were the first who experimentally verify the absence of functional TLR5 in several avian species and duplication of TLR7 in others. We finally resolved the origin of duplication in TLR1 and in TLR2 family. An important part of my research project focused on the prediction of potentially functionally important positions in TLRs. We have outlined an investigation strategy universally applicable to any coding genes. Moreover, we found that some of the positively selected positions importantly affect the surface charge distribution. In passerine birds, we also attempted to find ecological factors determining the adaptive evolution in TLRs. However, this attempt was unsuccessful. Besides that, our methodological research improved the knowledge of the molecular background of the PHA-skin swelling test used for assessing the inflammatory responsiveness related to the TLR function. Since further research is highly needed to test the real functional effect of the TLR genetic variation, at the end of this thesis I outline several possible future directions.

Abstrakt

Toll-like receptory (TLR) patří mezi klíčové a evolučně staré součásti imunitního systému zvířat. Jakožto receptory vrozené imunity tvoří první obrannou linii proti nejrůznějším patogenům. Správné navázání ligandů receptorem je přitom zcela zásadní pro bezchybné rozpoznání patogenů a následné spuštění přiměřené imunitní reakce. Protože zde dochází k přímé interakci mezi povrchem daného receptoru a patogenními strukturami, můžeme předpokládat molekulární koevoluci mezi hostitelem a jeho patogeny. TLR jsou tudíž díky variabilitě svých ligandů vystaveny značnému selekčnímu tlaku, který může být detekován jak na genetické, tak i proteinové úrovni. I tak je ale míra variability, kterou jsme popsali u ptačích TLR, překvapivě vysoká. Dokonce vyšší, než se dalo očekávat z dříve publikovaných prací u ostatních obratlovců, především savců. Ve své doktorské práci shrnuji výsledky, kterými jsem přispěla k výzkumu TLR u ptáků. Jako první jsme například experimentálně ověřili absenci funkčního TLR5 u několika ptačích druhů, či duplikaci genu pro TLR7 u druhů jiných. Také jsme definitivně rozřešili původ genové duplikace v rodině TLR1 a TLR2. Důležitá část mého výzkumného projektu se týkala predikce potenciálně funkčně významných pozic u TLR. Navrhli jsme postup identifikace těchto pozic, který je univerzálně použitelný i pro ostatní kódující geny. Navíc jsme zjistili, že některé z těchto pozic významně ovlivňují distribuci povrchového náboje. U pěvců jsme se pokoušeli najít ekologické faktory určující adaptivní evoluci TLR, nicméně bez úspěchu. Kromě toho jsme prohloubili znalosti o molekulární podstatě kožní zánětlivé odpovědi vyvolané aplikací PHA, která může být použita k ověření funkčních rozdílů mezi různými variantami TLR. Jelikož je však navazující výzkum v tomto směru stále potřeba a to hlavně, abychom otestovali skutečný funkční význam genetické variability popsané u ptačích TLR, na konci této práce navrhuji několik možných směrů, kudy se ubírat dál.

Introduction

My PhD thesis focuses on the **host-pathogen coevolution** as one of the most widespread evolutionary associations in the living world. Dealing with such a broad topic I decided to start my research with the hosts and their defence against possible threats from various pathogens, where birds may serve as model hosts and toll-like receptors (TLRs) as model components of the host's immune defence system. The coevolution is nicely expressed by the Red queen's metaphor (van Valen 1973), where in our case a never-ending evolutionary **arms race** is going on between host receptors and their pathogen-derived ligands (Woolhouse et al. 2002). This dynamic process is based on the adaptation in one species that is followed by **selection** acting in the second species giving rise to the adaptive structural coevolution. The mechanism of TLR-ligand recognition may represent a useful model system, namely because there occurs a direct contact between host and pathogen surface variants (Gay and Gangloff 2007). The proper receptor binding of a ligand is crucial for its correct recognition and for subsequent triggering of an appropriate immune response. TLRs are thus exposed to extensive selective pressure from various pathogens affecting certain codons (rather than whole loci). This is supported by findings that even a single amino acid substitution may change the receptor capability to appropriately recognize the ligand (Keestra et al. 2008, Walsh et al. 2008, Resman et al. 2009, Meng et al. 2010, 2011).

As Pattern recognition receptors, TLRs form the first line of **innate immune defence** against various pathogens and belong among evolutionary most original components of animal immune system (Kawai and Akira 2010). After successful pathogenic ligand recognition, TLRs trigger intracellular signalling pathways leading to expression of immunomodulatory cytokines, which results in **inflammatory response** and subsequent clearance of the threat (Veerdonk et al. 2011). At the same time this signalling also initiates adaptive immune response (Iwasaki and Medzhitov 2015) and thereby TLRs build the virtual bridge between innate and adaptive immunity. All **TLRs** are transmembrane proteins composed from a horse-shoe-shaped ectodomain built-up by multiple leucine-rich repeat motifs that form the interface for the direct contact between the receptor and pathogenic ligands, transmembrane domain and intracellular TIR domain which enables the downstream signal transmission (Jin and Lee 2008, Kawai and Akira 2010). Although TLRs are structurally highly conserved proteins (PAPER II.), they exhibit high inter- and also intraspecific genetic variation in birds (PAPER I., Alcaide and Edwards 2011, Grueber et al. 2014). In its phylogenetically original state the **avian TLR family** consists of ten receptors which are situated on cell surface (TLR10[1A], TLR1[1B], TLR2A, TLR2B, TLR4, TLR5 and TLR15) or in various cellular compartments as are endosomes, lysosomes and endolysosomes (TLR3, TLR7 and TLR21; Brownlie and Allan 2011). Each TLR is responsible for specific ligand recognition (as are e. g. flagellin, LPS, di/triacylated lipopeptides, ssRNA, dsRNA, CpG DNA; Mikami et al. 2012). The nature and beauty of TLR recognition is based on the fact, that their ligands play essential role for the survival of pathogens, and, therefore, pathogens cannot avoid expressing these structures (Takeuchi and Akira 2010).

The associations between TLR genotypes and susceptibility to various infections, allergies, inflammatory and autoimmune **diseases** or cancers has been described in humans on many examples (summarised in Misch and Hawn 2008, Netea et al. 2012, Medvedev 2013). Contrary to humans and some other mammals, in birds the evidence currently available is scarce and moreover all our knowledge comes from studies in domestic chickens (Leveque et al. 2003). Therefore, we decided to focus on investigation of adaptive variability in immune genes in **free-living birds**, where the diversity is enormous, and the natural selection operates unbiased.

Aims of the study

The general aim of my doctoral thesis is to illuminate the adaptive evolution of Toll-like receptors in birds. To achieve this goal, my colleagues and I first decided to focus on various aspects of evolution of these key innate immunity receptors, aiming to provide the description of genetic and also protein variability of TLRs. We predicted variability both on intra and interspecific levels high in non-model free-living birds. Hypothesising the TLRs to be directly influenced by host-pathogen coevolution, we strived to identify sites under positive selection in avian TLRs. Expecting high levels of false-positive results, we proposed that key adaptive variants should differ in their functional effects. Given that TLRs enter into direct molecular contacts with the pathogenic ligand, we predicted especially the receptor surface to play an important role in receptor function. Furthermore, we were interested in answering the question, how various ecological patterns could influence evolution of avian TLRs. As a special part of the project, I also aimed to uncover the molecular regulation of the inflammatory process in skin, because measuring this response could be used to study the TLR functional variation in birds.

Therefore, as partial aims of my doctoral thesis I set:

1) **To describe the history and general patterns of TLR evolution on interspecific level in birds**
2) **To assess the level of avian TLR polymorphism on intraspecific level**
3) **To test for selection acting in avian TLRs**
4) **To predict the functional significance of adaptive changes in TLR structures in birds**
5) **To identify ecological aspects influencing evolution of avian TLRs**
6) **To characterize molecular regulation of inflammation in birds**

Material and methods

To achieve the goals, I determined in my doctoral thesis, I have used a wide range of different methods from various lab procedures to further bioinformation data analyses during my PhD studies. Consistent with the focus of my work, the **genetic samples** were used as the initial and crucial material for most aspects of my work in all of the studies. We analysed genetic samples we collected during sampling procedures in the field (PAPER VI., PAPER V.). Other tissues for further genetic analyses, especially from the species not inhabiting the territory of the Czech Republic, came as scientific loans from various genetic banks (PAPER I., PAPER III., PAPER IV.). Furthermore, whenever possible, we downloaded target sequences from publicly available resources, e.g. NCBI, GenBank or RCSB PDB (PAPER I., PAPER II., PAPER III., PAPER IV.). Especially in the case of the major paper of my thesis (PAPER I.) were most of the investigated sequences extracted from the whole-genome data generated by the Avian Phylogenomics Consortium (Zhang et al. 2014), genomes included also in the Bird 10,000 genomes (B10K) project (http://b10k.genomics.cn/; Avianbase; Eöry et al. 2015).

Most of the molecular-genetic work was done in laboratories of Institute of Vertebrate Biology, the Czech Academy of Sciences in the external research facility in Studenec. Among the main laboratory procedures belong: DNA/RNA extraction, primer design and PCR product amplification, target sequencing, gene expression and copy number analysis. For sequencing we used two different approaches in papers included in this thesis: Sanger sequencing and target amplicon next-generation sequencing (NGS). **Sanger sequencing** was used not only for gene description (PAPER IV., PAPER V.), but also as the first step before expression analysis (PAPER IV., PAPER V., PAPER VI.), copy number variation (CNV) analysis (PAPER I.), or NGS method (PAPER V.) to sequence the broader region surrounding the region of interest in all genes with the aim to ensure the specificity of any subsequently designed primers. For **target NGS** we used two 454 platforms (Roche): GS Junior (PAPER V.) and GS FLX+ (PAPER III.). We used the **gene expression analysis** to answer three different questions. First, in the case of TLR5 pseudogene in birds (PAPER IV.) we wanted to check for presence or absence of a functional copy of this gene in the genome based on mRNA expression of the sequence. Second, we performed a semi-quantitative PCR to measure TLR expression in various tissues of the grey partridge (PAPER V.). Third, more recently in the paper describing the differential expression in selected cytokines before and after PHA treatment (PAPER VI.), we quantified the gene expression using classical real-time qPCR. The **copy number variation** (CNV) analysis based on real-time qPCR (Weaver et al. 2010) was used to resolve the number of copies occurring the particular gene in the genomes of investigated species (PAPER I.).

During my PhD studies, I have used many different pieces of software, tools and approaches for data analysis. For **bioinformatic analysis** of the sequenced data for example SeqScape, Geneious, fastQC, BioConductor packages, BLAST, MAFFT, PHASE, PAL2NAL, GENECONV, LightCycler 480 SW, etc. We used **phylogenetic analysis** in our studies very often, mainly to reconstruct the evolutionary history of the target gene/protein (using PhyML and MrBayes; Guindon et al. 2010, Ronquist et al. 2012). For species tree reconstruction we used approaches: to reconstruct the

schematic consensus species tree based on up to date published avian phylogeny (e.g. Jarvis et al. 2014) but without the information about the length of individual branches, or to generate the phylogeny tree for all investigated species only from the global phylogeny of birds using a web-based tool application available at http://birdtree.org/ (Jetz et al. 2012). To test **positive selection** posing on individual residues on the interspecific level we used various codon-based maximum likelihood methods: PAML (Yang 2007), FUBAR (Murrell et al. 2013), SLAC (Kosakovsky Pond and Frost 2005a) and MEME (Murrell et al. 2012). Along with positive selection we tested gene recombination (GARD; Kosakovsky Pond et al. 2006) and the degree of dissimilarity of amino acid substitutions according to their physiochemical properties using the tool PRIME (Kosakovsky Pond and Frost 2005b). **Amino acid physicochemical properties** (chemistry, charge and hydrophobicity) at all positively selected sites were graphically visualised using a web-based application Weblogo 3 (Crooks et al. 2004). The analysis of evolutionary non-conservativeness of amino acid positions was made to estimate location of functionally variable regions (ConSurf; Glaser et al. 2003, Ashkenazy et al. 2016). Moreover, we focused also on **possible function** of detected positively selected sites, therefore the comprehensive review of already published literature describing residues with any function as well as other studies detecting positive selection on interspecific level was done (for the most recent version see PAPER I.). Afterwards the distances of detected positively selected sites from these already known functionally important positions were measured using 3D structural protein models in the PyMOL (python command iterate and plugin distancetoatom). For prediction of **protein structures**, we use two approaches: I-TASSER (Roy et al. 2010) and Modeller (Webb and Sali 2014). Protein electrostatic potentials were calculated using PDB2PQR server (Dolinsky et al. 2004) based on the PARSE force-field and electrostatic calculation on the APBS web (Baker et al. 2001). The UCSF Chimera software was used for aligning the 3D protein structures and such aligned dataset was uploaded to the web-based PIPSA tool (Richter et al. 2008) to acquire a matrix of species with the pairwise comparisons of their surface electrostatic potential distances.

Results and discussion

Although Toll receptors were discover and described more than two decades ago in insects (*Drosophila*; Medzhitov et al. 1997) and TLRs have been described in model vertebrate species few years after that (mainly in human, mouse, followed by other mammals; Smirnova et al. 2000, Ferwerda et al. 2007, Shen et al. 2010, 2012, Areal et al. 2011, Smith et al. 2012, Quach et al. 2013), in birds the situation remained for a long time largely overlooked. The avian TLR research oriented mainly on the domestic chicken (Leveque et al. 2003, Boyd et al. 2007, Temperley et al. 2008, Kannaki et al. 2010, Brownlie and Allan 2011), with only few non-chicken avian TLRs described later (de la Lastra and de la Fuente 2007, MacDonald et al. 2008, Vinkler et al. 2009, Cormican et al. 2009, Gopinath et al. 2011). In fact, from the evolutionary point of view it is preferable to focus mainly on the situation in free-living animals to better understand the co-evolution of hosts and their pathogens in the natural, non-constrained system. Although recently the situation is improving and the number of studies dealing with TLRs in free-living birds is slowly increasing (see e.g. Alcaide and Edwards 2011, Grueber et al. 2014, Raven et al. 2017), most authors still focus mainly on the description of the *TLR* genetic variability without exploring further functional effects. Therefore, in my PhD project I tried to go a little bit further and predict the impact of the variation detected on functionally important regions and positions (e.g. in PAPER I., PAPER II.). Thus, in the articles my co-authors and I created predictions which could be subsequently experimentally tested. In particular, we found interspecifically important physicochemical changes on the receptor surfaces in the ligand binding regions (PAPER II., PAPER III.), which functionally differentiate individual group of species, which serves as a base for formulation of wider ecological hypotheses on TLR adaptive evolution. We also tested experimentally the phenomena of copy number variation (gene duplication previously described based on sequence data only; PAPER I.) and pseudogenisation (PAPER IV.) and verified the gene functionality based on gene expression analysis (PAPER VI., PAPER V.).

Gene gain and gene loss in avian TLRs (Aim 1)

As shown by our comprehensive analysis of all members belonging to the TLR family covering all major clades of avian phylogeny (PAPER I.), the TLR family is typically composed from ten members in birds. There are, however, exceptions since some of the species possess only nine TLRs, **lacking functional TLR5** because of its pseudogenization (PAPER IV., PAPER I.), while others recently **duplicated their TLR7** (PAPER I., Cormican et al. 2009, Grueber et al. 2012, Raven et al. 2017). To both these phenomena we brought gene expression evidence. Interestingly, these gene loss and gain events occurred in the TLR family several times independently during avian evolution. This suggests that similar selection forces may have acted in different avian clades, leading to adaptive loss or gain of the receptors. The question waiting to be answered is what selective forces could be responsible for these adaptations. Future research should attempt to answer this question by focusing on immune responses in groups of species with and without functional TLR5 and duplicated TLR7. Also searching for shared ecological traits (e.g. presence of similar groups of pathogens, inhabiting similar environments, feeding on the same diet or exhibiting related life-

history traits) may bring light into this investigation. As a first step, however, it is necessary to experimentally test the potential functional effect of TLR5 lacking/TLR7 duplication on the capability of the immune system to correctly recognize their ligands. Further research involving experimental infections will face the problem of the limited knowledge of pathogens present in wild living birds. Nevertheless, before more specific investigation is conducted, the experiments in TLR5 could begin using currently known widespread flagellated bacteria (e.g. *Salmonella typhimurium*, which recombinant purified flagellin is already commercially available). With respect to more ancient duplications that occurred in TLR evolutionary history, we helped to resolve the history of main duplication events in the **TLR1 family**. The main question was, if the *TLR1* gene duplication had occurred before mammals diversified from the sauropsids (Huang et al. 2011), or this duplication followed only after this divergence, independently in avian and mammalian lineage (Temperley et al. 2008, Cormican et al. 2009, Mikami et al. 2012, Wang et al. 2016). Our results supported the first scenario that *TLR1* duplication arose even before sauropsids and synapsids split into two separate lineages (giving rise to TLR1 [avian TLR1B] and TLR10 [avian TLR1A]), while in the TLR2 family the duplication probably occurred in parallel to *TLR2* duplication in some mammals that resulted in pseudogenisation of the second *TLR2* copy (PAPER I.). This finding may help us to better search for functional and evolutionary parallels in vertebrate TLRs.

Genetic variability and selection acting on avian TLRs (Aim 1, 2, 3)

Generally, in birds, TLRs are highly polymorphic molecules. They exhibit potentially functionally important variability that is shaped by presumably pathogen-mediated selection. On the molecular level the host-parasite coevolution is acting between the amino acid residues of the host receptors exposed on the surface and the particular ligand structures (Gay and Gangloff 2007). As a result, most of the relevant variability is present at the **ligand-binding** interface at the extracellular domain (PAPER I., PAPER II.). TLRs exposed to the **cell surface** contain more positively selected sites (mainly in TLR1 [TLR1B], TLR2A, TLR2B, TLR4 and TLR5), than receptors expressed into the endosomes (PAPER I., PAPER II., PAPER V.), probably because they bind structurally more diverse ligands (Mikami et al. 2012). Our results in birds supported the hypothesis proposed previously in mammals (Wang et al. 2016) that more positively selected sites are present in the **three-domain TLRs** than in the single-domain ones (except for avian TLR5 that is under very strong positive selection; PAPER I.). We also detected **convergent evolution** acting in avian TLRs (PAPER I.) which probably resulted from similar pathogenic selective pressures acting in unrelated host species. Despite the generally high sequence variability detected in avian TLRs on interspecific and also intraspecific levels, we also found exceptions. We revealed only **limited *TLR* genetic polymorphism** present in a grey partridge population (PAPER V.). Apparently, this polymorphism has only a minor functional impact. The low genetic variability of grey partridge TLRs is probably caused by a recent population bottleneck and local inbreeding in this species, which may also explain the relatively high susceptibility of the grey partridges to certain infectious diseases (Vitula et al. 2011).

Functional effect of genetic variability (Aim 4)

Since, it is quite well described that even a single amino acid substitution may change the receptor capability to appropriately recognizing the ligand (Keestra et al. 2008, Walsh et al. 2008, Resman et al. 2009, Meng et al. 2010, 2011), we may predict that among the positively selected sited, those harbouring the **non-conservative amino acid substitutions** with potentially dramatic changes in the receptor **physicochemical properties**, may have the most important functional impact. On interspecific level, we detected most of such non-conservative positively selected sites in two bacteria-sensing avian TLRs (TLR2A and TLR4; PAPER I.) with highly variable ligands known in mammals. Interestingly, several potentially functional sites were shared between birds and mammals (PAPER I., PAPER II., PAPER III.). However, the real functional effect of each of these positions must be experimentally tested. One way, how to do this, is to measure signalling activation potential of the recombinant receptors (differing in only one target amino acid) expressed in model cells and stimulated with a standard ligand panel under *in vitro* conditions (Voogdt et al. 2018).

TLR protein evolution (Aim 1, 4, 6)

Furthermore, we focused on TLR protein phenotypic evolution that may mirror the imprints of the ongoing or past parasite-mediated natural selection. Although, we have confirmed that avian TLRs are interspecifically very conservative in both their secondary and tertiary structures (PAPER II.), we have described significant differences in various surface characteristics (PAPER I., PAPER II., PAPER III.), mainly in the **electrostatic potential**. The most important physicochemical changes we found in the ligand binding regions, which functionally differentiates individual groups of avian species. The intracellular part of TLRs responsible for molecular signalling remained interspecifically conserved. Based on our results, it also appears that there might be an ongoing convergent evolution in the TLR surface charges between birds and mammals. In **passerine TLR4** we revealed distinctive variation in the patterns of the surface charge distribution, which enabled us to divide all the investigated species into four clearly separated clusters (PAPER III.). Unfortunately, we did not succeed in identifying any ecological explanation for these clusters. While partially the clusters reflected the phylogeny, an important part of the variation remained unresolved.

Measure of inflammation (Aim 5)

Major disadvantage of studying host-parasite system in birds is, that we know only a little about wild bird pathogens. To test the responsiveness of the host's immune system regardless on any specific pathogens, ecological immunology is using various *in vivo* tests. The **PHA skin-swelling test** has been frequently adopted by researchers investigating free-living birds (Smits et al. 1999, Kennedy and Nager 2006, Vinkler et al. 2010). We have designed an improved protocol for this test involving a cytokine mRNA expression profiling in skin (PAPER VI.). Comparing expression of cytokines involved in distinct regulatory immunological pathways, we were able to show that the test is directed mainly towards Th17 inflammatory immune response, rather than towards adaptive T-cell proliferation, as was previously assumed. This test may be later adopted with specific TLR ligands to describe the relationship between structural variability in TLRs and their capability to trigger an appropriate immune response.

Conclusions

During my PhD studies I focused primarily on the patterns of **evolution in avian Toll-like receptors**. I contributed to uncovering the story of gene gain and gene loss in the avian TLR family (Aim 1; PAPER I., PAPER IV.), to description of the TLR sequence variability (Aim 1, 2; PAPER I., PAPER II., PAPER III., PAPER IV., PAPER V.) and diversifying selection acting in these genes (Aim 3; PAPER I., PAPER II., PAPER III.). Furthermore, I participated in investigation of the receptor phenotypes evolution on protein level (Aim 4; PAPER I., PAPER II., PAPER III., PAPER IV.), in attempt to find out ecological patterns related to TLR variability (Aim 5; PAPER III.) and in improvement of methods to study the immunological processes tightly connected with TLR evolution (Aim 6; PAPER VI.).

The results reported in my doctoral thesis indicate **strong positive selection** driving TLR evolution in birds on both gene and also protein levels. However, further research is highly needed to describe the precise selective pressures shaping the TLR variability and repertoire. To resolve this task the researchers will need to besides others **focus also on pathogens** in the wild birds. The current lack of the quality pathogen data may be the reason, why studies investigating the relationship between the TLR variability and susceptibility to various diseases are still missing in wild birds.

Taken all together, **TLRs are crucial molecules** of the avian immune system on which researchers should focus more of their scientific attention.

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Curriculum vitae

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Personal information

BORN in České Budějovice, The Czech Republic, 6th December 1985; NATIONALITY: Czech

RESEARCHER ID: I-3888-2012; ORCID ID: 0000-0002-4578-633X

RESEARCH INTERESTS: Ecological and evolutionary immunology, immunogenetics, bioinformatics, ornithology

Education

Since 2011 Doctoral study of Zoology, Faculty of Science, Charles University.
Dissertation: **Adaptive evolution of Toll-like receptors in birds.**

2008-2011 Master's study of Ecology, Faculty of Science, Charles University.
Diploma thesis: **The Influence of Toll-like Receptor 4 Polymorphism on Condition and Ornamentation in Great tit.**

2005-2008 Bachelor's study of Biology, Faculty of Science, Charles University.
Bachelor thesis: **Methodological Aspects of Cell Immunity Measurements in Evolutionary Ecology Studies.**

Foreign Research Visits

2016 Dr. Dana M. Hawley group, Department of Biological Sciences, **Virginia Polytechnic Institute and State University**, Blacksburg, Virginia, USA; supervised by Dr. Robert Settlage from Advanced Research Computing at Virginia Tech (1 month)
Aim: characterization of transcriptomic changes during *Mycoplasma gallisepticum* infection in house finch and after bacterial LPS stimulation in great tit by next-generation sequencing data analysis

2015 Dr. Vladimír Beneš, Genomics Core Facility, **European Molecular Biology Laboratory**, Heidelberg, Germany (1 week)
Aim: final preparation of samples for next-gen sequencing – for both target sequencing using Illumina MiSeq and also for RNAseq of whole transcriptomes using Illumina NextSeq

2013 Professor David Burt group, Department of Genomics and Genetics, The Roslin Institute and Royal (Dick) School of Veterinary Studies, **University of Edinburgh**, UK (1 month)
Aim: the bioinformatic analysis of genomic data with the focus on avian Toll-like receptors

2010 Professor Jan T. Lifjeld group, The National Centre for Biosystematics, Natural History Museum, **University of Oslo**, Norway (3 month)

Aim: assessing the population polymorphism of Toll-like receptor 4 in bluethroats

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Employment history

Since 2010 part-time job at the Department of Zoology, Faculty of Science, Charles University
2009 part-time job at the Department of Population Biology of Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic

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Awards

2014 „**Vakovlk Junior**" – the award for the best doctoral student at the Department of Zoology, Charles University

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Conference contributions

INTERNATIONAL MEETINGS: 23 contributions in total, 8 talks (meetings of: *The Avian Immunology Research Group*, AIRG; *The European Society for Evolutionary Biology*, ESEB; *The Global Genome Biodiversity Network*, GGBN; *The Society for Experimental Biology*, SEB; etc.)
NATIONAL MEETINGS: 26 contributions in total

Grant projects

2014-2016 **Bainová H.**: Trans-species polymorphism in selected genes of innate and aquired immunity in tits (Paridae). The Charles University Grant Agency, grant No. 540214.

Since 2008 Co-operated on other 5 projects (supported by the Czech Science Foundation or by the Charles University Grant Agency)

Trainings

Attended ca 15 workshops or trainings about NGS data analysis, or various molecular-genetic approaches, etc.

Pedagogical praxis

Participating on teaching of four courses at the Charles University, mainly practical trainings (Genetic methods in zoology, Morphology of animals, Zoology of Vertebrates, etc.).

Supervisor or consultant of 3 successfully defended student's thesis.

Other skills

FOREIGN LANGUAGES: English (proficiency level: C2 – very proficient; 9th February 2016)

MOLECULAR GENETICS: DNA/RNA extraction, PCR, cloning, sequencing (Sanger, NGS), gene expression

DATA ANALYSIS: sequence data analysis, transcriptome data analysis, expression analysis, protein structure analysis, selection analysis, phylogeny analysis, UNIX

LICENCES: licence for animal-handling and animal experiment planning (since 2015), licence for bird-ringing (since 2008), driving licence B (since 2005)

HOBBIES AND INTERESTS: volleyball playing, horse riding, photography, hiking

Selected publications

The thesis consists of the following papers:

I. **Velová H.**, Gutowska-Ding M. W., Burt D. W. & Vinkler M. (2018): Toll-like receptors in birds: gene duplication, pseudogenization, and diversifying selection. Molecular Biology and Evolution, 35(9): 2170–2184. (IF_{2018}= 14.797)

II. Vinkler M., **Bainová H.** & Bryja J. (2014): Protein evolution of Toll-like receptors 4, 5 and 7 within Galloanserae birds. Genetics Selection Evolution 46:72. (IF_{2014}= 3.821)

III. Králová T., Albrecht T., Bryja J., Hořák D., Johnsen A., Lifjeld J. T., Novotný M., Sedláček O., **Velová H.** & Vinkler M. (2018): Signatures of diversifying selection and convergence acting on passerine Toll-like receptor 4 in an ecological context. Molecular Ecology, 27(13):2871–2883. (IF_{2018}= 5.855)

IV. **Bainová H.**, Králová T., Bryjová A., Albrecht T., Bryja J. & Vinkler M. (2014): First evidence of independent pseudogenization of Toll-like receptor 5 in passerine birds. Developmental & Comparative Immunology 45(1):151-155. (IF_{2014}= 2.815)

V. Vinkler M., **Bainová H.**, Bryjová A., Tomášek O., Albrecht T. & Bryja J. (2015): Characterization of Toll-like receptors 4, 5 and 7 and their polymorphism in grey partridge. Genetica 143(1):101-112. (IF_{2015}= 1.343)

VI. Vinkler M., Svobodová J., Gabrielová B., **Bainová H.** & Bryjová A. (2014): Cytokine expression in phytohaemagglutinin-induced skin inflammation in a galliform bird. Journal of Avian Biology 45(1):43–50. (IF_{2014}= 1.971)

Papers not included in the thesis:

VII. Vinkler M., **Bainová H.** & Albrecht T. (2010): Do we know what we measure?: Functional analysis of the skin-hypersensitivity response to phytohemagglutinin in Zebra Finch (*Taeniopygia guttata*). Functional Ecology 24(5):1081-1086. (IF_{2010}= 4.645)

VIII. Kropáčková L., Pechmanová H., Vinkler M., Svobodová J., **Velová H.**, Těšičký M., Martin J.F. & Kreisinger J. (2017): Variation between the oral and faecal microbiota in a free-living passerine bird, the great tit (*Parus major*). Plos One. (IF_{2017}= 2.766)

IX. Svobodová J., Bauerová P., Eliáš J., **Velová H.**, Vinkler M. & Albrecht T. (2018): Sperm variation in great tit males (*Parus major*) is linked to a haematological health related trait, but not ornamentation. Journal of Ornithology 159(3): 815-822. (IF_{2018}= 1.472)

X. Tešický M., **Velová H.**, Novotný M., Kreisinger J., Beneš V. and Vinkler M.: "Positive selection and convergent evolution shape molecular phenotypic traits of innate immunity receptors in tits (Paridae)." Submitted to Evolution. (IF_{2019}= 3.573)

XI. Bauerová P., Krajzingrová T., Těšický M., **Velová H.**, Hraníček J., Musil S., Svobodová J., Albrecht T. and Vinkler M.: "Lifetime bioaccumulation and health effects of heavy metals in longitudinally monitored urban bird blood." Submitted to Science of the Total Environment. (IF_{2019}= 5.589)