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Life history variation, immune response and survival: evidence for causality?



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Abstract

Life-history theory assumes that organisms face a trade-off between current and future reproduction to maximize their fitness. Life-history theory also assumes that an increase in the current reproductive effort will lead to additional fitness costs. One of these fitness costs can arise when an organism faces the trade-off between reproductive effort and investment in the maintenance of its immune system. Decisions based on environmental and life-history related circumstances during the reproductive period, might therefore influence the functioning of the immune system and ultimately the survival of the organism. In this literature study I focus on the question whether we can find evidence for causal relationships between life history variation, immune response and survival in birds during reproduction. Available data provide causal evidence for relationships between life-history traits and survival in some studies but not in others. Exploration of the relationship between life-history traits and aspects of the immune response revealed causal relationships in a number of studies. Because none of the studies manipulated the immune function itself the causal link between immune function and survival remains unproven. To unravel the causality of relationships between life-history traits, immune responses and survival we need more experimental work to provide a better insight in these complex relationships.

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Introduction

Life-history theory assumes that organisms face a trade-off between current and future reproduction (Holmes & Austad 2004; Korpimäki & Rita 1996; Nordling *et al.* 1998; Tieleman *et al.* 2008). An increase in the investment of current reproduction is presumed to be costly (Deerenberg *et al.* 1997; Hanssen *et al.* 2005; Korpimäki & Rita 1996). This can either result in reduced current reproduction in the short-term or in reduced survival of the individual in the long-term (Hanssen *et al.* 2005). For ecologists, survival has long been the most neglected attribute of fitness, due to the difficulty of quantifying it in the wild. Reproduction, on the other hand, has been monitored and manipulated in various ways and it has become the cornerstone of research effort on developing life-history theories (Lochmiller & Deerenberg 2000). I have chosen to focus on studies carried out on adult birds during their reproductive stages. These birds have some advantages for experimental research due to the defined start and ending point of reproduction and the easy evaluation of relative success, reproductive effort and reproductive costs (Lochmiller & Deerenberg 2000). To estimate the effect of an increase in the investment of reproduction on immune response and survival several manipulations are performed. In this literature study I focus on experimentally challenged adult birds but I will also discuss three studies where no manipulations were performed. Studies without manipulations can be important to estimate the amount of naturally occurring variation in a certain factor. Mechanisms involved in these life-history related processes are complex and hard to get grip on. I will focus on the role of immune function in relation to life-history traits and survival, because it has become increasingly clear that the mechanisms regulating both factors are far from mutually exclusive (Lochmiller & Deerenberg 2000). Life-history theory states that the development of immune function should be somehow balanced between the allocation of resources to reproduction and mechanisms that contribute to individual survival (Deerenberg *et al.* 1997; Tella *et al.* 2002).

The immune system evolved to protect the body against different kinds of pathogenic challenges. It consists of a complex network of overlapping and interlinked defense mechanisms. Based on their temporal dynamics immune responses are generally classified in two extremes: constitutive and induced responses. Constitutive immune responses are constantly maintained and therefore provide a system of surveillance and repair. The induced immune response is triggered after a pathogen has established itself in the body. In general we can state that constitutive immune responses are non-specific whereas induced immune responses are specific for a certain pathogen (Buehler 2008; Holmes & Austad 2004; Saino *et al.* 2003). Immunocompetence can be viewed as the ability of an organism to minimize pathogenic infections via constitutive and induced immune responses. Mounting immune responses and maintaining the complex immune system are thought to be nutritionally demanding processes that require trade-off decisions between competing nutrient demanding processes like growth, reproduction and immunity itself (Lochmiller & Deerenberg 2000). Apart from nutritional status, many life-history traits as well as environmental circumstances show to affect immunity factors like geographical variation within species (Ardia 2005a), degree of sociality within species (Moller *et al.* 2001), senescence (Saino *et al.* 2003; Shanley *et al.* 2009), drought (Fair & Whitaker 2008) and reproductive effort (Ardia 2005a; Ardia 2005b; Deerenberg *et al.* 1997; Hanssen *et al.* 2005; Ilmonen *et al.* 2002; Nordling *et al.* 1998).

The studies described in this literature review show different measures of immune responses. The blood parasite *Haemoproteus* often causes infections in birds and it can be used as an indicator of infection status (Nordling *et al.* 1998). Lymphocyte concentrations in the blood are sometimes used as an indirect measure of cell-mediated immunity. High heterophile/lymphocyte ratios have shown to be indicative of stress. Decreases of the albumin concentration in the blood plasma can be found in the occasion of almost any disease as well as malnutrition. After chronic or acute infections and with inflammatory processes, an increase in the globulin concentration is expected which indicates the activation of the humoral immune system. As a result of that, healthier individuals show higher albumin/globulin ratios (Kilgas *et al.* 2006). The antigens sheep red blood cells (SRBC), diphtheria toxoids and tetanus toxoids are often used to test the specific antibody response of the immune system. SRBC have a large number of different recognition sites where the antibody binds to the antigen (epitopes) what results

in less specific immune responses. Diphtheria toxoids and tetanus toxoids contain only a few epitopes and therefore lead to more specific immune responses (Hanssen *et al.* 2005). The Newcastle disease vaccine (NDV) is used to test the ability of the immune system to produce specific antibodies (Nordling *et al.* 1998). The functioning of the innate immune system can be evaluated by the ability of the blood to kill or phagocytise different pathogens.

In this literature study I will try to find an answer to the question whether we can find evidence for causal relationships between life history variation, immune response and survival in birds during reproduction.

This literature study focuses on three different patterns, all with a potential relationship based on causalities (figure 1). The hypothesis for pattern 1 holds that manipulation of life-history traits results in a negative consequence for survival probabilities. The hypothesis for pattern 2 holds that manipulation of life-history traits results in a reduction of the immune response. The hypothesis for pattern 3 holds that survival is influenced by the current state of immune responses.

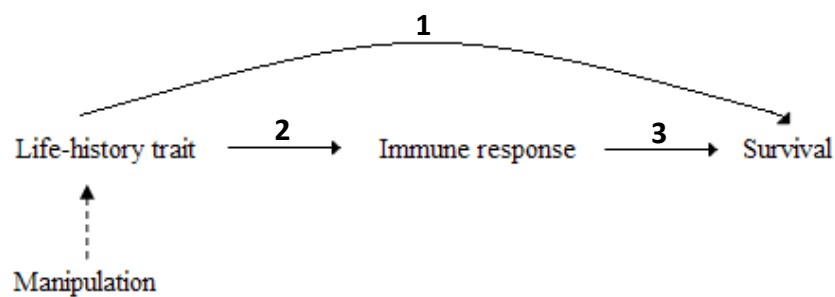


Figure 1 Scheme of possible causal relationships tested in this literature study. Numbers indicate the three patterns. Manipulations performed in studies used in this literature study were only conducted on life-history traits.

1. Relationship between life-history traits and survival

Many experiments tried to establish a relationship between a life-history trait and next-years survival in terms of reproduction in birds. In this chapter I will discuss four studies (table 1).

Table 1. Summary of studies exploring pattern 1 with overall conclusion of research per study.

Study	Conclusion	Authors
1.1	Effect of increased female reproductive effort on annual survival	Visser & Lessells (2001)
1.2	No effect of increased female reproductive effort on return rates	Hanssen <i>et al.</i> (2005)
1.3	Effect of brood enlargement on mortality during winter	Daan <i>et al.</i> (1996)
1.4	No effect of brood enlargement on local survival	Korpimäki & Rita (1996)

Visser & Lessells (2001) performed an experiment to explore the relationship between female reproductive effort and annual female survival in the Great Tit (*Parus major*). The researchers experimentally created three different groups: *full costs* where the females had to lay and incubate additional eggs, *free eggs* where the females only had to incubate additional eggs and *free chicks* where the females paid no extra cost of producing and incubating additional eggs. The *control* females raised the same number of chicks as the number of eggs laid. Visser & Lessells (2001) found a clear effect of female reproductive effort on female survival where *full cost* females have the lowest, and *free chicks* females have the highest survival (one-tailed OH-test testing the directional hypothesis that the fitness components vary between treatments as $full\ costs \leq free\ eggs \leq free\ chicks$, $P=0.025$; figure 2).

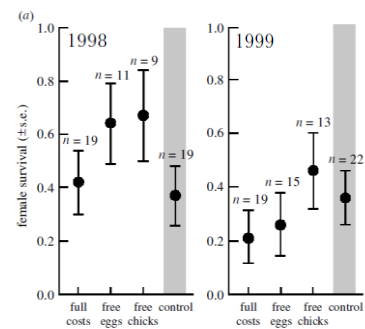


Figure 2 Female survival in relation to experimental treatments differing in the experimentally induced cost of egg production and incubation. Sample sizes are given above each error bar. (after Visser & Lessells 2001)

Hanssen *et al.* (2005) performed a study to investigate the relationship between female reproductive effort and annual return rates. In this study the researchers performed nest manipulations in the Common Eider (*Somateria mollissima*) to create high demand and low demand clutches. Females with *low incubation demand* incubated three eggs, and females with *high incubation demand* incubated six eggs, which reflects the naturally occurring variation in clutch size of the Common Eider. The researchers found no difference in return rates between females with *high demand* clutches and females with *low demand* clutches (logistic model with year as fixed factor $\chi^2=0.12$, $P=0.73$).

Daan *et al.* (1996) studied the relationship between increased parental effort and local survival in adult European Kestrels (*Falco tinnunculus*) in the Netherlands. The researchers were able to create reduced and enlarged broods by using two nests with equal numbers of nestlings of the same age. Subsequently, two nestlings were transferred from their natal nest to a foster nest which created reduced and enlarged nests. Kestrels of enlarged nests accepted the nestlings as if they were their own and adjusted the food provisioning to keep up with the changed food demand from the increased number of nestlings. As mentioned before, a trade-off is expected between reproductive effort and survival. Daan *et al.* (1996) came up with a scientific basis for this trade-off by

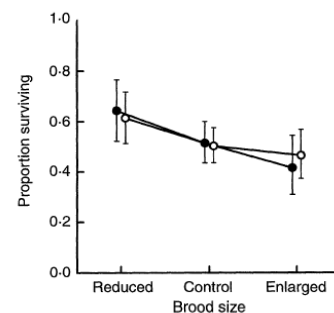


Figure 3 First year survival for kestrel parents after raising a modified brood (proportion surviving \pm 95% confidence interval based on binomial distributions). Open symbols=all birds, closed symbols=only the first experiment for each individual bird was taken into account (Daan *et al.* 1996).

showing that increased mortality during the winter months is due to the experimental enlargement of broods ($\chi^2 = 5.74$; $P=0.017$). Figure 3 shows the annual survival of the three experimental groups.

Korpimäki & Rita (1996) investigated the relationship between increased parental effort and local survival. The researchers studied a breeding population of the European Kestrel (*Falco tinnunculus*) in western Finland where the main food source consist of voles which fluctuate in 3-year population cycles. During three consecutive years, nest manipulations were performed by removing or adding one chick to a nest, creating reduced and enlarged nests respectively. Control nests experienced an exchange of one nestling of the same age between two control nests where the total number of nestlings remained the same. Korpimäki & Rita (1996) did not find an effect of treatment nor year on female survival (Logit analysis $G^2=3.47$, $df=2$, $P=0.18$ and $G^2=2.03$, $df=2$, $P=0.36$, respectively). Survival rates of females of experimentally increased broods appeared to be lower, though not significantly, than for females of control broods with an odds ratio being 0.58 (95% c.i. 0.18 to 1.90) (When the odds ratio equals 1 there is no effect; values > 1 indicate an increase in survival probability; values < 1 indicate a decrease in survival probability).

2. Relationship between life-history traits and immune response

This chapter will start with a general study on the relationship between life-history and immune defences across species, followed by four other studies concerning this relationship.

Table 2 Summary of studies exploring pattern 2 with overall conclusion of research per study.

Study	Conclusion	Authors
2.1	General explaining strongest predictors for immune response	Lee <i>et al.</i> (2008)
2.2	Effect of increased female reproductive effort on immune response	Hanssen <i>et al.</i> (2005)
2.3	No effect of increased female reproductive effort on immune response	Ilmonen <i>et al.</i> (2002)
2.4	Effect of increased female reproductive effort on immune response	Nordling <i>et al.</i> (1998)
2.5	No effect of increased reproductive effort on immune response	Tieleman <i>et al.</i> (2008)

Lee *et al.* (2008) performed a study to investigate the influence of reproduction on naturally occurring antibodies and complement activity in birds. The method used in this study is somewhat different from the ones described above. During the breeding season of two consecutive years, the researchers caught over 70 different species of Neotropical birds. Blood samples taken from each bird enabled the researchers to investigate the naturally occurring antibodies and complement activity in the plasma. To estimate relationships between species, Lee *et al.* (2008) created a phylogenetic tree from published phylogenies based on molecular data. Incubation period came up as the strongest predictor of antibody titre. Species with long incubation periods show high levels of circulation natural antibodies (conventional model $F=21.96$, $P<0.001$ and phylogenetic analysis $F=16.07$, $P<0.001$). Clutch size was the only life-history variable that was a significant predictor of complement activity (conventional analysis $F=5.28$, $P=0.025$ and phylogenetic analysis $F=7.03$, $P=0.01$).

Hanssen *et al.* (2005) studied the relationship between a life history trait and the humoral immune response. In this study, clutch size manipulations were performed in nests of the Common Eider (*Somateria mollissima*) to create nests with low and high incubation demand. Females with *low incubation demand* incubated three eggs and females with *high incubation demand* six eggs. Five days after clutch completion, the females were caught and the first blood sample was taken. After blood sampling, females were injected with 150 μ l of diphtheria-tetanus vaccine in the pectoral muscle and 1 ml of a 2% suspension of sheep red blood cells (SRBC) intraperitoneally to induce the immune response. Fifteen days after the first injection birds were recaptured and the researchers took another blood sample. The results show that *high incubation demand* caused a reduction in the number of females responding against tetanus and diphtheria, but it did not affect the tendency to mount a response against SRBC injection (figure 4). However, among the individuals that responded to the injection, females with *high* and *low incubation demand* did not show any difference in the magnitude of antibody response to the antigens SRBC, diphtheria and tetanus (ANCOVA $F=0.12$, $P=0.56$; $F=0.00$, $P=0.96$ and $F=0.15$, $P=0.70$ respectively). The researchers concluded that lymphocyte levels were lower in females with *high incubation demands* ($F=5.46$, $P=0.02$).

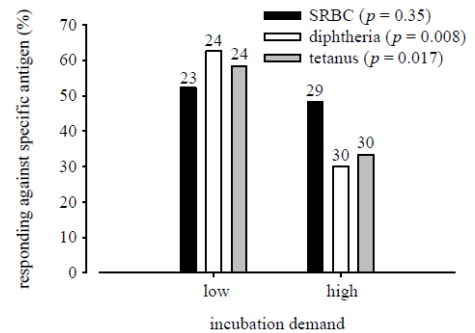


Figure 4 Percentage of individuals with measurable antibody responses against SRBC, diphtheria and tetanus. Sample sizes are given above each bar. (Hanssen *et al.* 2005)

Ilmonen *et al.* (2002) studied the relationship between a life history trait and immune response. In this study incubation manipulations were performed by adding or removing eggs from the clutch of females of the Pied Flycatcher (*Ficedula hypoleuca*) which manipulated the incubation effort of females. Two or three days after clutch completion, two eggs were transferred from the natal nest to the foster nest. At this time, females were caught and blood sampled prior to being immunized for the first time with a diphtheria-tetanus vaccine injection in the pectoral muscle. After two weeks (2-3 days after hatching of the chicks), a second blood sample was taken to measure the primary humoral immune response and females were immunized for a second time. Eleven to twelve days later, when the chicks were about to fledge, a third blood sample was taken to measure the secondary immune response. Incubation manipulations show no effect on the females primary or secondary immune response (primary response to diphtheria $F=0.53$, $P=0.59$; primary response to tetanus $F=0.40$, $P=0.67$; secondary response to diphtheria $F=0.52$, $P=0.60$ and secondary response to tetanus $F=1.79$, $P=0.18$ respectively).

Nordling *et al.* (1998) investigated the relationship between a life history trait and immune response. For this study the number of nestlings was manipulated to increase the reproductive effort of females in two populations of the Collared Flycatcher (*Ficedula albicollis*). Two days after hatching, two nestlings were transferred from their natal nest to the foster nest. This resulted in two experimental brood-size groups: reduced and increased brood-size. Exchange of two nestlings between two nests was applied in the control group. The two breeding populations of Sproge and Oggesänget (Baltic island of Gotland, SE Sweden) differ in habitat and are separated by a distance of about 30 kilometers. Females were immunized three days before the expected hatching date of their clutch by means of an injection containing Newcastle disease vaccine (NDV). Two weeks later, the females were recaptured and a second blood sample was taken. The female population in Sproge showed a clear negative relationship between brood size manipulation and the level of NDV-specific antibodies (ANOVA: $F=3.42$, ordered heterogeneity (o.h. test: $r_sP_c=0.955$, $P=0.0075$) whereas the population in Oggesänget showed no relationship at all (ANOVA: $F=0.01$, o.h. test: $r_sP_c=0.004$, $P=0.49$). Researchers also investigated the parasite prevalence and intensity of *Haemoproteus* in relation to the manipulation of the number of chicks. For this experiment, only non-immunized females were screened for infections by *Haemoproteus* to rule out any possible effects of vaccination on parasite resistance. Infected females were found to show a positive relationship

between experimentally increased reproductive effort and the intensity of infection with *Haemoproteus* (Kruskal-Wallis: $H=6.05$, $df=2$, o.h. test: $r_s P_c=0.951$, $P=0.008$).

Tieleman *et al.* (2008) investigated the relationship between a life-history trait and immune response. In this study, the wingspans of adult tropical house wrens (*Troglodytes aedon*) were manipulated by clipping primary feathers to increase the energy costs of flapping flight during chick rearing. The level of handicap reflects the naturally occurring variation in wingspan of tropical house wrens. To estimate the effect of increased energy costs during chick rearing on the innate immune response, researchers looked at three different aspects of the innate immune system. Increased energy costs during chick rearing showed no effect on the ability of fresh whole blood to kill *E. coli* bacteria, to phagocytise *E. coli* bacteria or to phagocytise *S. aureus* bacteria (bactericidal ability *E. coli* $F=0.17$, $P=0.67$; phagocytosis *E. coli* $F=2.97$, $P=0.10$ and phagocytosis *S. aureus* $F=0.04$ and $P=0.85$).

3. Relationship between immune response and survival

Only few studies have been performed on the relationship between immune response and survival. In this last chapter I will discuss two studies (table 3).

Table 3 Summary of studies exploring pattern 3 with overall conclusion of research per study.

Study	Conclusion	Authors
3.1	Effect of reduced immune response (by natural infection) on mortality	Nordling <i>et al.</i> (1998)
3.2	Effect of haematological parameter status on annual survival	Kilgas <i>et al.</i> (2006)

Nordling *et al.* (1998) investigated the relationship between immune response and survival. In this study researchers used female collared flycatchers (*Ficedula albicollis*) that were not subjected to any experimental manipulation. Every female was caught and bled while feeding young and the blood was screened for the presence of *Haemoproteus* infections. Females were then classified as either infected or not infected and survival rates could be compared between both groups. Because of high site fidelity, females who did not return to their breeding area within two consecutive years were classified as non-survivors. The results showed that females suffering from *Haemoproteus* infections indeed had higher mortality levels than females without *Haemoproteus* infections (all ages: $\chi_c^2=4.98$, $df=1$, $n=272$, $P=0.026$; figure 5). When the researchers statistically separated the one-year-old females from the older females they found an even more pronounced difference in mortality. Juveniles showed a significant increase in mortality levels when infected with *Haemoproteus* (juveniles: $\chi_c^2=6.73$, $df=1$, $n=97$, $P=0.0095$; figure 5) while older infected females show no significant increase in

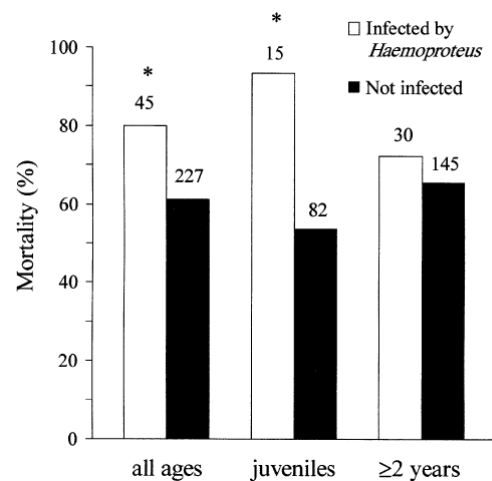


Figure 5 Difference in annual mortality rates between females who are infected by *Haemoproteus* and uninfected control females. First pair of bars shows all females in the test sample (all ages), the second pair of bars shows the one-year-old females (juveniles) and the third pair shows the older birds (≥ 2 years). Asterisks indicate significant differences in mortality rates. Sample sizes are given above bars. (Nordling *et al.* 1998)

mortality (≥ 2 years: $\chi^2=0.38$, $df=1$, $n=175$, $P=0.54$; figure 5)

Kilgas *et al.* (2006) investigated the relationship between haematological condition indices and survival. This study was performed on reproducing great tits (*Parus major*) not subjected to any experimental treatment. Adults were captured on the nest when nestlings of the first brood were at least eight days old and blood samples were taken to measure several haematological parameters. Because of high site fidelity and low breeding dispersal, those birds that were not recorded to be breeding in the year following the measurements were categorized as non-survivors. The researchers found that local survival did not differ between the sexes ($\chi^2=0.05$, $P=0.82$). In males surviving birds showed higher lymphocyte levels than non-survivors, while in females this relationship was not found (males: Wald=5.14, $P=0.023$; females: Wald=0.69, $P=0.406$). High albumin/globulin ratios during breeding enhanced the survival probabilities of great tits (Wald=7.95, $P=0.005$) whereas surviving individuals showed lower globulin ratios than non-survivors (Wald=6.33, $P=0.012$). Surviving individuals also had significantly lower heterophile/lymphocyte ratios than non-survivors (Wald=4.98, $P=0.026$).

Discussion

Relationship between life-history traits and survival

In the first study by Visser & Lessells (2001), the researchers emphasize that the negative effect of increased female reproductive effort on survival can only be found in their population when the females have to pay additional costs of egg-production and incubation. Female survival of *free chicks* females compared to *control* females does not show the same result whereas females with larger clutches (*free chicks* females) show higher survival rates than *control* females.

The study by Hanssen *et al.* (2005) shows no direct effect of manipulation of incubation demand within the naturally occurring variation in clutch size on female survival. However, the future reproductive investment is found to be reduced for females with high incubation demands. This might indicate a long-term consequence of increased reproductive effort on survival.

The study by Daan *et al.* (1996) shows that parents with enlarged broods increase the amount of daily time spent hunting and increase their daily energy expenditure (DEE) in response to an increased number of nestlings. The mechanisms that cause the relationship between increased parental effort and mortality are yet poorly understood. The fact that increased mortality is found during the winter months instead of the breeding season, suggests that the experimental increase in parental effort during the breeding season did not affect the predation risk and immunocompetence at that certain time. Daan *et al.* (1996) came up with a second explanation: the 'rate of living' theory, which states that degenerative cell degradation which underlies aging, senescence and death is related to the rate of metabolic energy turnover (Daan *et al.* 1996 and references therein; Saino *et al.* 2003). An experimental increase in DEE might indicate a disproportional relationship with increased mortality.

Korpimäki & Rita (1996) state in their study that it might be expected that the long-term brood manipulations show an obvious repercussion on subsequent parental survival. The breeding kestrels of western Finland face unpredictable changes in food abundance in subsequent years. This might influence the amount of experience the

birds possess of the local trends in food abundance. If food abundances are unpredictable, it might be beneficial to invest in the birds own survival, instead of the survival of their offspring. A study of Dutch kestrels by Dijkstra *et al.* (1988) shows that they experience food abundances that vary in a more predictable way during the breeding season. Korpimäki & Rita (1996) state that these birds therefore had better experience with local food conditions whereas the Dutch voles do not show such 3-year population cycles and steep crashes in vole numbers only rarely occur. Nutritional conditions during the breeding season may play an important role in the trade-off between parental effort and survival.

Is there a causal relationship between life-history traits and survival? Based on these four studies I did find evidence for causal relationships. However, I did not find a univocal answer to the question. Increased costs of reproduction by means of egg production show a negative effect on survival. On the other hand, when increasing the incubation demand by clutch size manipulations, researchers did not find an effect on survival. Increased reproductive effort by means of clutch size manipulation during chick rearing shows an effect on survival in one study but not in the other. Both studies deal with the same study species but in two geographically different places. The differences found may be related to the amount of manipulation which differs between the studies. The studies indicate that birds do increase their reproductive effort when challenged with experimentally increased clutch sizes. However, the increase in reproductive effort does not automatically result in a decrease in survival probabilities. The timing of manipulation in these experiments seems to be an important factor whereas manipulation during the incubation period shows no effect and manipulation during chick rearing does show an effect on survival. Many life-history and environmental factors influence birds during their reproductive periods. Adults face the trade-off between self-maintenance and reproductive investment. Decisions based on behavioural differences as well as nutritional status can severely influence the outcome of this trade-off. The conclusion by Korpimäki & Rita (1996) that the experience of the birds with respect to the local food abundances shows to be important for survival looks a bit far-fetched. I think that the food availability and nutritional status during reproduction are more important than the experience of the birds with local food-abundances over the years. Considering these studies I conclude that there are indications for causal relationships between increased reproductive effort and survival but more research has to provide better insight in these complex and probably time-related relationships.

Relationship between life-history traits and immune response

The study by Lee *et al.* (2008) shows that 'slow-living' species with long developmental times rely more on their antibody-mediated immune defence. The conclusion is based on the fact that incubation period is the strongest predictor of natural antibody levels. Long-living species are expected to favour adaptive immunity because overall they will face greater numbers of infections and the chance to face the same pathogen again is more likely. 'Fast-living' species are thought to emphasize a developmentally inexpensive innate immune defence. This has found to be true whereas Lee *et al.* (2008) show a positive relation between clutch-size and complement activity.

Hanssen *et al.* (2005) show in their study that a high incubation demand was found to be related to reduced humoral immune responsiveness against two antigens (tetanus and diphtheria). Increased incubation demands can partly be compensated for in breeding passerines by increasing the food intake. Common Eider females do not eat during the entire incubation period and therefore they are less able to compensate for increased incubation costs.

In the study by Ilmonen *et al.* (2002) an increase in reproductive effort on immune response showed no effect. One possible explanation given by the authors is that even if incubation is costly it is still likely to be less

energy-demanding than nestling provisioning. This would result in the ability of females to increase the incubation effort without immediately risking their immune functioning. Life-time reproductive success of females might also be favoured by investment in self-maintenance and immunity during the early breeding phase, whereas the costs of incubation are paid later during the chick rearing phase by reducing the parental effort when parental duties can be shared with the male.

The study by Nordling *et al.* (1998) shows that an increase in the reproductive effort within the naturally occurring variation in clutch size, causes a decrease in a specific immune response to a potential pathogen. The fact that the females from Sproge show a negative effect of increased reproductive effort on NDV-specific antibodies and the females of Oggesänget show no effect, could be due to differences in habitat quality and the small sample size in Oggesänget (n=10). The authors state that reproductive suppression of the immune system might be influenced by environmental factors in natural populations. Based on the findings of the intensity of *Haemoproteus* infection, Nordling *et al.* (1998) suggest that there is a reproductive trade-off with parasite resistance and that this will presumably work via suppression of the immune system. Another general statement made by the researchers is that birds infected with one pathogen (e.g. *Haemoproteus*) have an increased probability of being infected with yet another one. This poor immune function might be considered as a common cause of infection for numerous potential pathogens. This study, concentrating on only one pathogen, might therefore be an indicator of low parasite resistance in general and the increase in the intensity of infection as a measure of that resistance.

In the study by Tieleman *et al.* (2008) the authors show that tropical house wrens adjust their behaviour in response to a handicap-treatment, which results in a constant energetic work effort during reproduction. Different measures of innate immunity proved to be unaffected. This study shows that tropical house wrens do not sacrifice their body condition and immune function when facing increased energy demands during chick rearing.

Is there a causal relationship between life-history traits and immune response? On the basis of these five studies I believe that we can speak of causal relationships. Again, I did not find a univocal answer to the question. High incubation demand by means of clutch size manipulation shows reduced humoral immune responses. When incubation demand is increased soon after clutch completion, no effect can be found in the ability to mount an immune response against certain pathogens during early and late chick rearing. Another study shows that increased costs of reproduction by manipulating the clutch size in the early nestling phase causes a decrease in the level of disease specific antibodies and an increase in the intensity of parasite infection. The last study shows that an experimental increase of energetic workload during chick rearing shows no effect on the innate immune response. The studies that experimentally increased the incubation demand as well as the studies that experimentally increased the reproductive effort during chick rearing show contradictory results. The factors that influence immune functioning are not clear yet. These studies indicate that immune functioning differs between species and is related to life-history traits and environmental circumstances. Birds face trade-offs during reproduction with regard to their energy investments when they have to choose between self-maintenance (including immunity) and reproduction. Food availability seems to be an important factor in this aspect. Birds that are unable to forage while incubating have to pay costs of increased reproductive effort, while other birds seem to be able to compensate for the costs by increasing their food intake. The behavioural aspect is hard to quantify but it seems to play an important role in the energy distribution over the reproductive period. Based on these five studies, it is hard to say that there is a clear causal relationship between increased reproductive effort and reduced immune response due to the different parameters measured. The results do show that relationships can be found between life-history traits and survival within studies. More research has to provide better insight in this complex relationship and identify the factors influencing immune response.

Relationship between immune response and survival

In the study by Nordling *et al.* (1998) the authors show evidence of a relationship between increased mortality and *Haemoproteus* infection but they do point out that reduced parasite resistance cannot be defined by *Haemoproteus* alone. This study does not show a causal link between *Haemoproteus* and mortality because this would require manipulation of the immune system itself. The fact that only young females showed an increase in mortality as a consequence of *Haemoproteus* infection could have two explanations. The first suggests that selection sorts out individuals who are incapable of immunological control of previous infections. The second suggests that parasite-mediated costs of reproduction decrease with age.

The study by Kilgas *et al.* (2006) shows that several haematological parameters influence the survival probabilities of great tits. The researchers state that values of lymphocyte concentrations are not always easily to interpret. Decreased lymphocyte concentrations may indicate a lack of parasite infections but might also signal stress-induced immunosuppression. Kilgas and co-workers (2006) state that the observed sex difference in the relationship between lymphocyte concentration and survival might be caused by differences in the interactions between the endocrine and immune system found in both sexes. Males generally show a higher concentration of testosterone during breeding than females, whereas high concentrations of testosterone can sometimes suppress immunity (Verhulst *et al.* 1999). Kilgas *et al.* (2006) believe that despite the correlational nature of their study, the observed relationships indicate a causal link between some aspects of immunological condition during breeding and future survival.

Can we find evidence for a causal relationship between immune response and survival? Kilgas *et al.* (2006) state that they found a causal relationship between the naturally occurring state of several haematological immune parameters and survival in their study, while Nordling *et al.* (1998) state that they did not find a causal relationship between naturally occurring parasite infection and survival. Both studies have not manipulated the immune system itself, which is a crucial point in quantifying the result of an impaired immune response. Manipulations of the immune system are hard to perform as long as we are not able to quantify the factors that influence the generation and development of the immune system and the interaction and cooperation between these factors. Parts of the innate immune system are already formed in the embryo stages (Ardia *et al.* 2010). This indicates that the timing of the manipulation is very important for the long-term effects. More experimental research has to provide a better view of the factors that influence the immune system and the way in which they do this. From there we might be able to manipulate specific parts of the immune system and measure the consequences in terms of life history variation. These studies show that the age of the individual is important when we consider the relationship between immune response and survival. This finding is not unexpected, whereas the development of the immune system is ongoing and every encounter with a pathogen adds to the state of acquired immunity. Older birds have quite likely experienced more pathogenic challenges than young ones. Apart from the age of the bird, sex also plays an important role due to the difference in hormonal regulation that in turn can influence immune responses. Based on the results of these two studies I conclude that we can speak of consistent patterns between at least some aspects of the immune system in relation to survival but due to the lack of manipulation in both studies, I cannot speak of causal relationships.

Conclusion

Can we speak of *causal* relationships between life-history traits, immune responses and survival? I would say that we can but only in particular cases. Different studies measured different parameters for different bird species of different ages in different habitats and in different stages. The studies shown here came up with a variety of factors influencing immune function and survival. Those factors are not taken into account or they differ between studies in such a way that they become incomparable. So the patterns between studies are not that clear yet. Returning to the hypotheses scheme presented in the introduction (figure 1) I found causal evidence within studies that matches with my hypotheses. Causal evidence can be found for the relationship between the manipulation of life-history traits and negative consequences for survival. Based on the inconsistent results of these studies, hypothesis 1 cannot be fully confirmed. Discussion of hypothesis 2 came up with some causal evidences but also some contradictory results for the relationship between manipulation of life-history traits and reduced immune response. These contradictories make it also hard to formulate a firm conclusion about hypothesis 2. Hypothesis 3 comes up with consistent patterns between naturally occurring immune parameters and parasite infection in relation to survival. Because of the lack of manipulation of the immune system itself, I cannot speak of causal relationships. Definite proof of such causal relationships requires manipulation of the immune system itself. Because of the complexity, the lack of knowledge and the enormous amount of internal and external factors influencing different parameters of the immune system, it is hard to come up with a conclusive way of manipulating it. To start the exploration of this topic, one could try to incubate eggs in a sterile environment to exclude external factors triggering the immune system in the most early stages of development. However, internal factors like hormones and behavioural patterns are hard to control for but have found to be important in the formation and maintenance of the immune system. The laboratory looks like the most suitable place to control various external factors and discover the ones influencing the development and functioning of the immune system. I believe that we have to start with exploring the factors that influence the immune system and the way in which they do this before we can start manipulating it. I conclude that I did find several causal relationships in relation to life-history traits, immune response and survival, but only in particular cases. The exploration of this topic is still 'scarcely out of the egg' and more long-term research has to be performed to get insight in this complex but fascinating topic.

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