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# Associations between immune system genes of the bank vole (*Clethrionomys glareolus*) and outer surface protein genes of *Borrelia afzelii* spirochetes

## Abstract

Interactions between hosts and their parasites drive a coevolutionary dynamic, shaping genetic diversity, population structure, and disease spread. This dynamic can promote the maintenance of high levels of polymorphism, often exceeding expectations under neutral evolution. While coevolutionary theory assumes specific genetic associations between hosts and parasites, direct evidence for such relationships, especially in wild vertebrate populations, remains scarce. This thesis aims to contribute to addressing this gap by studying the bank vole (*Clethrionomys glareolus*) and its pathogen *Borrelia afzelii*, a bacterium that causes Lyme disease in humans. The research builds on the fact that bank voles are natural reservoir hosts of *B. afzelii* in Europe. To explore the genetic basis of host–pathogen interactions, the thesis investigates specific gene pairs in both host and pathogen, as well as the bank vole's transcriptomic response to *B. afzelii* infection. Together, these two approaches aim to uncover both specific genetic associations shaped by coevolution and broader patterns of immune activation that may underlie infection tolerance in this natural reservoir host.

The first chapter focused on a pair of host and pathogen candidate genes whose products are likely to interact directly and drive reciprocal selective pressures. Specifically, it investigated associations between the major histocompatibility complex (MHC) of the bank vole, a component of the adaptive immune system, and the ospC gene of *B. afzelii*, which encodes an immunogenic outer surface protein used to manipulate the host's immune response. Twelve variants of OspC were detected in the studied populations of bank voles. The results of Tajima's test suggested that OspC is evolving under balancing selection. Redundancy analysis revealed that neither MHC class II alleles nor supertypes (functional groupings) predicted infection status or infection intensity, as measured by qPCR, with *B. afzelii* strains carrying distinct OspC variants.

Nevertheless, variation in IgG antibody levels against two OspC variants common in the examined populations was significantly associated with specific MHC class II alleles among seropositive individuals. These findings imply that MHC alleles may shape variant-specific immune responses to *Borrelia* OspC, consistent with host–pathogen coevolution.

The second chapter examined another pair of genes involved in host–pathogen interactions: the bank vole complement factor H (CFH), an essential component of the innate immune system and a central regulator of the alternative complement pathway, and the ospE gene of *B. afzelii*, which encodes a surface protein that binds CFH to facilitate immune evasion. The results from PAML and MEME analyses provided evidence that the ospE gene is subject to positive selection. However, redundancy analysis revealed no associations between factor H family genes and OspE variants. Additionally, a haplotype network based on OspE sequences showed no geographical differentiation in the frequency of OspE variants, in contrast to the patterns previously observed for CFH, and co-correspondence analysis did not detect any spatial co-structure between CFH and OspE. Previous research has shown that CFH exhibits stronger population structure than the genomic average in bank voles, likely due to local adaptation. In contrast, findings presented in the thesis indicate that OspE does not reflect this structure, suggesting that the evolution of these genes may be shaped by distinct and possibly unlinked selective forces.

The third chapter utilized RNA sequencing to investigate the transcriptomic response of bank voles to *B. afzelii* infection, with the aim of identifying additional genes involved in host defense and examining how the response of this natural reservoir host differs from that reported in accidental hosts, such as humans. Differential gene expression analysis and weighted gene co-expression network analysis (WGCNA), followed by functional enrichment, revealed activation of genes involved in adaptive immune processes, particularly those related to B-cell function and antibody production. In contrast, the inflammatory response was subdued compared to that reported in humans or mice. Additionally, shifts were observed in the expression of genes related to extracellular matrix, whose protein products are exploited by *Borrelia* to facilitate adhesion and persistence within host tissues. These changes may reflect an evolved tolerance strategy that limits tissue damage and reduces the risk of chronic, systemic

manifestations of the disease. Notably, several enriched pathways were associated with neurodegenerative diseases and included genes involved in energy metabolism, oxidative stress responses, and protein turnover. These findings suggest that *B. afzelii* infection may have broader physiological effects in its natural reservoir host than previously recognized.

In summary, this thesis provides insights into genetic mechanisms underlying host-pathogen interactions in natural bank vole-*B. afzelii* system. It explores how coevolution shapes both specific gene-level associations, such as between MHC and OspC, and broader transcriptomic responses to infection, highlighting immune modulation consistent with evolved infection tolerance. Together, these findings advance our understanding of host–parasite coevolution and the ways in which natural reservoir hosts manage persistent infections.

Keywords: host-pathogen interactions, bank vole, *Borrelia afzelii*, Lyme disease