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Host-Parasite Interactions in Natural Populations

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Abstract

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Parasitism is one of the most common ways of living and it has arised in many taxa. Parasites feed and live inside or on their hosts resulting in both long and short term consequences for the host. This thesis is exploring the phenotypic and genotypic effects of animals living with parasitic infections. I have been studying three different parasite groups and their associated host species: the great snipe, a lekking freshwater wader bird that migrates between Africa and Northern Europe; the tree sparrow, a stationary passerine found close to human settlements and lastly the water vole, a large rodent living in riparian habitats.

Avian malaria is one of the most commonly studied parasites affecting birds. *Atoxoplasma*, an intestinal protozoan parasite is less studied but is thought to be endemic in free-ranging birds. Given the freshwater habitat great snipes inhabit, a prevalence of 30% avian malaria infections is not high and that the prevalence fluctuated among years. Sequencing of the avian malaria cytochrome b gene revealed that parasites are similar to avian malaria parasites found in African birds suggesting that they were infected on the wintering grounds in Africa. Tree sparrows had few malaria infected individuals, a result that is consistent with other studies of stationary birds at high latitudes. *Atoxoplasma* infections were common in tree sparrows and capture-recapture analyses show decreased survival in infected compared to uninfected birds and signs of lower mating success among infected.

Genetic analyses comparing the transcriptome between mated and unmated great snipe males revealed that the genotype is important for mating success and health status for some of the expressed genes. That variations in some of these genes are involved in maintaining a good health status and mating success supports handicap models for sexual selection in this lek mating system.

The major histocompatibility complex (MHC) is a part of the immune system and it contains genes involved in immune response. In water voles, a number of new MHC alleles were identified. Based on their *in silico* phenotype they were grouped into supertypes to facilitate studies on how helminth infections affect the MHC diversity in the water voles. Some of these MHC supertypes provided resistance to one helminth species, but the same supertype caused the opposite effect for other helminth parasites. Overall, parasites are a driving force for maintaining genetic diversity and parasite infections lowers survival rate, which would lead to a lower lifetime breeding success.

Keywords: Arvicola terrestris, avian malaria, balancing selection, Major histocompatibility complex, parasitetranscriptome

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List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Halvarsson, P., Kålås, J.A. and Höglund, J. Avian Malaria Prevalence Affects Survival in Great Snipe (*Gallinago media*). (Manuscript)

II Höglund. J., Wang, B., Sæther, S.A., Blom, M.P.K, Fiske, P., Halvarsson, P., Horsburgh, G.J., Burke, T, Kålås, J.A., Ekblom, R. Blood transcriptomes and *de novo* identification of candidate loci for mating success in lekking great snipe (*Gallinago media*). (Resubmission to Molecular Ecology)

III Svensson, M. and Halvarsson, P. Fitness Effects of Coccidian and Avian Malaria Parasites in Tree Sparrows. (Manuscript)

IV Relationship between helminth infections and MHC class II supertype diversity in natural water voles (*Arvicola amphibius*) populations. (Manuscript)

The cover illustration was kindly provided by Alex Richter-Boix

Contents

Abbreviations

BCI	Body Condition Index
cDNA	complementary DNA
DNA	Deoxyribonucleic acid
HCA	Hierarchial Cluster Analysis
MHC	Major Histocompatibility Complex
p	Observation probability in capture-recapture studies
p	p-value
PBR	Peptide Binding Region
P/H	*Plasmodium/Haemoproteus*
RNA	ribonucleic acid
SNP	Single Nucleotide Polymoprhism
ST	Supertype

In the case of the misseltoe, which draws its nourishment from certain trees, which has seeds that must be transported by certain birds, and which has flowers with separate sexes absolutely requiring the agency of certain insects to bring pollen from one flower to the other, it is equally preposterous to account for the structure of this parasite, with its relations to several distinct organic beings, by the effects of external conditions, or of habit, or of the volition of the plant itself.

The author of the 'Vestiges of Creation' would, I presume, say that, after a certain unknown number of generations, some bird had given birth to a woodpecker, and some plant to the misseltoe, and that these had been produced perfect as we now see them; but this assumption seems to me to be no explanation, for it leaves the case of the coadaptations of organic beings to each other and to their physical conditions of life, untouched and unexplained.

It is, therefore, of the highest importance to gain a clear insight into the means of modification and coadaptation.

Charles Darwin, 1859

Introduction

The interaction between parasites and their hosts have always been a captivating subject in evolutionary biology. In the beginning, studies were focused on macroparasites, parasites visible to the naked eye, such as helminths and arthropods and their associated diseases but with the advent of the microscope, unicellular parasites were discovered and later on, the sometimes complex parasite life cycles were disentangled (Power 2001). A clear example of parasitism was described in the first pages of 'The origin of species' by Darwin (1859) where he reported the parasitic plant mistletoe and the need of understanding the adaptations between vector, parasite and host (see epigraph). In a later chapter, he also described the interactions with its tree hosts and how many mistletoe plants could kill their host tree. Ever since, if not before, biologists have turned their attention to host-parasite interactions.

Parasitism has evolved among many different organisms: viruses, prokaryotes, protists, helminths and arthropods, thus establishing itself as a common way of living. Although parasites can be lethal for the host, it is in the interest of the parasite to keep its host alive (Esch & Fernández 1993; Power 2001). Parasite infections generally cause a negative fitness effect on the host and the intensity depend on the severity of the infection (Sheldon & Verhulst 1996). This negative effect can result in an increased cost in mounting the immune response against the parasite, especially under stressful situations, such as host's breeding behavior or adaptation to harsh environment, when the host can allocate fewer resources to fight off parasitic infections. (Ilmonen et al. 2000; Zuk & Stoehr 2002). Moreover, the impact of a parasitic infection can affect individuals and species differently depending on if they co-evolved or being exposed to the parasite for the first time (Boughton et al. 2011).

Allocating life resources between growth, reproduction and maintenance is a balancing game that favors individuals that can maximize their lifetime reproductive success (Sheldon & Verhulst 1996). In environments where parasites are frequent, individuals with rare alleles and genetic makeup tailored for parasite resistance show a selective advantage (Hamilton 1980). This should lead to a fixation of beneficial alleles, but as a parasite also can adapt to its environment, there will be an evolutionary arms-race with host and parasite trying to overcome each other thus maintaining genetic variation (Van

Valen 1973). The importance of genetic diversity in the interaction between hosts and their parasites is one of the fundamental cornerstones for the co-evolutionary arms race between them (Carius et al. 2001; Radwan 2008).

In natural animal populations, the major histocompatibility complex (MHC) is frequently studied as it is tightly linked to individual fitness, mate choice and conservation (Edwards & Hedrick 1998; Piertney & Oliver 2005). The MHC is an essential component of the so called acquired immune system and contains genes which mediate the presentation of antigens on the cell surface in order to be recognized by T- and natural killer cells. When a pathogen antigen has been recognized it will trigger the immune response where the antigen is presented in the MHC peptide binding region (PBR) and it is here where most of the allelic variation can be found. Individual MHC alleles have been associated to parasite resistance (Westerdahl et al. 2005; Hedrick 2006). On the other hand, the huge allelic richness can pose a problem when studying parasite effects, a way to overcome this is to group different MHC alleles into functional supertypes based on the similarities in the PBR allowing an easier association between the different MHC haplotypes response to parasite infections. This has successfully been applied in a number of MHC studies (Trachtenberg et al. 2003; Schwensow et al. 2008; Sepil et al. 2013; Lillie et al. 2015; Meyer-Lucht et al. 2010; Pilosof et al. 2014). The evolutionary consequences mediated by parasites do not apply selective pressure only on genes involved in the immune defense. A classic example in humans is the case of sickle cell anemia, where the individuals that are heterozygote for a hemoglobin gene have higher protection from severe cases of malaria caused by *Plasmodium falciparum* compared to the homozygotes (Hedrick 2011).

Akin to human malaria, avian malaria parasites belong to the genera *Plasmodium*, *Haemoproteus* and *Leucocytozoon,* which often exhibit a strong host specificity. They can be found in a wide variety of birds and are spread by dipteran vectors (Bensch et al. 2000). Many studies of haematozoans demonstrated modest effects on their hosts, while other have shown more dramatic effects (Höglund et al. 1992; Siikamäki et al. 1997; Merino et al. 2000; Samuel et al. 2015; Bosholn et al. 2016). It has been shown that after the first infection avian malaria leaves the cardiovascular system for the liver where it become latent and asymptomatic and can give rise to a new outbreak when the animal is under stress conditions, for example during breeding or migration (Valkiūnas et al. 2002; Scheuerlein & Ricklefs 2004).

The correlation between stress levels in birds and parasitic infections have been confirmed by several studies. *Atoxoplasma* is another protozoan parasite of the genus *Isospora* commonly found in birds. Although the effects of Atoxoplasmosis are not yet well understood in captive birds (Schrenzel et al. 2005; Adkesson et al. 2005), the disease has been associated with negative effects, such as decreased activity and weight loss. Symptoms are most

common in juveniles and stressed birds (Adkesson et al. 2005). House sparrows (*Passer domesticus biblicus*) infected with *Atoxoplama* show high mortality when under stressed, especially when the individual also have an accessory *Leucocytozoon* infection (Gill & Paperna 2008). *Atoxoplasma* is transmitted via feces contaminated food and it colonizes epithelial cells in the intestine spreading from there to liver, spleen, lungs and other organs via leucocytes (Schrenzel et al. 2005; Hõrak et al. 2004; Gill & Paperna 2008).

Just like avian malaria parasites and *Atoxoplasma*, helminths can be found in the liver. However, helminths (often referred to as intestinal worms) are most often found in the gastrointestinal tract where they are generally asymptomatic, but can cause symptoms in severe cases. Helminths found in other tissues are considered more harmful and can cause tissue damage and death (Ferreira & Andrade 1993; Soveri et al. 2000; Fuehrer et al. 2011). Helminths represent a diverse group of parasites that can have quite different effects on the host, for example, *Capillaria hepatica* can cause liver disorder and death (Ewing & Tilden 1956; Ferreira & Andrade 1993) while individuals infected with *Taenia taeniaeformis* can develop immunity (Lightowlers et al. 1984).

Parasites affect the hosts in various ways. The impact can be found at the genomic level (Edwards & Hedrick 1998; Hedrick 2006) which translates into changes in gene expression and immunitary responses or at a phenotypic level with changes in host behavior and phenotype. In this thesis I have been investigating parasite frequencies in different wild populations and combined them with observational and genetic data to study the interactions between parasites and three host species: Great snipe (*Gallinago media*), Tree sparrow, (*Passer montanus*) and Water vole (*Arvicola amphibious*).

Research aims

Specific research aims explored in this thesis are:

1. Prevalence of parasites in different populations (Paper I, III, IV)
2. How parasite infections affect life span (Paper I, III)
3. How parasite infections influence mating success (Paper II, III)
4. Associations between mating success, heterozygosity and polymorphism in expressed genes (Paper II)
5. How multiple helminth parasites infections drive the genetic diversity in MHC class II (Paper IV)

Methods

Species studied

Great snipe

The great snipe is a wader bird that migrates between Africa and the breeding grounds in northern Europe (Fiske & Kålås 1995). On the breeding grounds males are gathering on leks where they display and defend small non-resource based territories that the females visit only for mating (Höglund & Lundberg 1987; Höglund & Alatalo 1995). Breeding habitats have a disjoint distribution from the Scandinavian mountain range to the Yenisei River in Russia with a gap in lowland Scandinavia and Finland. In the Scandinavian mountains, leks are situated on wet mountain slope areas near the tree limit (Kålås et al. 1997) and in the east on costal meadows and flood plains (Kuresoo & Leibak 1994). Mating is highly skewed and only a few males reproduce (Höglund & Alatalo 1995).

On the lek females can be seen copulating with the same or other males on multiple occasions during several nights. After mating the female take responsibility of nesting, incubating and feeding the chicks while the males are attracting more females. After the mating period, the birds return to Africa (Fiske & Kålås 1995).

Field work was conducted between May and June in the study populations during various years, especially mentioning Gåvålia, Norway where the long time study is conducted, in this thesis covering the years between 1993 and 2010. Birds were caught at onset of breeding season using mist nets during lekking. Blood samples were taken from the brachial vein and stored in 5% DMSO. Behavioral observations were made each night during the mating

season mating success of individual males was estimated as described in Fiske & Kålås (1995) and Sæther et al. (2000). Only males observed in detail for at least 5 days during the mating season were assigned mating success.

Tree sparrow

The tree sparrow is a resident passerine bird, abundantly found close to farms, gardens and parks throughout Asia and Europe. It nests in holes or nest boxes (Cramp & Perrins 1994; Summers-Smith 1995). Although it has been considered to be monogamous, molecular screening of parenthood reveals occasional polygynous mating (Cramp & Perrins 1994), and about 30% of the chicks have been fathered by other males than the social male (unpubl. data).

For this study, sampling took place between 1999 and 2004. The data consist of blood samples and demographic information from two Swedish populations situated in farmed areas, Uppsala-Ekhaga (59º50'N, 17º48'E) and Ösmo (58º59'N, 17º58'E). In Uppsala-Ekhaga, the study area was located within an organic farm, while Ösmo used conventional farming. At these study sites males are about 5% larger than females (Svensson 2006).

After being caught with mist nets or trapping, the tree sparrows were individually ringed with color rings, measurements and weight was taken as well as blood samples. Catching took place in winter prior to or during breeding season during the study period. Tree sparrows were monitored regularly to determine breeding success and matings. Birds rearing the chicks were determined by the color rings using spotting scopes. Observed polygynous matings were controlled for in subsequent analyses as it is known to affect brood survival (Svensson, unpublished data; Reid, Monaghan, & Ruxton, 2002).

Water vole

Water voles (*Arvicola amphibius* formerly *A. terrestris*) are large semi-aquatic microtine rodents (~300g) found in riparian habitats, where they have their own territory in small colonies. They are vegetarian and eat grass and herbs close to the water body (Stoddart 1970; Lawton & Woodroffe 1991). Its natural range is from Europe to Western Asia.

Water voles were sampled from three *A. amphibius* populations in Sweden (Katrineholm 58°59'16"N, 16° 8'8"E; Östermalma 58°57'22"N, 17° 9'0"E; Uddevalla 58°21'3"N, 12° 0'10"E) using underground traps in at least 5 field sites per population. After catching, they were euthanized and brought to the lab where they were dissected and parasites were collected from the liver.

Molecular methods

DNA extraction (I, II, III & IV)

DNA was extracted using a standard high salt precipitation protocol (paper I, II & III) (Paxton et al. 1996), a standard phenol-chloroform procedure (paper III) (Sambrook et al. 1989) or Qiagen DNeasy Tissue Extraction Kit for Blood and Tissue (paper IV). To verify DNA quality prior to parasite PCR, samples have been used to amplify various markers, including microsatellites and MHC class II alleles (paper I & II) (Unpubl. data; Ekblom et al. 2004; Ekblom et al. 2007). Quality control for tree sparrow DNA was performed with sexing primers P2/P8 described in Griffiths et al. (1998). Concentrations have been measured using Nanodrop (Thermo Scientific).

Molecular parasite screening and sequencing (I, II, III)

To amplify parasite DNA, a nested PCR approach was used to separate two groups of hematozoan parasites (*Plasmodium*/*Haemoproteus* and *Leucocytozoon*) (I, II & III). The external PCR step was performed to amplify a section of the mtDNA *cyt b* with the primer pair HaemNFI/ HaemR2L (Hellgren et al. 2004) and inner PCR for *Plamsodium/Haemoproteus* with HaemF/HaemR2 (Bensch et al. 2000) and the inner for *Leucocytozoon* primers Haem FL/HaemR2L was used (Hellgren et al. 2004). . Nine positive avian malaria samples were sequenced on an ABI PRISM™ 377 with Big Dye 3.1 chemistry (Life Technologies).

Atoxoplasma parasites (III) was screened using a nested PCR method described by Adkesson et al. (2005). This method amplifies a region of the 18S ribosomal RNA gene and it was designed to amplify *Isaspora* parasites but has proven successful for *Atoxoplasma* parasites (Adkesson et al. 2005). *Atoxoplasma* belong to the genus *Isospora* and the term is only used for extra-intestinal stages in birds.

RNA extraction and sequencing (II)

RNA was extracted using RNeasy Protect Animal Blood Kit (Qiagen) and cDNA was synthesized using the MINT kit (Evrogen). cDNA was purified through QIAquick PCR purification columns (Qiagen) prior to sequencing. Tagged sequencing libraries of each individual were prepared and sequenced on one full plate of GS FLX Titanium (Roche 454) at the Uppsala SNP&SEQ Technology Platform (www.sequencing.se).

Great snipe transcriptome (II)

The great snipe transcriptome was *de novo* assembled in gsAssembler utilizing data from all individual simultaneously after removing sequencing adapters, low quality reads and cDNA synthesis primers. Sequences longer than 100 bp were functionally annotated using BLAST (Altschul 1997). BLAST searches ware performed against the chicken (*Gallus gallus*) genes (International Chicken Genome Sequencing Consortium 2004) and zebra finch (*Taeniopygia guttata*) genes (Warren et al. 2010).

To investigate gene expression levels, each individual sequencing library was mapped back to the isotigs generated from the assembly. Reads that mapped to alternative splice variants of the same gene were only counted once and reads that mapped equally well to two or more genes were removed. In order to compare the gene expression profiles of successful and unsuccessful males, the 14 individuals were clustered into two groups, mated and unmated. Read counts per each gene and individual were extracted from the '454GeneStatus' files from the mapping results. To identify SNPs in the transcript sequences, reads from all individuals were mapped to the contigs using gsMapper with the 'cDNA' mode and its default settings. The sequencing data for each individual were then mapped back onto the assembled contigs to estimate the SNP genotypes for each individual using gsMapper. All computations were performed using the computational resources provided by SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (Uppmax).

Water vole genotyping (IV)

DNA extracts of water voles were sent to SNPsaurus (http://snpsaurus.com/) for genotyping by sequencing. SNPsaurus uses a nextRAD technique with selective primers for binding and amplifying genomic DNA. SNP markers were obtained from the nextRAD sequencing for sequences with a minimum read depth coverage of 10. SNP markers were extracted with a minimum minor allele frequency of 5% if the amplification success across all samples was at least 90% per loci. After the SNPsaurus protocol there were had exactly 4,000 SNP markers left for downstream analysis.

For genotyping the MHC, primers JS1 and JS2 (Schad et al. 2004) were used to amplify a 171 bp fragment of MHC class II DRB exon 2 that includes the largest part of the peptide binding region (PBR). The primers were originally designed for the Gray mouse lemur, *Microcebus murinus*, but have successfully been applied to *A. amphibius* and other rodents (Meyer-Lucht & Sommer 2005; Oliver & Piertney 2006; Bryja et al. 2007; Tollenaere et al. 2008). Forward and reverse primers were modified at the 5' end for Illumina MiSeq sequencing with an individual 8 bp barcode and a sequence of three N (to facilitate cluster identification). Amplicons were marked with an individual combination of a forward and a reverse barcode for individual identification. PCR was conducted according to the protocol given in Schad et al. (2004). Purified samples were quantified and combined to 4 equimolar pools. Library preparation and sequencing was performed on an Illumina MiSeq at the National Genomic Infrastructure (NGI), hosted at SciLifeLab in Uppsala, Sweden (Lampa et al. 2013).

Data analysis and statistical methods

Paper I

Observational data for each bird was used from the same year as the DNA sampling. Age was determined based on tail feather analysis (Fiske 1994). The minimum estimate of age was used for each adult individual and birds were assumed to be at least two years old if they were caught as adults. To test for differences in parasite prevalence a GLM with binomial distribution and

logit link function was used. Statistical analysis was conducted with the software R 2.4.0 (R Development Core Team 2006).

Paper II

Differential gene expression analyses were conducted using the R/Bioconductor (Gentleman et al. 2004) and edgeR (Robinson & Oshlack 2010) packages. The effect on mating success (number of females mated) of the genotype for each SNP were tested with Generalized Linear Models, fitted with a Poisson error distribution and a log link function in R. Differential expression levels (mated-unmated) were plotted against the effect size for each locus. Significance threshold (p-value (*p*)) was set to 0.01 to avoid false positives. Genetic differentiation was investigated between mated and unmated males using the 'population differentiation' option in GenePop (http://genepop.curtin.edu.au/) (Raymond & Rousset 1995) testing for both genic differentiation and genotypic differentiation for each locus separately using Fisher's exact test.

The genotypes of 9 candidate SNP loci and 28 non-candidate loci were tested for associations with mating status (mated or not) in a data set consisting of 146 males caught and studied at the Gåvålia site during several years and mating seasons (see Fiske & Kålås 1995; Sæther et al. 2000; Sæther et al. 2005). Multiplex primers were designed for the 37 SNP markers flanked by regions of, at least, 100 bp on both sides. To test for geographical and spatial genetic structure, three methods were used: outlier analyses in LOSITAN (Beaumont 2005; Storz 2005; Antao et al. 2008), STRUCTURE analyses (Falush et al. 2003) and DAPCA analyses (Jombart 2008).

Birds from the Gåvålia site was used in tests for associations between parasitism and mating success and birds from all sites for looking at relationships between genotype and malaria. All standard statistical analyses and handling of large datasets were performed using R 2.15 and later versions.

Paper III

Body condition index (BCI) was calculated for each bird, which is an index based on body weight relative to body size (Schulte-Hostedde et al. 2005). To test differences in survival rates between infected and uninfected individuals, software MARK 4.2 was used to estimate survival rates (Φ) and re-capture probabilities (p) using maximum likelihood methods (White & Burnham

1999; Cooch & White 2004). The models were then compared based on the best AIC score. Effects on breeding success was calculated using GLMMs. All statistical calculations and analyses were made using R 2.4.0 (R Development Core Team 2006).

Paper IV

Genetic clustering was performed on water vole individuals with the STRUCTURE software across our sampling sites. STRUCTURE incorporates multi-locus genotype data to assign individuals to a range of populations (Pritchard et al. 2000). After identifying the population structure, population summary statistics were calculated within and among populations using SPAGEDI (Hardy & Vekemans 2002). Wright's F_{ST} statistic was calculated across and between all populations to determine the degree of population differentiation

MHC sequences were demultiplexed and filtered for artifacts and chimeras with software jMHC (Stuglik et al. 2011) after extraction with software FLASH (Magoč & Salzberg 2011). For each individual PCR, all alleles were visually inspected to remove any combination of two sequences that could be classified as chimeras and template switching. Only sequences with open reading frames were considered and compared to sequences deposited to Genbank. Any sequences containing insertions or deletions were discarded. Identified MHC alleles were grouped into functional supertypes based on their physicochemical properties (Sandberg et al. 1998). Therefore, the codons in the functionally important PBR (according to Bondinas et al. (2007)) were described with five z-descriptors defined by Doytchinova and Flower (2005). With these data, the alleles were grouped with a Hierarchical Cluster Analysis (HCA) in R (R Core Team 2016).

Associations between parasite infections and MHC supertypes were tested using co-inertia analysis with R-package 'ade4' (Dray & Dufour 2007) and linear mixed models with automatic model selection using R-packages 'Lme4' (Bates et al. 2015) and 'MuMln' (Bartón 2016).

Results and discussion

Avian Malaria Prevalence Affects Survival in Great Snipe (*Gallinago media*) (I)

The total parasite prevalence was 30.0% among the adult individuals. 28.6% tested positive for *Plasmodium/Haemoproteus* (P/H), 4.9% for *Leucocytozoon* and 3.5% were double infected. Two of the twelve populations studied stand out with higher prevalence than the others: Separated by 3 km, Bekkelaegret showed twice the prevalence observed at Gåvålia. In Hemavan all eight male birds were infected by P/H parasites.

Mendes et al. (2005) studied several wader species in their breeding habitats and winter habitats (two and 12 species respectively) and found no infected birds in summer habitats and 18% infected in the winter habitats. The 30% reported in this study lay within the range that is normally reported for other bird species, although the great snipe is a wader living in wet habitat and is expected to be exposed to more malaria vectors.

Contrasting to our study, studies on marine and costal waders show very low prevalence or absence of malaria parasites (Peirce 1981; Earlé & Underhill 1993; Valkiūnas 2004; Mendes et al. 2005; Yohannes et al. 2009). Furthermore, low infection rates can be seen in birds overwintering and migrating through marine/saline habitats (Figuerola 1999). Differences in prevalence can also be explained with the higher sensitivity of the nested PCR for low parasitemia (Hellgren et al. 2004) compared to the single PCR used by Mendes et al. (2005).

In the main study site Gåvålia, there was no trend in prevalence for either P/H or *Leucocytozoon* among years through 1993-2001 (**Fig 1**). While some studies report temporal fluctuations in parasite prevalence (Allander & Bennett 1994) others do not (Siikamäki et al. 1997). The samples from these studies were collected during a limited number of years, so long term fluctuations may have gone undetected. Not only can the prevalence fluctuate, but also different malaria parasite strains can fluctuate in prevalence as reported in great reed warblers (*Acrocephalus arundinaceus*) (Westerdahl et al. 2005; Bensch et al. 2007). In our long-term data series, we found

fluctuations in parasite prevalence for both parasite groups at the main study site, however there were no trend showing an increase or decrease. Absence of trends in malaria prevalence is not strange as the great snipe habitat has plenty of water and potentially steady abundance of parasite vectors and is also a demonstration of the importance of long time studies.

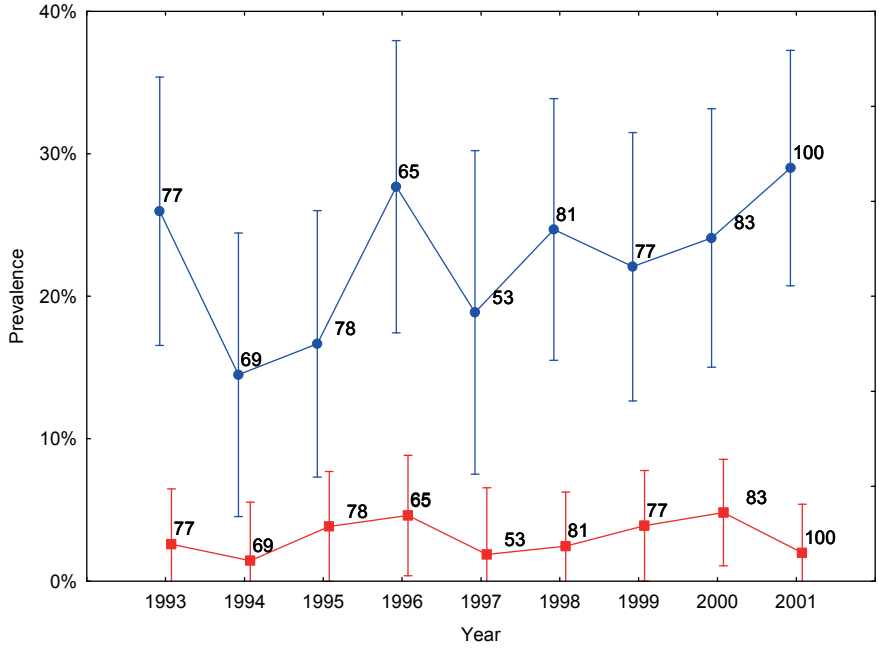

Fig. 1. Variation in *Plasmodium/Haemoproteus* prevalence (blue circles) and *Leucocytozoon* prevalence (red squares) in Gåvålia. Numbers indicate sample size and bars denote 95% confidence interval.

Since the snipes can be found in two distinct habitats, we divided them into flood plain and mountain groups respectively, but found no difference in prevalence among them (P/H: $\chi^2 = 1304.5$, df = 1, $p = 0.48$, *Leucocytozoon*: $\chi^2 = 428.9$, df = 1, $p = 0.49$). There was no difference in prevalence between the sexes for either of the two parasite lineages, P/H ($\chi^2 = 732.9$, df = 1, $p = 0.061$) and *Leucocytozoon* ($\chi^2 = 186.6$, df = 1, $p = 0.33$).

Nine of the positive samples were sequenced, four for *Leucocytozoon* and five for *Plasmodium/Haemoproteus*, and they yielded one sequence for *Leucocytozoon spp*. and three sequences for *Plasmodium spp*. The *Leucocytozoon* sequence had a 99% BLAST match to infected birds in southern Europe, Africa and South America, while the *Plasmodium* sequences had a 99% match to *Plasmodium* found in African birds.

From the sequences we can deduce that most of the infections occur in their winter habitat. However it cannot be ruled out that infections also occur at the breeding grounds, especially for females that stay longer. We might expect a difference if females spend a longer time in a habitat with less parasites. However, no difference was found between sexes. The absence of sex differences is consistent with other studies (Krone et al. 2001; Pérez-Tris & Bensch 2005)

In the main study site, Gåvålia, P/H prevalence decreased with age (χ^2 = 945.93, df = 4, p = 0.01), but not so for *Leucocytozoon* (χ^2 = 334.90, df = 4, p = 0.20). P/H infected individuals have a lower mean age than uninfected individuals (χ^2 = 328.99, df = 1, p = 0.02), but this pattern was not found for *Leucocytozoon* (χ^2 = 333.29, df = 1, p = 0.33).

The results show that *Plasmodium/Haemoproteus* infected birds have lower mean age than uninfected and that the prevalence in older birds is lower. In contrast to our study, other bird studies report an increase of parasitized birds with age (Höglund et al. 1992; Krone et al. 2001; Scheuerlein & Ricklefs 2004). In accordance with Arriero & Møller (2008) we found that birds infected with P/H had a higher probability of being infected with more parasites (in our study *Leucocytozoon)*, and we saw no change in this relation among age classes. A common pattern in observed host-parasite relationships is that young birds have higher intensity of parasites compared to adults (Allander & Bennett 1994; Sol et al. 2003), and the mortality is higher for young birds with high parasite loads (Sol et al. 2003).

Blood transcriptomes and *de novo* identification of candidate loci for mating success in lekking great snipe (*Gallinago media*) (II)

Males infected with avian malaria parasites had lower mating success than uninfected males. Our results thus suggest that physically exhausting lek displays could be used by females as indicators of 'good genes' while selecting their mates and that genetic variation for such traits is present in the population. The lek mating system of the great snipe requires that males are in good health, condition and physical status for them to be able to secure

matings. The frequent territorial fighting and exhausting display behavior would be very demanding for unhealthy individuals and, since males are forced to work, any diseased individuals would either not be able to maintain their territories or would be discriminated against by females.

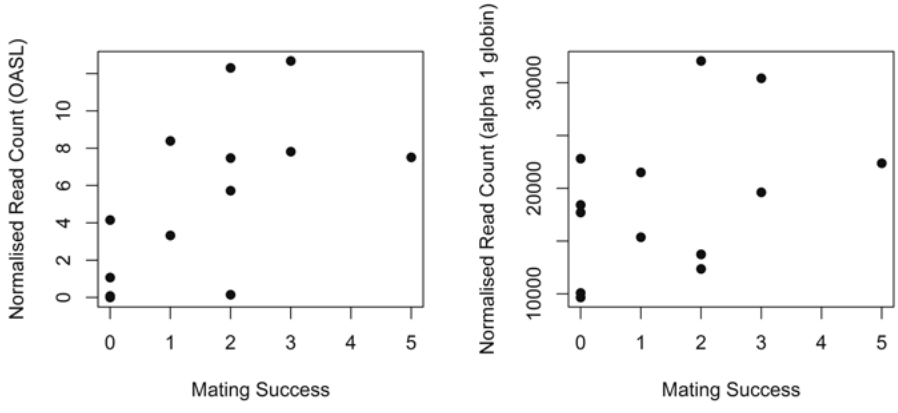

Fig. 2. Correlation between mating success and individual gene expression levels plotted for the three transcripts with significant differential expression. Left: *OASL* (r_s=0.68, n=12, *p* =0.004). Right: *HBAA* (r_s=0.37, n=13, *p* =0.09).

The most highly-expressed transcript in peripheral male great snipe blood cells encodes for an orthologue to α-1 globin chain in chicken and zebra finch (*HBAA*). HBAA is a protein involved in oxygen transport (Richards et al. 1979). *HBAA* also tended to be overexpressed in in the males who obtained matings than in the unmated males and the normalized read count of individual males seems to be correlated with mating success (**Fig. 2**). Genetic polymorphisms for the genes encoding haemoglobin have been detected among a large number of species and many of these polymorphisms have been shown to correlate with, and explain local adaptations to hypoxia (Jessen et al. 1991; Storz et al. 2007; Weber 2007), physical work ability (Gardner et al. 1977), anemia (Ingram 1957) and malaria resistance (Allison 1954). Thus, the literature support evidence that hemoglobin molecules are subjected to selection and that different variants may be favored in different situations.

We found significant relationships between mating success and higher expression of the 2'-5'-oligoadenylate synthetase-like gene (*OASL*). OASL has a primary function in RNA degradation and has been found to mediate resistance to flaviviruses, such as the West Nile virus in chicken (Tatsumi et al. 2003; Tag-El-Din-Hassan et al. 2012). Genetic variants of this gene in humans have also been shown to be associated with various cardiovascular-related traits (Middelberg et al. 2011) and displays antiviral activity against encephalomyocarditis virus and hepatitis C virus (Rebouillat et al. 1998; Marques et al. 2008; Ishibashi et al. 2010). Thus, there is a possibility that the observed variation in expression in this gene between mated and unmated males are related viral infection resistance. A possible explanation is that mated males, in good physical condition, can upregulate certain genes (like *OASL* and possibly *HBAA*) (**Fig. 2**) and allow them access to females and matings while unmated males may have been forced to upregulate other genes.

Fitness Effects of Coccidian and Avian Malaria Parasites in Tree Sparrows (III)

Atoxoplasma was found in 56.5% of the adult tree sparrows, varying from 53% in Ösmo to 61.6% in Uppsala-Ekhaga. Among juveniles, *Atoxoplasma* was found in 12.5% of the screened individuals. 5.9% were infected with the avian malaria parasites *Haemoproteus*/*Plasmodium*, 4.0% in Uppsala-Ekhaga and 2.7% in Ösmo. *Haemoproteus*/*Plasmodium* was found in 3 of 311 screened juveniles (<1%) and *Leucocytozoon spp.* was only found in two juvenile birds and one adult bird sampled in Uppsala, of a total of 306 screened adult birds The prevalence is relatively low in comparison to many other studies of avian malaria in wild birds (Valkiūnas et al. 2003; Ishtiaq et al. 2007; Scheuerlein & Ricklefs 2004; Westerdahl et al. 2005), but it also follow the low prevalence House sparrows (*Passer domesticus*) at high latitudes (Marzal et al. 2011). The prevalence of *Atoxoplasma* in blood in wild bird species has not been thoroughly examined previously, although it is believed to be relatively abundant (Schrenzel et al. 2005; Adkesson et al. 2005; Gill & Paperna 2008).

After between 6 and 24 months, 40 birds had a new blood sample taken were analysed for *Atoxoplasma* and of these, 35% were negative at the first occasion and positive on the seconds. 15% were infected at both occasions

while 27.5% of the positive birds were found negative the second occasion. Hence, 60% acquired an *Atoxoplasma* infection between sampling occasions. The analyses of re-captured individuals also showed that individuals positive at the first capture can be negative at later occasions or that the parasites in the blood are blow PCR detection level.

When both parents reared the chicks, parasite infections did not appear to affect the breeding success significantly. When taking only the male parent into account (Coeff. = -1.317, s.e. = 0.637, $\chi^2 = 4.264$, $p = 0.039$) A negative effect of *Atoxoplasma* was found. Broods parented by birds infected by *Atoxoplasma* or *Plasmodium*, neither had different nestling survival or hatching success compared with uninfected birds. There might possibly have been a negative effect on the nestling survival in cases when the male parent had *Atoxoplasma*, though this could be ambiguously concluded. A possible explanation can be that polygynous fathers had lower nest attendance and chick feeding (Svensson. personal observation; Reid et al. 2002).

Table 1. Estimates of survival rates (Φ) from the four most parsimonious survival models are shown. The first column shows to the estimated monthly survival rate for the different groups included in each model respectively. The standard error of the estimate and a calculated yearly survival rate is also shown. (*Atoxoplasma* and *Plasmodium/Haemoproteus* infected groups pooled).

Model	Φ estimate (monthly)	s.e.	Φ(yearly)
Φ(const) p(pop,t)	$\Phi_{All} = 0.944$	0.006	50.01%
Φ(U-status^2, Ö-const) p(pop,t)	$\Phi_{U\text{-}ninf} = 0.960$	0.008	61.54%
	$\Phi_{U\text{-}inf} = 0.935$	0.011	44.36%
	$\Phi_{Ö} = 0.930$	0.014	41.49%
Φ(status^2) p(pop,t)	$\Phi_{ninf} = 0.953$	0.008	55.91%
	$\Phi_{inf} = 0.936$	0.009	45.24%
Φ(pop) p(pop,t)	$\Phi_{U} = 0.948$	0.007	52.97%
	$\Phi_{Ö} = 0.929$	0.014	41.49%

U = Uppsala, Ö = Ösmo, ninf = not-infected, inf = infected. Status^2 denotes a grouping in which birds were grouped as uninfected or infected (*Atoxoplasma* and *Plasmodium/Haemoproteus* pooled).

In the capture-recapture analysis, parasite infections had a negative impact on the survival, although it was only apparent in one of the study populations (**Table 1**). In Uppsala-Ekhaga the overall survival was 50%, but when taking parasite prevalence into account, uninfected individuals had a 66% yearly survival compared to 44% for infected. Telfer et al. (2002) discussed the underestimation of pathogen mortality: when the data consists of point samples it is not known when an individual becomes infected and dies relatively fast after infection and thus remain in the uninfected group while others survives and appear in the infected group when sampled. This might have led to an overestimation of the infected state and an underestimation of a pathogens negative effect.

Relationship between helminth infections and MHC class II supertype diversity in natural water voles (*Arvicola amphibius*) populations (IV)

Population Katrineholm had 21% *C. hepatica* infected individuals, while it was absent in the other two populations. Also, *T. taeniaeformis* was more frequent in Uddevalla and Östermalma populations than in Katrineholm. In total three individuals were double infected with two parasites. One individual from Uddevalla was infected with *E. multilocularis* and this parasite was excluded from subsequent analysis.

Among the three populations defined by STRUCTURE, 42 MHC alleles were identified, three of them were identical to previously reported alleles and three were two codons shorter than the rest. indicating up to four loci (three excluding the 165bp alleles) while other water vole studies have reported five alleles and maximum two alleles per individual (Oliver & Piertney 2006) and an allelic richness of 16 alleles in Bryja et al. (2007). Number of loci can also vary between populations (Barbisan et al. 2009). The use of high-throughput sequencing has enabled us to find more alleles within each individual compared to Sanger sequencing.

After defining the codon positions in the PBR for each allele, according to Bondinas et al. (2007), with the five z-descriptors, we classified them into 8 supertypes (ST1-ST8). Supertypes ST4, ST6 and ST7 were associated to a reduced *C. hepatica* and *V. mustelae* infection probability and there was no

supertype associated to an increased infection probability for *C. hepatica* (**Table 2**). In contrast, in both *T. taeniaeformis* and *V. mustelae,* there where supertypes associated to an increase in infection probability and ST1 and ST5 a decrease in *T. taeniaformis*. Opposite effects of ST5 was found between *T. taeniaeformis* and *V. mustelae.*

The most striking pattern in the co-intertia analyses of the parasites was that none of the supertypes were associated with a higher infection rate of *C. hepatica*, while there were supertypes both reducing and increasing infection rate/probability of *T. taeniaeformis* and *V. mustelae*. In humans *C. hepatica* causes serious liver disorders (Ferreira & Andrade 1993) that in many cases are lethal (Ewing & Tilden 1956; Fuehrer et al. 2011). The lethal effects of *C. hepatica* in other species should also be present in water voles; none of the MHC supertypes are associated with an increase in infection rate suggesting selection against alleles causing susceptibility. The positive and negative association between different parasites and MHC together with population genetic differences and differential parasite fauna are a driving forces for maintaining MHC variation in water voles.

Table 2. Comparison of positive and negative effects from co-inertia (A.) and GLMM model averaging analyses (B.). For example; in the co-inertia analysis, individuals with supertype 6 and supertype 8 (ST6 & ST8) are more likely to be infected with *T. taeniaformis,* and indivuduals with ST6 less likely to be infected. Co-inertia effects are manually extracted from the co-inertia vectors and the GLMM effects are taken from the effects table independent of effect size. In B. Supertypes not designated a positive or negative value were not included in the most parsimonious models (dAIC<2).

A. Co-inertia

Parasite	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8
Capillaria hepatica				-		-	-	
Taenia taeniaeformis	-				-	+		+
Vesteria mustelae		(+)		-	+	-	(-)	

B. GLMM

Parasite	ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8
Capillaria hepatica		+		-	-		-	+
Taenia taeniaeformis	-	-		+	-	+	-	+
Vesteria mustelae	-	+		-	+	-	-	

Conclusions and further directions

Parasitism is a common way of living. Almost all living beings experience a parasitic infection during their lifetime and the three study species in this thesis are no exception. In this thesis, together with co-authors, I have studied how parasites affect their hosts in their natural environments.

In papers I, III and IV, we studied the chance of been infected by parasites. Not only does the chance of getting infected differ between the environments the animals are living in, but also the length and time of exposure are critical factors. The great snipe is a bird living in fresh water flood plains and marshlands, where blood sucking dipterans can transmit malaria parasites, so it is hardly surprising to find a high percentage of malaria infected birds. Thanks to a long observational data series, we could identify that there are fluctuations in prevalence between different years, but no statistically significant increase or decrease. It seems likely that weather conditions can influence dipteran vector abundance for example in Africa, where the snipes winter. Another explanation for this could be that different strains of parasites are more successful in different years, and this is something that needs further exploration in the future.

All of the avian malaria parasites sequenced showed similarities to parasites found in African birds, suggesting they have mostly been infected on their wintering grounds. During winter in Europe, the dipteran vectors die so there's no reservoir of parasites that can be transmitted. In general, stationary birds at high latitudes have a low avian malaria prevalence and the tree sparrows we studied in paper III follow this pattern. Together with malaria parasite sequencing, it supports the theory that most of the great snipes get infected in Africa.

The tree sparrows are frequently infected by *Atoxoplasma* parasites. These parasites are transmitted via feces contaminated food. This leads to the question on how the food gets contaminated. Tree sparrows frequently eat seeds and they can abundantly be found on farms handling grains, and the birds gather to feed and defecate on the same place. This is also the case for water voles that ingest helminth eggs via contaminated food. Among the water vole populations, we also found a difference in the parasite fauna, where *Capillaria hepatica* was found in only one of the populations.

The helminth effect on the MHC diversity was studied in paper IV. While some MHC supertypes cause resistance to one parasite, they may not effectively protect against other parasites. The variation in parasites fauna drive the selection for allelic diversity in the MHC.

When the host suppresses or clears an infection, it needs to allocate resources that could have been used for other functions to the immunity response. In paper II we studied the transcriptome of mated and unmated great snipe males. We found a few genes that are differently expressed in mated and unmated males. These genes are involved in immune function and durability in other species. Mating success is definitely condition dependent and the lek mating system require males in good health that can spend a maximum of energy during mating season. We showed that malaria infected males have lower mating success, which could indicate that they have to spend resources to fight infection instead of displaying. For some genes the genotype is important. We found that variation in some of these genes are involved in maintaining a good health status and supports handicap models for sexual selection in this lek mating system.

In the case of the great snipe, the females are solely responsible for rearing the chicks and thus responsible for breeding success. In tree sparrows (Paper III), where both parents can rear the chicks, did not find clear evidence that *Atoxoplasma*-infected birds had lower brood survival than uninfected. However, if we put it in the context of lifetime reproductive success, the results indicate an effect of *Atoxoplasma* infection. Older great snipes, (paper I) are more seldom found infected by avian malaria parasites compared to young birds. This pattern can often be seen among different bird species and need be investigated further to understand the mechanism behind this pattern.

Our different studies in three distinct animal species led to a general conclusion confirming that parasites are a driving force for maintaining genetic diversity and that parasite infections lowers survival rate, which would lead to a lower lifetime breeding success: the ultimate cost of being parasitized.

Sammanfattning

Parasitism är ett av de vanligaste levnadssätten och det finns exempel på parasiter i många olika organismgrupper, bland annat malaria och plattmaskar. Samspelet mellan parasiter och deras värdar har fascinerat oss människor i generationer och de flesta organismer kan infekteras av minst en typ av parasit. Inom biologin studeras förhållandet mellan parasiter och värdar för att ta reda på hur parasiter påverkar olika aspekter av värdens liv. I den här avhandlingen har jag analyserat hur tre olika värdar påverkas av de parasiter som de bär på.

Fågelmalaria, som inte infekterar människor, är en av de vanligaste parasitgrupperna som går att hitta hos många fågelarter. Beroende på om parasiterna och fågelarterna utvecklats tillsammans under en lång tid eller inte, kan de drabbas olika hårt. Till exempel har många fågelarter dött ut eller minskat kraftigt efter att fågelmalaria introducerats på Hawaii. Efter den första infektionen finns parasiterna latent och fåglarna kan få ett nytt utbrott om de blir stressade, till exempel under häckningssäsongen eller under flytten mellan sommarområden och övervintringsområden.

I avhandlingen har jag studerat två fågelarter, Dubbelbeckasin (*Gallinago media*) och pilfink (*Passer montanus*) samt vattensorkar (*Arvicola amphibious)*. Dubbelbeckasiner är en vadarfågel som flyttar mellan Afrika och norra Europa. Med hjälp av blodprov tagna under häckningssäsongen har jag studerat fåglar från olika ställen i Skandinavien och Österuropa. Under häckningen samlas hanarna på lekar där de spelar mot andra hanar och försvarar små territorier som saknar resurser. Honor ansluter sig till leken i ett parningssyfte. Pilfinkar är stannfåglar som man ofta hittar i parker, gårdar och i människors närhet. De häckar i hål och holkar. Länge har de ansetts vara monogama, men de är i själva verket ofta polygama.

Undersökningar i avhandlingen (manus I) visar på att fågelmalaria är vanligt förekommande hos dubbelbeckasinerna. Ungefär 30 % av dem var infekterade med någon av de typer malaria jag studerat med hjälp av molekylära metoder. *Plasmodium* och *Haemoproteus* är de vanligaste malariaparasiterna och de påträffades oftare hos unga fåglar som kommer tillbaka från Afrika för första gången än hos äldre fåglar som övervintrat i Afrika många gånger. Trots att fåglarna lever i en miljö där malariaspridande myggor existerar, varierade

andelen infekterade fåglar mellan olika år. Sammantaget går det dock inte att urskilja något mönster där andelen smittade individer ökade eller minskade med tiden.

En annan påtaglig effekt av att ha malaria visades hos hanarna. Dubbelbeckasinhanarna parade sig inte lika ofta om de var infekterade med malaria. En hypotes är att hanarnas parningsspel är uttröttande och att honorna märker att de inte spelar lika intensivt som oinfekterade. För att undersöka orsakerna sekvenserade vi alla gener som användes under parningen och kom fram till att det fanns gener som användes olika mycket oavsett om hanen var infekterad eller inte. En av dessa gener, *HBAA*, är människans motsvarighet till hemoglobin och parade hanar hade högre genuttryck än oparade. En annan gen som uttrycktes mer hos parade hanar var *OASL* som är inblandad i att skapa motståndskraft för virus. En förklaring till varför parade hanar uttryckte dessa gener i högre uträckning än oparade är att de är i bättre fysisk kondition, medan oparade hanar kan behöva använda andra gener i större omfattning.

Fågelmalaria förekom hos 6 % av pilfinkarna, och det är en vanlig observation hos fåglar i norr. Däremot fanns det en annan parasit som påträffades oftare, och 56 % av pilfinkarna var infekterade av *Atoxoplasma*. Det är en vanligt förekommande encellig parasit som sprids via förorenad mat. *Atoxoplasma* har endast varit föremål för fåtalet studier och de kunskaper som finns kommer främst från tama fåglar. Parasiten påverkar fåglar negativt när de är stressade och då ofta med en dödlig utgång. Pilfinkar som har atoxoplasmos uppvisar högre dödlighet än oinfekterade fåglar. För att jämföra hade infekterade fåglar 45 % chans för överlevnad till nästföljande år, medan motsvarande siffra för de friska fåglarna var 61 %. Dessutom var de infekterade fåglarnas parningsframgång är inte lika stor som hos de friska individerna. Det är främst hos hanarna som effekten på parningsframgång är påtaglig och en förklaring kan vara att polygama hanar inte orkar mata ungarna lika mycket.

Precis som *Atoxoplasma* sprids band- och plattmaskar med förorenad mat. Maskar som förekommer i mag-tarmkanalen är ofta mindre farliga än de som återfinns i andra vävnader, som till exempel levern. I avhandlingen har jag även studerat vilka effekter några av dessa parasiter har på vattensorkarnas genetiska variation i MHC klass II, dvs. en del av immunförsvaret som styr utvecklingen av antikroppar. MHC är en av de delar av genomet som har högst genetisk variation. Den stora variationen gör att det är svårt att studera effekter av en viss allel och för att kunna studera parasiternas påverkan har jag gjort supertyper av allelerna. En supertyp baserar sig på alleler som ger antikropparna ett liknande utseende i området där antigenen binder till, för att sedan presenteras för andra delar av immunförsvaret. I de tre populationer av vattensorkar som jag har studerat påträffades 42 alleler, varav 39 hittills varit okända. Allelerna kunde grupperas i 8 supertyper och vissa supertyper gav

motståndskraft mot en viss parasit, men gjorde samtidigt individen känsligare för en annan parasit. En av parasiterna, *Capillaria hepatica*, hade ingen supertyp som gjorde individen mer mottaglig. *C. hepatica* orsakar stor dödlighet hos olika däggdjur, inklusive människor. Tack vare att artrikedomen bland parasiter varierar mellan populationer och att det finns genetisk variation i olika populationer resulterar i att den genetiska variationen i MHC bibehålls.

De olika delstudierna i avhandlingen pekar på parasiternas dubbla roll och leder till följande slutsatser: även om parasiter har negativa effekter på de djurarter som studerats, vilket i slutänden leder till lägre parningsframgång genom en för tidig död, är parasiter också en av orsakerna till att genetisk variation bibehålls.

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