Bird-Parasite Interactions

*Using Sindbis Virus as a Model System*

BY

KARIN LINDSTRÖM
Abstract


This thesis focuses on the evolutionary interactions between birds and a parasite, the mosquito-borne Sindbis virus (Togaviridae, Alphavirus). In conclusion, the results show that the Sindbis virus is widespread among birds, and that the fitness of infected hosts may be reduced by the virus. Furthermore, virus clearance ability was revealed by male plumage traits, and viraemia was related to hormonal- and social status.

The distribution of Sindbis virus infections among passerine birds was examined in five areas in Sweden. Almost all species tested were infected, and three species of thrushes were identified as the main hosts. In a series of experimental infections, greenfinches (Carduelis chloris) kept in aviaries were used as hosts. First, the behavioural consequences of an infection were investigated. During the infection, birds tended to reduce their spontaneous locomotion activity, and when escaping from a simulated predator attack, infected birds had reduced take-off speed. Furthermore, when comparing virus clearance rate between male greenfinches, I found that males with large yellow tail ornaments had faster virus clearance rates as compared to those with smaller ornaments. Thus, male virus clearance ability was honestly revealed by the size of an ornament. Moreover, males with experimentally elevated testosterone levels experienced a delayed, but not increased viraemia as compared to controls. When the relationship between male social rank and viraemia was examined, I found no evidence that high-ranked males suffered reduced rank during the infection. Nevertheless, viraemia patterns of males were related to their social rank, so that low-ranked birds had a delayed viraemia as compared to high-ranked birds.

Key words: Carduelis chloris, Sindbis virus, host-parasite interaction, ornament, social dominance, testosterone, sexual selection.

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ISSN 1104-232X
ISBN 91-554-4773-2

Printed in Sweden by University Printers, Uppsala 2000
You can’t always get what you want, honey
you can’t always get what you want.
You can’t always get what you want,
but if you try, sometime you just might find
you get what you need!

Jagger/Richards

To the memory of my mother
This thesis is based on the following papers, which will be referred to in the text by their Roman numerals I-V.

I. Lundström, J.O., Lindström, K.M., Olsen, B., Dufva, R. & Krakower, D.S. Prevalence of Sindbis virus antibodies among Swedish passerines indicate that thrushes are the main amplification hosts. Submitted manuscript.

II. Lindström, K.M., van der veen, I., Legault, B-A. & Lundström J.O. Behavioural alterations of Greenfinches (Carduelis chloris) during a virus infection: are avian virus infections costly? Submitted manuscript.


IV. Lindström, K.M., Krakower, D. Lundström J.O. & Silverin, B. The effects of Testosterone on a viral infection in greenfinches (Carduelis chloris): an experimental test of the immunocompetence handicap hypothesis. Submitted manuscript.

V. Lindström, K.M. Social rank and virus resistance in greenfinches. Submitted manuscript.

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The order of the authors reflects their involvement in the papers. I have personally written and performed all analyses of papers II, III, IV and V. Co-authors in papers II, III and IV collected data, discussed ideas, analyses and commented on the text. In paper I, I collected part of the field data, performed most of the laboratory tests, discussed the analyses and commented on the text.
INTRODUCTION

Most animals share their environments with a rich fauna of parasites. The need to survive and reproduce successfully in these environments has therefore forced birds and other animals to evolve a wide array of defences against parasites (Clayton and Moore 1997). Host-parasite interactions have recently become a central concept in studies of evolutionary ecology due to their impact on sexual selection (Hamilton and Zuk 1982, Andersson 1994). Also, by integrating life-history theory and sexual selection, an interesting new area of research has developed. With this approach it may become possible to understand which factors may limit an individuals investment in immunity (Sheldon and Verhulst 1996, Norris and Evans 2000). Although parasites increasingly have become the focus of much scientific interest, many aspects of the interactions between hosts and parasites are still poorly understood. The aim of this thesis has been to investigate one host-parasite relationship in more detail, by testing some of the ideas put forth to understand the complexity of the evolutionary interactions between hosts and parasites.

Birds and parasites in nature

A parasite can be defined as an organism that lives in, or on another living organism, obtaining from it part or all of its organic nutrients, commonly exhibiting some degree of structural modification, and causing some degree of damage to its host (Price 1980). Viruses are small and simple organisms with an obligate parasitic lifestyle, that utilise host cells for their own reproduction. Parasites in general, and viruses in particular, often have short life-cycles. This gives them a capacity to change in genetic composition between generations, and hosts have to be able to respond to these changes. On an evolutionary time-scale, the interactions between hosts and parasites may therefore be viewed as a never-ending arms race (Morse 1994, Domingo and Holland 1994, Ewald 1994). The composition of the parasitic fauna may also change over time. Birds in nature are subject to infections by many different parasites, some of which appear and disappear in cycles (Clayton and Moore 1997). It is often assumed that parasitic infections are costly for the
hosts, but in practice, these costs can be difficult to measure. Parasitic infections may affect host fitness by causing disease symptoms, which may lead to death of the host. Mild infections may cause morbidity that reduces the host’s ability to avoid predators or even other parasitic infections. Parasites may also have negative impact on host reproduction or competitive ability (Møller et al. 1990, Hudson and Dobson 1997). Paper I describes the distribution of Sindbis virus infections among avian hosts in nature, and paper II investigates if the Sindbis virus can impair infected birds ability to escape from predators.

**Parasite-mediated sexual selection**

In order to explain the evolution of extravagant male ornaments Darwin (1871) proposed theory of sexual selection. Darwin reasoned that males with large ornaments could be favoured by sexual selection if females preferred to mate with such males. He, however, did not provide an explanation as to how females could benefit from such preferences. In order to resolve the benefits of female choice, a series of handicap-models of sexual selection were developed (Zahavi 1975, Grafen 1990). These models proposed that male ornaments were costly to maintain, and therefore only high-quality males should be able to afford these costs. Following this argument, if male quality and ornament size are positively related, and heritable, females would benefit from their choice through increased survival of their offspring. Hamilton and Zuk (1982) developed the handicap-models further, and suggested that it was the ability to resist the currently prevailing fauna of parasites that was the quality revealed by male ornaments. The hypothesis predicts that within species there should be a negative correlation between ornament expression and parasite load. It was mainly this hypothesis proposed by Hamilton and Zuk (1982), that placed parasites in a central position in studies of sexual selection.

To date, a large number of studies on birds and other animals have demonstrated that within species, male ornaments can be used as indicators of parasite load, or immunocompetence, i.e. the ability of an individual’s immune system to resist and control infections (Møller 1990, Andersson 1994, Hamilton and Poulin 1997). Although the Hamilton-Zuk hypothesis has gained much empirical support, their idea has been controversial. It has been argued that the hypothesis is impossible to falsify, as several
factors may explain why the predicted association was not found (Read 1990). It can for example be argued that the cruzial parasite taxa were not included in the analyses. Also, for most studies, the underlying assumption that parasite resistance ability is inherited by the offspring has not been tested, and alternative hypothesis have often not been excluded (Read 1990). Although females may use male ornament to select parasite free males, they may also do so to obtain non-inherited direct benefits. These direct benefits could for example include an increased feeding effort by a male in good condition (Hoelzer 1989). Alternatively, females may choose highly ornamented males to gain indirect benefits through the production of highly attractive sons (Fisher 1930, Jones et al. 1998). Previous tests of the Hamilton-Zuk hypothesis, have also received criticism due to the somewhat opportunistic approaches ecologists have adopted to evaluate immunocompetence (Siva-Jothy 1995). Antibody response to novel or familiar antigens, level of serum globulin and the size of immune organs have all been used to estimate variation in immunocompetence. For several reasons, these measurements may not accurately reflect an individual’s immunocompetence (Sheldon and Verhulst 1996, Penn and Potts 1998). So far, few studies have attempted to test if expression of male ornament can be related to their ability to control an experimental infection (Møller 1990, Hamilton and Poulin 1997). Also, although viruses are common parasite for birds (Nuttall 1997), no study have related male ornament expression to his ability to control a virus infection. This relationship is examined in paper III.

The immunocompetence-handicap

The relationship between ornament expression and parasite resistance is physiologically poorly understood. Several ideas have been put forward to explain how such a relationship could evolve and be maintained. Carotenoid pigments have been proposed to function as the physiological link between immunity and ornament expression (Olsen and Owens 1998). According to another influential model, the immunocompetence-hypothesis, the relationship between male quality and ornament size could be guaranteed if the expression of male ornaments required the presence of an immunosuppressive hormone, such as testosterone (Folstad and Karter 1992). Although it was initially believed that testosterone
was costly in terms of immunosuppression (Grossman 1985), several recent studies on
birds have now led behavioural ecologists to question this assumption (Hillgarth and
Wingfield 1997, Hasselqvist et al. 1999, Evans et al. 2000). It has also been argued that
testosterone does not regulate the expression of male plumage ornaments (Owens and
Short 1995). Although testosterone may have no direct influence of ornament expression, a
positive relationship between ornament expression and testosterone levels could be
mediated by social dominance (Poiani et al. 2000). It is also possible that interactions
between males \textit{per se} can induce costs associated with male ornament expression
(Johnstone and Norris 1993). Following the same line of reasoning, several authors have
proposed that stress hormones like corticosterone could be the key relating ornament
expression to immune functions (Møller 1995, Hillgarth and Wingfield 1997, Evans et al.
2000). In paper IV, I investigated if increased levels of testosterone would suppress
immunity to the Sindbis virus infection.

\textbf{Life-history trade-offs}

Although investments in immunity are vital for survival, other investments are also
important. When resources are limited, individuals are unable to optimise all traits
simultaneously, so that each investment must be traded off against others. Within life-
history theory, several such trade-offs have been identified (Stearns 1992). The limiting
resources could be both energetic and structural components. The fact that mounting of
immune response can be costly has been demonstrated for mammals (Lochmiller and
Deerenberg 2000), but not for birds (Svensson et al. 1998). To understand why individuals
may invest differently in immunity, it has been suggested that immunity should be studied
from a life-history perspective (Sheldon and Verhulst 1996). So far, two important trade-
offs between immunity and other life-history traits have been identified. In birds,
investments in sexual ornaments and reproduction have been shown to limit the resources
available for parasite defence (Gustafsson et al. 1994, Nordling et al. 1998, Verhulst et al.
1999). The relationship between social status and immunity could represent another
potential trade-off for the individual (Barnard et al. 1994, Zuk and Johnsen 2000). If
maintenance of a high social status is costly, this may have negative effects on immunity.
The relationship between parasite resistance ability and male social status is investigated in paper V.

**METHODS**

The field study (I) was based on data collected between 1990-1998 from five study areas in Sweden. All other data presented in the thesis were collected between 1996 and 2000. For all experimental infections (II-V), I used greenfinches (*Carduelis chloris*) captured in the Uppsala area (59°50’N; 17°50’E). The birds were caught in the autumn or winter at feeding sites located in the Uppsala Botanical garden, at the Fyris River near the Uppsala sewage treatment plant, or at Bäcklösa with mist-nets and ground-traps. After capture, all birds were transferred to an aviary and kept in cages. Within a few days after capture, all birds were ringed, sexed and aged into two age classes i.e.; yearlings and older (Svensson 1992). During the experiments birds were kept isolated (II-IV) or in pairs (V) in cages (0.5 x 0.5 x 0.5 m). Birds were supplied with food (sunflower-seeds supplemented with hemp, millet, and vitamins) and water every second day and all cages contained bowls and sitting rods. The aviary was maintained on a natural daylength cycle.

**Natural history of the greenfinch**

Greenfinches are medium sized (ca 30g) passerines that occur in most parts of Europe. It is a sexually dimorphic species: males have distinct yellow colour patches on the breast, primary wing feathers, and side of the tail (Merilä et al. 1999). The yellow pigments in the plumage consist of carotenoids (Stradi et al. 1995). Females and juvenile birds have less pronounced colour patches, and are therefore more cryptic. When nesting, the female builds the nest and incubates. The male feeds the female during the incubation, and after the chicks have hatched, both parents feed the chicks. Greenfinches lay an average of 4.83 eggs/clutch and can produce several clutches per year (Eley 1991). Due to a heavy predation of eggs and nestlings, only half of the eggs produce offspring that survive to the fledgling stage. Fledgling period is 14 days, and age of first breeding is one year (Cramp...
and Perrins 1994). Greenfinches are seedeaters that feed gregariously on the ground, or in bushes. In greenfinch flocks, access to food is influenced by dominance status: thus a few dominant birds have priority access to the food, and subordinate birds may have to wait for their turn or may eventually leave the flock if they are not successful (Cramp and Perrins 1994). Thus, an individual's social status may influence survival. Greenfinches communicate their social status through plumage signals and through social interactions (Fig. 1). Plumage brightness in greenfinches has been shown to function as a badge of social status, and brighter males have higher status (Rohwer 1975, Maynard Smith and Harper 1988, Eley 1991, Cramp and Perrins 1994).

Fig. 1 Illustration of a male greenfinch, showing the position of the yellow tail patches on wings and tail. During social interactions, greenfinches may display threats by a head forward gaping posture with wings and tails slightly lifted.

The mating system of greenfinches has previously been studied in an English population during a three year field study (Eley 1991, also in Cramp and Perrins 1994). This study found that while the majority of greenfinch males were monogamous, polygamy (24%) was frequent. Among polygynous males, most were bigamous (74%) but nestings with up to five females per male were recorded. Apparent non-breeders in the population (28-34%) were mostly males (Eley 1991). Male plumage brightness was related to mating success, and polygamous males had brighter than average plumages. Among polygynous males, brighter males paired with more females, and males that were involved in extra-pair copulations were also brighter than average (Eley 1991). These data imply that females prefer to mate with the most colourful males, although this study was unable to distinguish between female choice and male-male competition (Eley 1991). Among male greenfinches
infected with blood parasites (*Haemoproteus* sp.) in Sweden, Finland and Spain, plumage brightness was negatively related to blood parasite load (Merilä et al. 1999). For these greenfinches, male plumage brightness was also found to be positively related to testis size in the breeding season (Merilä and Sheldon 1999). Together these studies imply that plumage brightness in male greenfinches can be an indicator of phenotypic quality.

**The Sindbis virus**

The Sindbis virus is a RNA virus that belongs to the Alpha viruses, and the virus family Togaviridae (Lundström 1999). The virus consist of a 12kb RNA strand, that has been completely sequenced (Strauss and Strauss 1994). It is an arthropod-borne virus (arbovirus), transmitted to birds by blood-sucking mosquitoes. It has a wide host-range and infects almost all species of Swedish passerines (I). The virus is common in bird populations during the summers when vector mosquitoes are numerous.

During a Sindbis virus infection, the virus enters the host cells through cell surface receptors (Wang et al. 1992). It replicates by budding through the plasma membrane and can be detected in the bloodstream of infected bird hosts 2-7 days after initial infection (Strauss and Strauss 1994, Fig. 2). The infection triggers an immune response, and the most important component for clearance is thought to be the production of neutralizing antibodies against protein E2 (Griffin et al. 1997). The complement system is important for clearance of the virus, and depletion of complement results in prolonged viraemia (Hirsch et al. 1980). Sialic acid can influence the activation of the complement system, and it has been demonstrated that the sialic acid content of hosts cell tissues can affect viraemia. Thus, natural immunity is higher in hosts with less sialic acid in their tissues (Hirsch et al. 1983). For chicks with undeveloped immunity, the infection can be lethal (Lundström and Turell, unpublished study). The virus can also infect humans. In humans the infection can lead to rash and a persistent joint ache (Espmark and Niklasson 1984, Niklasson et al. 1988). After the infection has been cleared, antibodies can be maintained several years in humans (Niklasson et al. 1998). For birds, antibodies may persist at least for several months, and once an antibody response has been produced, birds appear to be immune to subsequent infections (Lundström, Krakower, Lindström, unpublished study).
In this thesis, I have used the clearance rate of the Sindbis virus infection as an estimate of immunocompetence. By measuring the clearance rate of a natural infection, I have been able to avoid the problem of determining which component of the immune system is the best estimate of immunocompetence (Siva-Jothy 1995, Westneat and Birkhead 1998, Norris and Evans 2000). Nevertheless, the assumption that has to be made is that the ability to control the Sindbis infection is positively related to the ability to control other infections. Clearance of the Sindbis virus infection is a complex process that requires the activation of both general and specific components of the immune system. Thus, at least some components of the immune response are general. Also, studies on poultry have demonstrated that immunity to one type of infection can be positively related to immunity to other infections (e.g. Cheng and Lamont 1987).

**Fig. 2** Example of viraemia and antibody response from three greenfinches (823, 830 and 824) after a Sindbis virus infection. The left part of the figure shows virus titres (day 1-7) from a plaque tests expressed as the number of plaque forming units (PFU)/ml of blood in each day. The right part of the figure shows antibody titres (day 7-90) from a PRNT-test expressed as the reciprocal of the blood dilution giving an 80% reduction in plaques numbers. During the infection, infectious virus particles can be detected in the blood of infected host up to one week after infection. Around this time, specific antibodies against the virus first appear.
Greenfinch measurements

For all greenfinches included in the experiments, body mass was measured with a Pesola spring balance, to the closest 0.5 g, and tarsus length was measured with digital callipers to the closest 0.1 mm. For males, the brightness of the plumage was scored by measuring the length of the yellow tail, and wing patch with callipers to the closest mm (Fig.1). I also counted the number of yellow feathers on the tail, primaries and alula on the right side of the bird. The coverage of yellow on the breast was estimated on a scale between 1-5. The repeatability of these measurements were moderate to high. Two principal components of plumage brightness (PC1 and PC2) were extracted, and these two components were used as plumages brightness scores (III). The first component (PC1) mainly described the brightness of the wings, and this brightness was closely related to the birds age. The second component (PC2) mainly described the size of the tail patch, and was unrelated to age. Both components were adjusted for age-dependant variation before the analyses. Since the brightness of primarys and breast were uninformative of virus clearance rate (IV, V), the length of the tail patch was used as a predictor of viraemia in later experiments (IV, V). Two studies (II and V) include behavioural observations of greenfinches. In the first of these studies (II), spontaneous locomotion activity was measured by counting the number of hopping or flying movements each bird made during 25 minutes each morning. Furthermore, the body mass and speed and angle of take-off flights after a simulated predator attack was measured, as described by van der Veen and Lindström (2000). In the last study (V), dominance hierarchies within pairs of males was measured by observing their interactions around a feeding site from a hide. Males with priority access to food and water were classified as dominants following the methods described by Senar (1990). In one experiment (IV), blood testosterone concentrations were measured. A detailed description of the sampling procedure and analyses is presented in paper IV.
Virus infections and assays

In all experimental infections (II-V), greenfinches were injected with infectious Sindbis virus diluted in 0.1 ml saline solution (0.09 % NaCl) subcutaneously into the breast. In two studies (II & III), I prepared a solution with 10^3 plaque-forming units (PFU)/100 µl and infected birds immediately after preparation. The amount of virus injected was based on the estimated amount of virus delivered by infected mosquitoes during blood feeding (Chamberlain and Sudia 1961). When a large number of birds were infected (III-V), or when birds were infected on different days (II), a virus solution was prepared with the concentration of 10^4 PFU/100 µl. This solution was frozen in separate vials until the infection. Freezing and thawing reduced the amount of virus about tenfold, so that the inoculated dose became equal to the one obtained during a natural infection when thawed. The Edsbyn 82/5 strain of the Sindbis virus was used in all infections (II-V). This strain represents the north European genotype of Sindbis virus (Norder et al. 1996), and was taken through three passages in Vero cells before being used in the experiments.

To measure daily virus concentrations (III-V), birds were sampled for blood at 24h intervals during seven days after inoculation. To measure the amount of specific neutralising antibodies, blood samples were taken once a week until four weeks after the infection, and then at two and three month post infection (III-IV). All blood samples (100µl) were taken from the jugular vein using a fine syringe (27G). After sampling, blood was either diluted in 1:10 in Hanks’ balanced salt solution (I, III, IV and V) (Life technologies), or stored as undiluted plasma (III). Samples were kept on ice until storage, and thereafter kept in -70°C until assayed. Blood samples from day one to seven were assayed for virus content (III-V), with a plaque-test (Lundström et al. 1990). In the plaque-test, serial 10-fold dilution’s of the blood samples were seeded in duplicates on a monolayer of Vero cells, cultured on 24-well plates. Cells and blood-samples were then overlaid with a nutrient solution in agar. After one day, live cells were visualised by neutral red staining, and infectious virus particles were counted as plaques of dead and unstained cells. The lowest detectable level of virus in the assay was 10^2 PFU/ml blood. The amounts of Sindbis-specific antibodies in the samples were measured by a plaque-reduction neutralisation test (PRNT) on heat inactivated (30 min in 56°C) samples (I, III, IV). In
this test, blood samples were serially diluted four-fold starting at 1:20, using the same buffer solution, cell cultures and plates as used in the plaque assay. In the PRNT, each well was seeded with 30 - 80 PFU of Sindbis virus and the neutralising antibody titres of the samples were expressed as the reciprocal of the dilution giving 80% reduction in plaque numbers (Early et al. 1967, Francy et al. 1989).

**Statistics and data analyses**

I used parametric tests where data met the required assumptions of normality (Sokal and Rohlf 1995). When data was non-normal, either transformed data, or non-parametric statistical tests were used (Siegel and Castellan 1989). To test for differences in virus clearance ability between individuals, total viraemia, defined as the sum of the log_{10} transformed daily virus titres, were used (III, IV and V). I also used peak viraemia, defined as the highest recorded virus titre, and virus clearance rate, defined as the number of days with detectable virus titres (Fig. 2., III). To analyse time differences in viraemia patterns between individuals, I compared early and late viraemia (IV and V), as the sum of the log_{10} transformed daily virus titres for the first days (early viraemia); corresponding to the proliferation phase, and for the last days (late viraemia); corresponding to the clearance phase. The borders between early and late were arbitrarily chosen. To test for differences in antibody production (III and IV), I compared antibody titres at 21 days post infection, at the antibody peak: defined as the highest detected antibody titre of all samplings, and the antibody production rate: defined as the antibody peak divided by the time it took to reach this peak (Fig. 2).

Since the Sindbis virus is a naturally occurring virus, some wild caught birds may have developed immunity after previous exposure. Thus, to be able to compare the ability to clear a primary infection, I wanted to exclude all birds that had encountered the virus in nature. These birds could sometimes be identified by detectable amounts of specific antibodies before the infection (III). However, even after experimental infections antibodies can not always be detected at all samplings. Thereby, this method could not be used to reveal all birds with previous exposures. In a pilot experiment in which birds where challenged with a second Sindbis virus infection, I found that all birds that had been
previously exposed had become immune to the virus, and produced no detectable viraemia. Also, as their secondary antibody responses were both faster and stronger compared to a primary response (Lindström, Lundström, Krakower, unpublished study). In experiments with viraemia and antibody data available (III, IV and V), I identified and removed birds that had previously been exposed to the virus.

RESULTS AND DISCUSSION

Distribution of Sindbis virus infections in the field

In the first study of the thesis (I), the distribution of Sindbis specific antibodies among free-ranging Swedish passerines in five areas was investigated. Antibodies occurred in almost all passerine species (13/15, or 87%) sampled in large numbers (≥20 individuals). The average prevalence across species was 7.7%. This and other studies indicate that the Sindbis virus has a very wide host range (McIntosh et al. 1969, Lundström et al. 1993, Lundström 1999). Although it seemed likely that all species of passerine birds could be utilised by the virus as hosts, antibodies were unequally distributed between species. Higher than average antibody prevalences were found in three thrush species, the fieldfare (*Turdus pilaris*) (43.3%), the redwing (*Turdus iliacus*) (37.0%), and the song thrush (*Turdus philomelos*) (22.2%). The distribution of antibodies in relation to bird body mass was analysed for Sindbis virus and a related North American alphavirus (eastern equine encephalomyelitis virus). Both these viruses are transmitted by mosquito-vectors. It could be concluded that medium sized birds were the most common hosts of both these viruses. A factor that potentially could create differences in infection prevalence between species is that species may have differential exposure to vectors, or differ in anti-mosquito behaviour (Anderson and Brust 1995). It has previously been demonstrated that animals of large size exhibit less behavioural defences against mosquitoes (Edman and Scott 1987, Scott and Edman 1991). Within species, there was no differences in infection prevalence between sexes. Early in the summers (June and July), adult birds were more often infected than hatchling year birds, but later in the summer (August), the infection prevalence was equal between birds of different age classes.
Costs of the virus infection

No attempts have been made to describe symptoms of disease associated with Sindbis virus infections in passerines. Thus, the study presented in paper II represents a first attempt to examine whether the Sindbis virus infection was indeed costly for infected birds. To estimate the potential costs of infection, the behaviour of Sindbis virus infected greenfinches was measured. First the spontaneous activity of greenfinches before and during an infection was compared. There was a tendency for birds to reduce their activity during an infection. To investigate if infected birds are more vulnerable to predation, I measured the body mass, and the speed and angle of take-off flights from a simulated predator attack on both morning and evening. When comparing differences in take-off speed from before to after infection, between a virus-infected group and a control-treated group, it was found that take-off speed in the virus treated group was reduced in the evenings, but not in the mornings (Fig. 3). There was also a significant increase in body mass in the virus treated group compared to controls (Fig. 3). When healthy, greenfinches can maintain the speed of take-off flights, although their body mass increases throughout the day (van der Veen and Lindström 2000). However, during the infection the increased body mass may lead to reductions of flight speed. An impaired take-off ability could reduce the fitness of infected hosts. In conclusion, the behavioural alterations observed imply that the infection was energetically costly, and that birds responded to the increased energetic costs by altering their behaviour during the infection. The behavioural alterations observed could also result from direct symptoms of disease, such as cell or tissue damage (Holmes and Zohar 1990).
**Fig. 3** Bars in the upper graph illustrate the mean (± s.e) change in take-off speed of greenfinches in the morning and the evening, from before until after a saline treatment (unfilled bars), or an infection with the Sindbis virus (filled bars). Positive values on the x-axes result from a mean within-individual increase. The lower graph illustrate mean (± s.e) change in body mass. Sample sizes are $n = 10$ for saline group in all cases, and $n = 9$ for virus group in all cases. Significant differences in post-hoc paired $t$ tests are illustrated with bars, * $p < 0.05$. 
Sexual selection

According to the intra-specific prediction of the Hamilton-Zuk hypothesis, a negative relationship between parasite load and ornament size should be expected (Hamilton and Zuk 1982). In paper III, this prediction was tested using greenfinches infected with the Sindbis virus. During an infection, greenfinches may vary in viraemia, virus clearance rate and antibody production rate, although infected with equal virus doses (Fig. 2). Greenfinches also vary in plumage brightness, and for males, plumage brightness may be a sexually selected trait (Eley 1991, Merilä and Sheldon 1999, Merilä et al. 1999).

Two principal components (PC1 and PC2) were used to describe plumage brightness of males. For the second principal component, there was a positive relation between virus clearance rate and plumage brightness. Thus, males with large tail patches had a faster clearance of the infection (Fig. 5). Males with large tail patches also had lower virus titres during the infection-period, this was revealed by the negative relation between total viraemia and tail patch size (Fig. 5). This is the first study to demonstrate that the clearance rate after a virus infection can be predicted by male ornament size. I found no clear relationship between male plumage characters and antibody titres produced after the infection. Also, there were no clear relationships between viraemia and antibody production rate (Table 1). Thus, at least for this virus infection, the amount or speed of antibody production after infection was a poor predictor of parasite load or parasite clearance rate.

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Table 1  Pearson correlation coefficients ($r$) of virus clearance and total viraemia against antibody titres 21 days after the infection (AB day 21), antibody peak levels (AB peak) and the antibody peak level/peak week (AB production rate). The sample size was 28 in all cases.
Fig. 5 Virus clearance rate ($r = 0.58$, $p = 0.001$, $n = 28$) and total viraemia ($r = -0.46$, $p = 0.01$, $n = 28$) as a function of plumage brightness. Plumage brightness is measured as PC2 scores, adjusted for age dependent variation. Males with large yellow tail patches had higher virus clearance rate and lower total viraemia compared to males with smaller tail patches. There were no clear relationships between any measurement of antibody production and plumage brightness.
Testosterone and virus clearance

In paper IV, I tested if the immunocompetence-handicap model (Folstad and Karter 1992) could be applied to the greenfinch-Sindbis virus system. According to this model, increased testosterone levels are expected to suppress immunity (Folstad and Karter 1992). If the model holds, an increased viraemia or reduced antibody production of testosterone-implanted males would be expected. Also, if the quality signal discovered in paper III is honest, the cost of increased testosterone should be lower for males of better quality (Grafen 1990). I therefore predicted that males with large tail patches should have lower costs associated with the elevated testosterone levels. Thus, a birds response to the treatment should be related to his tail patch size. I tested these predictions by introducing either a testosterone- or control-implant into males, that were matched in pairs according to their age and tail patch size. All birds were then infected with Sindbis virus after which viraemia and antibody production rate were compared between treatments. Following the implantations, testosterone concentrations differed between treatments. Still, I found no differences in either antibody production rate or total viraemia between treatments. Furthermore, there was no evidence that males with small tail patches reacted differently to the treatment compared to males with large tail patches. Thus, I could not find support for the assumption of the immunocompetence-handicap hypothesis. However, there were differences between treatments in the timing of the infection (Fig. 6). Early in the infection (Day 1-3), testosterone treated males had lower viraemia compared to control treated males. Late in the infection (day 4-7) testosterone treated males had highest viraemia. Thus overall the implant caused a delay, but not an increase, in viraemia.

In other recently published studies on birds investigating the immunosuppressive effect of testosterone, results have been equivocal (e.g. Hasselqvist et al. 1999, Evans et al. 2000, Peters et al. 2000). Apparently, testosterone can interact with immunity, but these interactions are not straightforward (Marsh 1992). The experiment with greenfinches shows that increased testosterone concentrations can result in both increased and decreased virus titres during an infection. A factor that further complicates the interpretation of these results is that increased levels of testosterone can cause a release of stress hormones (e.g. corticosteroids). In two recent studies on house sparrows (Passer domesticus), the effects
of testosterone implants on immunity were entirely explained in terms of an accompanying release of corticosteroids (Evans et al. 2000, Poiani et al. 2000). Thus, it is possible that the effect on viraemia resulted from a release of stress hormones rather than differences in levels of testosterone (Apanius 1998). It has also recently been argued that testosterone redistribute immune cells, rather than inhibits immune functions (Braude et al. 1999). Consideration of these point suggests that the immunocompetence-handicap model may need to be revised.

Fig. 6 Mean (± s.e) of daily virus titres of testosterone and control implanted male greenfinches. Plotted values are log_{10} transformed Sindbis virus titres (PFU/ml blood) for day 1-7 after infection. The sample size was 15 in each group. Early in the infection (sum of titres day 1-3), testosterone implanted males had lower virus titres (t-test, t_{28} = 2.48, p = 0.020), and late in the infection (sum of titres day 4-7), testosterone implanted males had higher virus titres (t-test, t_{28} = -2.42, p = 0.022).

Social status and virus clearance

The final experiment (V) focuses on the relationship between the Sindbis virus infection and social dominance. I tested if infected males would lose rank against an uninfected male and whether the virus clearance ability of males was related to their social status. First, I carried out daily observations of males kept together in pairs, in which one male in the pair was infected. During the infection, the number of altered ranks between subsequent days were no higher than what could be expected by chance (Fig. 7). Also, when an infected and
an uninfected male were first introduced, there was no association between infection status and the outcome of the dominance test. Instead, the outcome of the dominance test could be predicted from the outcome of a male’s previous encounters in all cases. Thus, none of the results indicated that social status decreased during the Sindbis virus infection.

When viraemia patterns between subdominant and dominant males were compared, it was found that dominant males had higher early viraemia (Fig. 8). There was a significant positive correlation between within-group rank and early viraemia, and birds with high rank had high early viraemia. Total viraemia did not differ between dominants and subdominants, nor was it related to within group rank. Late viraemia was higher among subdominant birds, and at this time, there was a significant negative relationship between social rank and late viraemia.

A bird’s social rank may be the trait that is most strongly connected to fitness. Dominant individuals may gain access to both food and partners, especially when these are in short supply and there is competition over these resources. Although I had previously found indications that other behavioural traits, such as take-off flight and locomotion activity were down-regulated during an infection-period (II), the results of this experiment (V) indicate that social status was maintained during the infection. The close relationship between social status and viraemia was an interesting and somewhat unexpected finding. The differences found in viraemia patterns between males of different rank could be due to a physiological trade-off. If males with high rank initially had invested less in immunity, they would become more easily infected, and thereby obtain initial high virus titres. However, rank may also be positively related to quality, so that high-ranked males respond to their heavy infection with an efficient and rapid virus clearance. Mechanistically, these differences could be due to differences in hormone levels. A potential hormone candidate that could create such a difference is testosterone. A high social status in birds and mammals is commonly associated with elevated testosterone levels, although this relationship does not always hold true (Wingfield et al. 1990, Evans et al. 2000). In this study, the viraemia pattern of subordinate males were similar to that of testosterone implanted males, and a more likely hormone candidate is therefore corticosteroids. Subordinate males in this study and testosterone implanted males in the previous study (IV) could both have experienced increased levels of stress hormones. Stress hormones,
like corticosteroids are potent regulators of immunity, and increased stress can function both as an enhancer and a repressor of immune responses or parasite load (Apanius 1998).

**Fig. 7** The proportion of pairs with stable ranks between subsequent test days. In each pair, one male was infected with Sindbis virus at day 0. On the following four days, 16 pairs of males were ranked daily. The solid line indicate the proportion of ranks (89%, \( n = 31 \)) that remained stable during the control week, when none of the males were infected. The area above the lower (hatched) line indicates the variation that was expected due to chance. Thus although one male was infected, ranks remained stable.

**Fig. 8** Mean (± s.e) of daily virus titres for dominant and subordinate males after an experimental infection. The sample size is 8 in each group. Early in the infection (sum of titres day 1-4), subordinate males had lower virus titres, and late in the infection (sum of titres day 5-7), subordinate birds had higher virus titres.
CONCLUSIONS AND PROSPECTS

The distribution of infections in nature may vary for many reasons. In the first paper of this thesis (I), data showed that passerine species sharing the same environment may differ in infection prevalence. Since exposure to infections may differ, this may create differences in infection prevalence between individuals. Although many infections are not directly lethal, the fitness of infected individuals may still be reduced if for example escape-behaviours are impaired during the infection (II).

Although infected with equal doses of the virus, greenfinches varied in their ability to control the infection. The reasons for this variation is not well understood. For this virus infection, there was no clear relationship between antibody-production and viraemia. Since much of the genetic variation in the immune system of vertebrates is located in the major histability complex (MHC) (Grahn 2000), an interesting prospect for the future would be to investigate if there is a connection between variation in viraemia and MHC genotype.

The results in paper III show that the size of the tail patch in males reveals information about his ability to control the Sindbis virus infection. If virus clearance ability is heritable, female greenfinches that prefer males with large tail patches could potentially gain indirect genetic benefits (Kirkpatrick and Ryan 1991). It remains to be investigated if the ability to control the Sindbis virus is heritable. In a study on bluethroats (Luscinia svecica), it was recently demonstrated that females enhance the immunocompetence of their offspring through extra-pair copulations (Johnsen et al. 2000). That virus resistance can be a heritable trait, have been demonstrated in studies of poultry (e.g. Bumstead et al. 1993).

It has become increasingly clear that male ornament expression can reveal aspects of quality, but it remains unclear how these factors are regulated. I found no support for the prediction that increased testosterone levels in males were costly in terms of immunosupression (IV). However, I did find that viraemia patterns could be modulated by testosterone, and similar differences in viraemia patterns were also related to an individuals social status (V). Since an individuals behaviour may be regulated by hormone levels, a model that could fully explain how quality signals are maintained would probably have to consider both the effects of testosterone and social stress on immunity (Poiani et al. 2000).
Many male traits can function both as cues in female choice and in male-male interactions simultaneously, and for the expression of such traits the costliness of female directed signals could be influenced by social interactions with other males (Berglund and Bisazza 1996, Qvarnström and Forsgren 1998, Poiani et al. 2000). Also, since plumage colour of greenfinches are carotenoid based, the interactions between carotenoids and parasite load is an interesting area that deserves further investigation (Olson and Owens 1998). The integration of these models will most certainly provide an interesting challenge for future research.
ACKNOWLEDGEMENTS

To become a doctor on greenfinches is somehow not exactly what I had expected…… Although I feel a bit surprised, I am very glad I did this, and this four years long journey into the scientific world has been both stimulating and rewarding. Of course, it would not have been possible for me to complete the project without the help and inspiration from many other people. First, I would therefore like to thank everyone at ‘Zootis’ for making it into the fun, friendly and interesting place that it is to work in. THANK YOU ALL! Although, that includes most of the people mentioned below, some persons deserves to be thanked at least twice:

The first one of these is of course my wonderful supervisor-roommate-coauthor Jan Lundström. Janne has taken the time to help with all sorts of practical things, we have had many interesting discussions over the years and he has read and commented on all my manuscript without loosing his good mood. I am most grateful for all time and support you have provided, for inviting me to your nice family, and for being a friend! Thank you!

I would also like to thank Jan Ekman for getting me started by hiring me, and Jacob Höglund for helping me to reach the finish line. Ingrid Ahnesjö is a great person that has helped me to maintain calm and organised. Douglas Krakower made a huge effort feeding and bleeding birds, and even sang encouraging songs while doing so. He also turned out to be an excellent English proofreader. I really enjoyed working with you Special-D! Ineke helped me a lot with both texts and statistics, and kept reminding me to take coffee breaks. You are a rock (or klippa in Swedish)! Anna K helped to improve many of my texts and she also kept my knowledge of nudibranchs and flower maintenance updated. These years would have been much less fun without you! Tina S kept my supervisor busy, so that I could occasionally rule the office. She also sometimes changed the channels on my TV. Boris-Antoine did his master project on greenfinches and patiently counted their jumps while learning Chinese. Arne and Pekka sometimes provided me with greenfinches from the garden. Reija analysed a lot of greenfinch blood-smears, Theresa J and Stein-Are helped me improve the text in the summary. Henk gave comments on my text and has been a good friend. Juha gave great comments on any manuscript that I gave him, Måns A taught me that answers can sometimes be found in Poultry Science, Christer H showed me
how to hold a bird and Lotta helped me (without knowing it) by writing a very good thesis. 
Also, a lot of other people like Maria S, Ane, Simon, Katrin, Sami, Fredrik W, Lisa, Johan 
W, Robert, Marcus, Anders, Jon and Olle has kept the party spirit alive at the department. 
A great deal of practical help was provided by Nisse. Over the years, Nisse taught me one 
or two things about carpentry and once was nice enough to give me a sandwich at 
Bäcklösa. Marianne and Ingela also helped with many practical things and kept the 
department organised. Karin Söderhäll did a great job keeping the Vero cells alive, and 
Jonas Blomberg and his colleagues at clinical virology kindly let me use their lab to grow 
Vero cells.

Other people have contributed to this work in a more indirect but equally important 
way. Pysselklubben and the Wednesday Clay-Day with Barbro kept my weekdays fun. On 
other days, all my wonderful friends have kept my life busy and happy. Kursargänget have 
been great party company at all times. The weekends with Västeråsbrudarna gave much 
important inspiration and always filled my batteries with new energy. Göran H is a special 
friend that deserves special mentioning. Lene fed me with tasty meals and much good 
advice. Sofia has been a great adventure and holiday companion. Thank you all for being 
so great friends!

Finally, I would like to thank my dear family: My sister Lena taught me that 
sometimes you need to take things one step at the time, she also helped by providing me 
with a working computer. Lena, Leif, my father Sam and Arja have provided much 
encouragement and personal support. Thank you all for being there! My last and biggest 
thanks goes to my wonderful mother Margit for believing so much in me, and for 
supporting all weird things that I have done in my life. You will always be there for me in 
my heart!

Financial support was provided by the Olle Engqvist Byggmästare Foundation, the 
Crawfoord Foundation, the Zoological Foundation and the Royal Swedish Academy of 
Science.
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Samspelet mellan fåglar och parasiter - med Sindbisvirus som modellsystem.


Den första artikeln i avhandlingen kartlägger utbredningen av Sindbisinfektioner hos vida fåglar i Sverige (Artikel I). För att ta reda på vilka fåglar som varit infekterade fångades fåglar in med slöjnät på sommaren vid fem olika platser under flera år. Blodprover från ca 1000 fåglar av ett 40-tal arter analyserades för förekomst av antikroppar mot Sindbisviruset. Förekomst av antikroppar visar att en fågel varit infekterad av viruset. Hos nästan alla fågelarter där jag undersökte mer än 20 fåglar fanns antikroppar mot viruset hos någon individ. Detta visar att Sindbisviruset verkar kunna utnyttja de flesta arter av tättingar som värdjur. Andelen infekterade individer skilde sig dock mellan arter, och tre trastarter; björktrast (Turdus pilaris), rödvingetrast (Turdus iliacus) och taltrast (Turdus philomelos), var oftare infekterade än övriga tättingar. Hos dessa trastar var mellan 22% och 44% av alla testade individer bärare av antikroppar, medan genomsnittet för samtliga arter var 8%. Tidigt under somrarna var äldre fåglar oftare infekterade än årsungar, men denna skillnad försvann under sensommaren. Hanar och honor hade
antikroppar i lika hög grad. Eftersom så stor andel trastar var infekterade kan förekomsten av trastar i ett område vara en betydelsefull faktor som reglerar Sindbisvirusets utbredning.

För övriga artiklar i avhandlingen användes grönfinkar (*Carduelis chloris*) som värdjur för viruset (Fig. 1). Jag valde att arbeta med grönfinkar eftersom de infekteras av viruset i naturen, och är lätt att hålla i bur. I den andra artikeln undersökte jag om fåglarnas beteende påverkades av en infektion (Artikel II). De beteenden som mättas hos fåglar var deras aktivitet och deras förmåga att fly undan en simulerad rovfågelsattack. Under infektionen fanns en tendens till att grönfinkarna var mindre aktiva. Infekterade fåglar hade också en högre kroppsvikt. När infekterade fåglar flydde undan en simulerad rovfågelsattack så lyfte de med en lägre hastighet, kanske för att de var tyngre än vanligt. Om fåglar som infekteras med viruset har svårare att fly från rovfåglar, kan detta försämra deras chanser att överleva. Detta kan i sin tur innebära att fåglar med god förmåga att motstå infektionen gynnas i det naturliga urvalet.

**Fig. 1** Hos grönfinken (*Carduelis chloris*) är hanar färggrannare än honor, och hanens färger utgör en signal om hanens kvalitet. Denna signal kan uppfattas av både honor och andra hanar. Cirka 10% av alla grönfinkar i Sverige blir på sommaren infekterade med Sindbisviruset, som överförs via infekterade myggor.


Grönfinkshanarnas fjäderdräkt används inte bara som en kvalitetssignal till honor; den kan även ge information om hanens rang, eller sociala status, i flocken. Tidigare undersökningar av grönfinkar visar att grönfinkshanar med färgrika fjäderdräkter är av hög rang. I den femte artikeln undersökte jag om hanarnas rang kunde påverkas av en virusinfektion, och om det finns något samband mellan en hanes rang och hans förmåga att motstå en virusinfektion (Artikel V). Genom att studera två grönfinkshanars beteende när de skulle äta vid en gemensam matskål kunde jag fastställa deras rang. Vid matsålen vek
nämligen hanar med låg rang undan för dominanta hanar. Under infektionen förlorade inte de infekterade grönfinkshanarna i rang gentemot oinfekterade hanar. Däremot fanns det ett samband mellan en hanes rang och de viruskoncentrationer de fick efter en infektion. Tidigt under infektionen (1-3 dagar) hade hanar med hög rang höga viruskoncentrationer i blodet. Sent under infektionen (4-7 dagar) hade istället de lågt rankade hanarna höga viruskoncentrationer. Av detta försök kan man dra slutsatsen att en hanes sociala status verkade påverka hans förmåga att kontrollera en infektion. Hanar med låg rang var de som var bäst på att motstå infektionen i början. Detta kan bero på att deras immunförsvar redan var mobiliserat för att vara redo att bekämpa eventuella skador. Högt rankade hanar blev snabbast virusfria. Detta kan bero på att högrankade hanar var i bättre kondition, och när immunförvaret väl mobiliserats så hade de inga problem med att hantera infektionen.


Att kunna försvara sig mot parasitangrepp är en egenskap som är nödvändig för att kunna överleva i naturen. Genom att studera hur försvar mot parasiter formas av evolutionen kan vi förstå vilka processer som måste få verka för att värddjur ska kunna upprätthålla ett naturligt skydd mot parasitangrepp.