The role of disease risk and life history in the immune function of larks in different environments

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CHAPTER 1

General introduction

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Preface

This thesis investigates the role of disease risk and life history in shaping the immune function of larks (Alaudidae) in different environments. I use data collected from birds living in the mountains of Afghanistan, in the deserts of Saudi Arabia, in remnant plateau grasslands in Kenya, and in a national park in the Netherlands. Birds in all these environments must overcome the universal challenges associated with survival and reproduction. Different species have evolved different means – that is, different life histories – to accomplish this. One essential component of self-maintenance and survival is defence against the diverse and unrelenting threats of infection and disease to which all species are continually exposed. It is primarily for this purpose that the immune system has evolved. Within and among species, the form and function of the immune system varies, which might be due to different life histories or the disease landscapes that different species inhabit. Investigating how these two possibilities contribute to immunological variation is a key aim of this thesis.

Ecological Immunology

All animals possess some form of immune system. This is the set of anatomical, chemical and physiological defences that together protect an animal from the foreign organisms and substances, including its own abnormal cells, that might do it harm. This highly complex, multi-layered, and often redundant system is essential to life and offers many benefits, including minimising the negative impacts on fitness exerted by infections and disease (Brown, Brown and Rannala 1995; Fitze, Tschirren and Richner 2004). However, immune systems are costly in terms of the energy and time required by their development, maintenance and usage (Schmid-Hempel and Ebert 2003; Klasing 2004) and because of the 'collateral damage' to the body's own healthy cells that may result from some immune responses (Råberg *et al.* 1998). This means that a maximal immune response is not necessarily an optimal one (Viney, Riley and Buchanan 2005). Depending on the levels of resource availability and disease threat, animals may respond differently in terms of the type and the extent of immune response (Sheldon and Verhulst 1996). Understanding and explaining this type of immunological variation within and among species are central goals of ecological immunology, which uses immunological measures to test ecological and evolutionary hypotheses (Sheldon and Verhulst 1996; Sadd and Schmid-Hempel 2009). Studies in ecological immunology are typically conducted in non-domesticated animals, often in free-living conditions, rather than in traditional model species maintained under controlled, laboratory settings. Understanding the causes of immunological variation, rather than the particular molecular mechanisms, is the goal.

A question of costs and benefits

Since the outset, ecological immunology has used a cost-benefit framework as a powerful means to explaining why and how immune systems should vary. One approach, taking a cost-based perspective, relies heavily on life history theory (Roff 1992; Stearns 1992) and considers immune function as a costly physiological trait of an individual that is involved in unavoidable trade-offs with other physiological processes such as growth or reproduction. As a trait that contributes towards self-maintenance, and hence survival and opportunities for future reproduction, immune function is expected to have evolved alongside other life history traits in order to maximise fitness. Ecological immunologists interpret trade-offs involving self-maintenance as one explanation for the variation in immune defences observed among animals (Sheldon and Verhulst 1996; Norris and Evans 2000; Schmid-Hempel 2003). While this approach to explaining immune variation has been productive, the conclusions may be incomplete. Indeed, many ecological immunology studies fail to appreciate the benefits that immune defences convey.

Thus, a second approach to understanding immunological variation considers the benefits of the immune system. The main benefit, of course, is the protection against infection by fitness-reducing foreign organisms such as parasites and pathogens (disease-causing agents). Given the costs associated with the immune system, immune defences are predicted to be higher or stronger when the risk of infection is greater (Lindström *et al.* 2004; Tschirren and Richner 2006; Horrocks, Matson and Tieleman 2011). High-risk environments might include the tropics (e.g. Møller 1998; Guernier, Hochberg and Guegan 2004) and freshwater (e.g. Piersma 1997; Mendes *et al.* 2005). A high degree of sociality, large group sizes or aggressive interactions between individuals are examples of behaviours that could increase the risk of disease transmission and infection (e.g. Semple, Cowlishaw and Bennett 2002; Snaith *et al.* 2008; Spottiswoode 2008). Despite these examples, finding appropriate measures to assess the disease risk encountered by an individual is not straightforward. For example, different species may encounter different types of disease risks as well as different levels of exposure, and measures applicable to one environment might not be appropriate in another. Instead, researchers have often quantified immune defence levels and used these as justification for prior assumptions about how disease risk may vary. Alternatively, the abundance and diversity of parasites on a host are used as indices of disease risk. These measures are likely already confounded by current host defences, both behavioural (e.g. grooming, parasite avoidance) and immune (Moyer, Drown and Clayton 2002). In chapter 2, my co-authors and I address some of these issues head-on. We outline a framework within which immune function and the selective pressures exerted by pathogens can be considered and highlight how a greater understanding of the threats posed by infectious agents can help put variation in immune defences into perspective. We introduce the concept of the immunobiome - all the living organisms that can live in or on a host and with the potential to evolve in response to immune defences. Interactions between the immune system and the particular immunobiome of an animal shape its immune defences, both over evolutionary and ecological time-scales. We also tackle the practical problem of how to measure pathogens and broader disease risk in different environments, by suggesting molecular and other methods that may be appropriate for use in ecological immunological studies.

A comparative approach using the lark family

Birds are often studied in ecology and ecological immunology because they are generally easy to observe and capture, and tend to occur in sufficient densities that large numbers can be studied. Individual birds can be non-invasively marked with colour rings, and life history variables such as clutch size can be relatively easily measured. In chapter 3 we first encounter the larks (Alaudidae), the family

of birds that provide the study system used in this thesis. At first glance, larks may appear an unusual choice of study species. These songbirds are relatively cryptic in appearance and conceal their nests on or close to the ground. These qualities might make larks less tractable to experimental manipulations than, for example, box-nesting species. Nonetheless, the lark family provides an ideal study system for the comparative approach that I took in this thesis. The comparative approach is most powerful when comparing related species, avoiding complications that arise from different evolutionary histories. All lark species eat similar foods and behave in similar ways, meaning that comparisons among species are not confounded by diet or behaviour. Despite these similarities and the universal preference of larks for open grassland habitats, different species experience a wide range of climatic and environmental conditions, ranging from hyper-arid to mesic and tropical. The behaviour, physiology and life history of several lark species living under these different conditions have already been intensively studied (reviewed in Tieleman 2005). However, until now the immune systems within and among lark species have been poorly characterized. Since the diverse climatic and environmental conditions experienced by larks are thought to reflect differences in terms of disease risk (i.e. hyper-arid habitats, low disease risk; mesic and tropical habitats, higher disease risk), larks provide a potent tool for studying the ecology and evolution of immune function. Overall, the lark family provides a powerful study system where behavioural and physiological aspects are well understood, where life histories and environmental disease risks among species vary, and where understanding of the causes and consequences of immune system variation represents the next frontier.

Immune defence along a gradient of predicted disease risk

I use lark species to address a question that is central to both my thesis and to ecological immunology: to what extent are environmental disease risk and life history responsible for influencing immune investment and driving observed patterns of immunological variation? Both disease risk and life history have been used previously to explain variation in immune responses among and within species. However, the two often co-vary, making it difficult to determine which factor is more fundamentally associated with immune variation. Furthermore, in certain circumstances, predictions about immune investment based on these factors can diverge (chapter 2). In chapter 3, I relate multiple immune indices, measured in 23 populations of 12 lark species, to proxies of environmental disease risk and indices of life history. Unlike some comparative study systems in which predicted disease risk and life history are positively correlated, these potential explanatory variables vary independently among lark populations. This independence provides an opportunity to disentangle their relative roles in shaping

immune variation. I found that in larks, indices of innate immunity were strongly and positively correlated with abiotic proxies of environmental disease risk. In contrast, life history traits related to reproduction showed little relation to investment in innate immunity.

I used the gradient of disease risk again in chapter 4 where, together with my co-authors, I explored variation in immune defence using similar analytical techniques, but from a slightly different perspective. I investigated patterns of the antimicrobial proteins ovotransferrin and lysozyme in the albumen of lark eggs collected along the gradient of disease risk. Eggs provide a simplified model of the immune system, characterised by fewer defence components and fewer infection risks. Furthermore, comparing and contrasting the relationships between disease risk and immune defences in birds and their eggs helps to identify the selective pressures that have shaped each type of defence. Declines in egg viability, microbial loads on eggshells, and trans-shell infection of eggs are higher in more humid environments (Cook *et al.* 2003; Beissinger, Cook and Arendt 2005; Cook *et al.* 2005a; Cook *et al.* 2005b; Wang, Firestone and Beissinger 2011). This led us to predict that if egg antimicrobial defences have evolved to match the risk of microbial infection, then concentrations of antimicrobial proteins in eggs should vary with environmental conditions. In that case, eggs from humid locations should contain higher concentrations of ovotransferrin and lysozyme than eggs from more arid environments. The results of chapter 4 show that ovotransferrin concentrations matched our prediction but concentrations of lysozyme showed opposite patterns and were highest in arid environments with low disease risk. This raises interesting questions regarding the function of lysozyme in avian eggs as well as suggesting possible trade-offs between antimicrobial proteins in the albumen. The study also revealed that precipitation, one proxy of environmental disease risk, had very little power to explain patterns of antimicrobial variation, despite experimental evidence pointing to the importance of moisture for trans-shell infection (Cook *et al.* 2005a; Shawkey *et al.* 2009; D'Alba, Oborn and Shawkey 2010). Temperature was better than precipitation at predicting concentrations of antimicrobial proteins in eggs, but temperature was a poor predictor of immune defences of larks along the disease risk gradient (chapter 3). This contrasting role of temperature perhaps reflects the ectothermic nature of eggs and the endothermic capacities of birds.

Environmental and seasonal variation in immune defence and disease risk

Both chapters 3 and 4 highlight the value of abiotic proxies for biotic variation in disease risk. Nonetheless, in part III of this thesis I followed up on a conclusion from chapter 2, that biotic measures of disease risk components are vital to

understand more fully observed patterns of immunological variation. I introduced a novel air-sampling technique to quantify the abundance of microbes shed from birds and in ambient air. These methods provide both host-dependent and host-independent measures of biotic disease risk. The de-coupling of host defences and potential host exposure represents a significant advancement in ecological immunology and provides a new avenue for future studies. We used these air-sampling methods to assess environmental and bird-associated microbial assemblages in larks living in the Arabian Desert and in the temperate Netherlands. We also measured indices of innate immunity and assessed these in light of predictions arising from disease risk and life history, which has been previously well studied. Supporting the results of chapter 3, environmental disease risk explained more of the variation in immune defences than did life history. Temperate larks, which were exposed to higher concentrations of airborne microbes, and carried denser microbial assemblages, also exhibited higher innate immune indices than their desert-living counterparts did. In contrast, the life history explanation of immune variation, which predicted higher immune investment in desert-living larks, was not supported.

Variation in disease risk is observed between environments (chapter 5), but variation in disease risk within an environment may also be a significant driver of immune variation. In chapter 6, I report that immune indices change seasonally in larks living in the Arabian Desert. I also show that these changes are accompanied by parallel modulations in the microbial densities shed by birds and contrasting modulations in the microbial concentrations in the wider environment. This study underscores the necessity of both host-dependent and host-independent indices when quantifying disease risk and the results raise interesting questions about the environmental scale at which animals respond immunologically to microbes.

A contribution to the ecologists' immunological toolbox

Despite considerable progress in the last fifteen years, the desire of ecological immunologists to study non-domesticated, free-living species is also their Achilles' heel. Reagents for non-model species are generally unavailable, and protocols suitable for laboratory-controlled animals may be unsuitable for animals that are living free. In the final part of this thesis, I contribute to the ecologists' immunological toolbox by validating a field-friendly assay for the measurement of ovotransferrin (chapter 7). Ovotransferrin is an acute phase protein that increases in the blood in response to inflammation and infection. The same antimicrobial protein is also found in the albumen of avian eggs, as we report in chapter 4.

Conclusion

In larks it is disease risk rather than life history that explains variation in investment in innate immune defences (chapters 3 and 5). This result provides a counter-point to earlier theoretical and empirical studies that emphasise the importance of life history as an explanation for immunological variation (Sheldon and Verhulst 1996; Norris and Evans 2000; Lee 2006; Martin II, Hasselquist and Wikelski 2006). This new insight has, in part, resulted from the development and application of a method for measuring host-dependent and host-independent axes of disease risk (chapters 5 and 6). These direct measurement methods revealed among-individual and among-population differences in disease risk and helped validate abiotic proxies for use when direct measurements are impossible. The direct measurement methods also revealed insights into and raised questions about the scale at which larks perceive threats and respond immunologically: data in this thesis suggest that this scale might be quite small (chapter 6). Abiotic proxies for environmental disease risk were relied upon when assessing the relative influence of disease risk and life history on immunological variation (chapters 3 and 4). Although these abiotic proxies are useful, well validated, logistically uncomplicated, and easy to understand, further application of direct measurement methodologies offers great promise for shaping our understanding of interactions between disease risk and immune investment. Molecular techniques in particular have the potential to revolutionise the direct measurement of pathogens and other immuno-reactive agents (chapter 2), particularly as their application becomes easier and more practical in diverse field settings. In a similar vein, new assays for measuring additional aspects of immunity (chapter 7) will allow for a more complete characterisation of the immune system. Since its inception the field of ecological immunology has raised and addressed many thought-provoking questions about how and why the immune system is so variable in its structure and its responses. I hope that my thesis contributes to that on-going process by answering some questions and posing many more.