Fitness consequences of immunocompetence, health status and parasitism of the Barn Swallow *Hirundo rustica*

PhD thesis

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1. INTRODUCTION

From life-history studies we know that the resources available for an individual are limited, and consequently the energy invested in one trait reduces the resources available for other characters (Stearns 1992). In order to maximize reproductive success, birds invest heavily in progeny. As a result, the high cost of reproduction reduces the condition and the survival of the individual. It has been pointed out several times during the last decade that in the trade-off between reproduction and parasitism, the physiological status of the individual may play an important role (Schantz et al. 1999). The results show that an increased investment in reproduction results in an elevated risk of infestation, which is related to a suppressed immune system (e.g. Ots 1999). Consequently, because the health status and immunocompetence of the host determine the quality of defense against parasites, and because maintaining an efficient immune system is costly, it is important to understand the mechanism and role of the physiology in this trade-off, as well as their role in determining the evolution of life history traits (Papers I, II). A prerequisite in the evolution of these traits is their heritability, namely the capacity of the individual to transmit genes determining traits under selection to the progeny (Paper III).

In recent years the study of the host-parasite relationship has received an outstanding amount of attention from behavioral ecologists and evolutionary biologists, because it is a subject of great importance in both practice (conservation biology) and in theory (evolutionary) (Bush et al. 2001, Clayton and Moore 1997, Ewald 1994, Poulin 1988, Wakelin 1996). This recognition is reflected in the large number of articles published and books written on the topic. With observation of the host on one hand and of the parasite on the other, we can study the adaptation of organisms to this cohabitation, as well as the evolution of traits. The parasitic life supposes that the organism diverts important resources from the host, thereby reducing its fitness (Lehman 1993, Moller 1997). As a consequence, a quick evolutionary response is expected from the host, leading to a decrease in the number of parasites. The huge diversity of immunological, ecological and behavioral forms of defense in birds support this theory (Clayton and Moore 1997). Among these various defense mechanisms, the periodic shedding of feathers (known as molting), by which the host "left" the ectoparasites and the parasite dependent secondary sexual characters serving for conspecifics as a cue to avoid infected individuals, are one of the most well known. The immune defense (see above) against blood sucking parasites is considered to be an effective method of purification against parasitic symbiotes. It is important to note that the same defense mechanisms evolve in the parasites, in order to evade the host defenses (Paper VI). As in the case of hosts, the defense arsenal of parasites has a broad range, namely physiological, morphological and behavioral defense. The result is the well-known host-parasite co-evolutionary arms race (Dawkins and Krebs 1979), in which the selection of an adaptive change in defense mechanism in the host is followed by a quick evolutionary response in the parasite.

From the theoretical point of view, it is important to study the life history of those parasites that depend completely on the host (obligate parasites) and are species-specific, namely they parasitize only one or a few host species. The survival of these parasites depends on their virulence, because if the host dies then the parasites perish, or at least their growing capacity is reduced. A generally accepted theory is that the virulence of the species-specific parasites is low, while that of the generalists is high (Bull et al. 1991, Ewald 1991, Herre 1993). In the study of host-parasite relationships, a special interest is devoted to birds. This animal group is ideal for studying the role of parasites on the evolution of life histories, since the life-strategies of the birds are variable, and they are easy to study in field conditions. Furthermore, it is important to mention that birds harbor a variable number of parasites, from the very virulent blood-sucking ecto-, endo- and blood parasites to the benign feather mites and lice.

Lice and feather mites are one of the most diverse arthropod groups living in symbiotic relationship with birds, with high prevalence and intensity of infestation among group-living species (Proctor and Owens 2000, Johnson and Clayton 2003). The virulence of these symbionts is low, which is related to their species-specific life (the transmission is generally between related individuals by body-to-body contact). This has been confirmed by a few previously published papers (e.g. Clayton and Tompkins 1995, Figuerola et al. 2003). Despite the abundance of these symbiotic groups, we have very little information about their role in the life history of the host. In order to investigate these deficiencies, I used the very abundant feather mites of the barn swallow *(Hirundo rustica)*, and I followed their effect on the condition, breeding performance and survival of the barn swallows by an indirect estimation of the number of lice (Papers IV, V).

2. OBJECTIVES, QUESTIONS AND HYPOTHESES

2.1. The role of health status and immunocompetence in the reproductive success of the barn swallows

In a correlative study, I followed the relationship between health status and immunocompetence of the birds in order to determine the role of condition indices in the breeding time of barn swallows. Until now, the role of physiological condition in the life history of the birds was studied mostly on sedentary species (e.g. Moreno et al. 1998, Ots 1999). Thus, we have only sporadic data on migratory birds (Gustafsson et al. 1994, Hasselquist et al. 2001). Furthermore, in a brood-size manipulation experiment I studied the predicted trade-off between reproductive effort and the physiological condition of female barn swallows, and specifically I was interested in the role of the different components of the immune system (e.g. leukocytes, T-cell immunity) in determining the reproductive effort of the birds. This was followed by determining the change in the different components of the immune system during increased stress.

2.2. Heritability of morphological and physiological traits

Considering the role of morphological and physiological traits in determining the fitness of the birds, I studied the heritability of different traits of the nestling barn swallows in a partial brood-size manipulation experiment, when half of the nestlings from a nest were exchanged. Furthermore, I investigated the less examined assumption that traits under strong natural selection (e.g. the immune system) realize lower heritability than traits under less intensive selection (e.g. morphological traits). By determining the additive and phenotypic variance of the traits, I could determine the components of the heritability.

2.3. The origin and the role of feather holes in the fitness of the barn swallows

The origin of feather holes, which are on the wing and tail feathers of the barn swallow is still unclear. In studies conducted primarily by Moller and his co-workers (Barbosa et al. 2002, Kose and Moller 1999, Kose et al. 1999, Moller 1994, Moller et al. 2004a,b, Saino et al. 1995), feather holes are considered to be produced by lice. However, except for a correlational study (Moller 1991), no rigorous experiment was conducted in this respect. In order to elucidate this problem, I provide additional data related to the origin of feather holes. Furthermore, in a five-year correlative study on the breeding population of barn swallows, I followed the relationship between the number of feather holes and the fitness of the birds, as measured by the breeding time, breeding success, survival and condition indices.

2.4. The relationship between feather mites and the barn swallow

In this host-symbiont relationship, I studied the effect of feather mites on the fitness of the birds on one hand, and followed the escape behavior of mites on molting barn swallows on the other. Based on the sporadic data we have on feather mites (Proctor and Owens 2000, Proctor 2003), various symbiotic life forms could be assumed, namely the commensal, mutual and parasitic nature of mites. In order to elucidate this problem, I conducted an experiment in which I manipulated the number of feather mites on birds, and then compared the condition and fitness of the birds in experimental and control groups. Data about the escape behavior of feather mites is also very scarce. Studying this behavior is very important, since the molt of birds was considered to be an adaptive behavior of the host to effectively sweep ectoparasites (Jovani and Serrano 2001, Moyer et al. 2002). In order to fill this gap, I studied the ability of feather mites to escape falling feathers, and the cues they used in order to detect the next to be shedding feathers. First, I followed the distribution of feather mites on the primary wings of molting barn swallows, and then conducted an experiment on breeding barn swallows where I tested two alternative hypothesis explaining the cues used by feather mites during escape. The window hypothesis states that feather mites sense the altered airflow caused by the gap of the fallen feather. Alternative ly, feather mites use the vibration of the next to be fallen feather in order to avoid falling down.

3. THE STUDY SPECIES, AREA AND METHODS

3.1. The barn swallows

The barn swallow is a small (~ 20 g), monogamous, semicolonial, long-distance migratory species, which is widely distributed in Europe (Cramp 1988). Advantageous traits of the species make it easy to study in different manipulation experiments. The barn swallow generally breeds solitary, less frequently in loose colonies, and its nests are located inside barns. The site fidelity of the species is very high, which is reflected in high philopatry of adults to the breeding sites. This is important for the present study, because it makes the estimation of the survival probability of the birds easy and accurate. The sexual selection and breeding biology of the barn swallow is well studied (Moller 1994), so we have detailed information about the role of the secondary sexual characters, as well as about the trade-offs between different traits related to breeding success. The breeding population of the European barn swallow has decreased significantly during the last decades, so in these experiments I had to take into consideration the laws of protection.

3.2. **The study site**

The study was conducted between 1999 and 2004 in the Hortobágy National Park at several horse and cow barns near Balmazújváros (47°37'N, 21°21'E), and at two other barns at Szálkahalom situated at a distance of 10 km from the first site. In 2004 I collected additional data on a post-breeding barn swallow population near Sic (46°55'N, 23°52'E) in the Transylvanian Basin of Romania, and in the same year on three molting populations in South-Africa near Bloemfontein, Free State (29°2'N, 26°24'E), at Creighton, Kwazulu-Natal (29°58'N, 29°49'E) and near Durban, Kwazulu-Natal (29°38'N, 31°5'E). The breeding area of the birds is surrounded by meadows, pastures and agricultural lands. Based on the short distance between colonies situated in different farms, birds were treated together as a single population. This is supported by the observation that breeding birds migrate between farms, confirmed by capturing birds ringed in other farms.

3.3. General methods

During the five-year-study I systematically controlled the breeding colonies during May-August, following each breeding pair from egg laying until fledging. In each year during the pre-laying period I captured as many birds as possible with mist nets, after which I individually marked them with aluminium rings, and with an individual color ring combination. This combination of color rings makes it possible to individually determine each bird at its nests. The identity of nest owners was later confirmed by capturing adult birds at their nest with a trap during feeding nestlings. During the study I consistently measured the same biometrical, parasitological and immunological parameters. The same biometrical measures were taken on the post-breeding birds in Romania and on the molting birds in South-Africa. For this latest group I also noted the molting of wing and tail feathers of the birds. In order to measure the reproductive effort of breeding birds, I counted the feeding activity of parents in three years (2000-2003).

3.4. Measures of the health state indices and immunocompetence (Papers I, II, III)

In bird ecology and behavioral ecology studies, several methods are used to measure the health status and immunocompetence of the birds, from the most simple and cheapest leukocyte counts based on blood smears, to the more sophisticated immunological tests (e.g. ELISA). The immune system of the birds, like that of other vertebrates, is composed of two arms. First, the innate immunity is the hereditary branch of the immune system. Second, the acquired immunity evolve during the ontogeny of the individual, and the immunity develop during the encounter of the immune system with antigens. Furthermore, the acquired immune system is composed of cellular and humoral immunity. The basic difference between the two is that the cellular immune system acts via the activity of different cells, while the humoral defense system is based on immunoglobulins produced by immune cells (e.g. B-lymphocytes).

The health status of the birds was measured by several physiological parameters. After measuring birds, I collected approximately 80 ul blood from the brachial vein in heparinised capillaries, and after centrifugation I calculated the hematocrit. In order to determine the relative and absolute number of leukocytes, I smeared a drop of blood on a slide. After counting the different types of leukocytes, I used only the number of lymphocytes and heterofiles because of the low proportion in the blood of other leukocytes (eosinofiles, monocytes and basofiles). Leukocytosis (increase in the number of leucocytes) is most commonly due to infectious diseases (Davis 1981, Fudge 1989). Heterophils are non-specific phagocytic immune cells, which are important components of the innate immune system together with monocytes, basophils, eosinophile granulocytes and natural killer cells. The heterophile/lymphocyte ratio is widely used as an indicator of stress (Maxwell 1993), and it is known to increase under various stressful conditions. The adaptive immune system is comprised of the lymphocytes that can be classified into two main types: T-lymphocytes and B-lymphocytes. The T-lymphocytes are considered to be the main part of cell-mediated immunity and comprise around 70% of the lymphocytes. Lymphocyte concentration in peripheral blood can be an indirect indicator of adaptive immune system activity, while the T-lymphocyte responses to an antigen measure the cell mediated immune response of an individual.

I assessed the cell-mediated immunity of breeding female barn swallows and nestlings by T-lymphocyte activity to the phytohemagglutinin antigen in response to brood size manipulation. Based on studies conducted on poultries and wild birds (Alonso-Alvarez and Tella 2001, Goto et al. 1978, Lochmiller 1993), I supposed that the stronger the response to the antigen the more immunocompetent the individual is. Lastly, based on the blood samples collected in 2000 and 2001, I determined the plasma carotenoids concentration. The spectrophotometric method used did not allow me to separate the different carotenoids, as a consequence I determined the total carotenoids concentration. I used carotenoids as condition indices, since these biomolecules are important antioxidants regulating the physiological homeostasis and the immune system (Malter et al. 2000).

3.5. Brood size manipulation (Papers II, III)

In order to measure the cost of reproduction, I manipulated the reproductive effort, and concurrently followed the change in health status and immunocompetence of the birds. I conducted the manipulation in 2000 and 2001, when I partially transferred half of the nestlings between pairs of broods increasing and decreasing by one nestling the brood size. A third group was held as control. Considering the design and advantages of the partial cross-fostering, the environmental and genetic components of traits can be separated, making it possible to determine the heritability of different morphological and physiological traits.

3.6. Counting the number of feather holes and feather mites (Papers IV, V, VI)

During the six-year study of the breeding population of barn swallows, I counted the number of feather holes on both primary and tail feathers after capturing and measuring adult birds. The pooled data from this counting was used to analyze the relationship between feather mites and the condition of the barn swallows by collecting data on the intensity of infestation from the breeding populations during 2000 and 2004, and from the post-breeding Romanian and molting South-African populations in 2004.

3.7. Manipulation of the number of feather mites (Paper V)

In order to investigate the effect of feather mites on the fitness of the breeding birds, I fumigated half of the adult barn swallows, and the following year I compared the traits of surviving birds with that of the control group sprayed only with water. In order to repel mites, I used an insecticide found in commerce, containing 0.17% permetrin and 0.07% biorezmetrin (Insecticide 2000, Waldner GmbH Wien, Austria). The fumigation conducted in 2003 had a significant effect on the number of mites from the birds, since after several weeks following the manipulation, birds fumigated with insecticide had less mites than swallows in the control group (Mann-Whitney U test, $Z = 3.89$, $n1 = 10$, $n_2 = 17$, $P = 0.0001$), and among birds which survived the following year this difference remained significant ($F1,_{59} = 34.84$, $P < 0.0001$).

3.8. Testing the avoidance of molting feathers by feather mites and the simulation of molt (Paper VI)

The escape behavior of feather mites was first studied on molting barn swallows, on which I determined the dynamics of mites on individual primaries. Second, it was studied in an experiment conducted on non-molting birds, in which I simulated the molt by cutting the rachis or by pulling out the 6^{th} primary (counting proximally), and I followed the movement of mites from the manipulated primary to the distal neighboring feathers. Both studies were conducted in 2004, the first on South-African birds and the second on breeding barn swallows. I manipulated the birds during the first half of the breeding period, and then approximately three weeks later I recaptured them and I re-counted the number of mites on each feather. For the reference I kept a control group, in which no manipulation was made and all measurements were the same as in the first two group s.

4. RESULTS AND CONCLUSION

4.1. The health status, immunocompetence and breeding performance of the barn swallows

4.1.1. The variation in condition indices during the breeding season (Paper I)

In the study of the relationship between breeding time, health status indices and immunocompetence I showed that the physiological indices are strongly correlated with the breeding time for both males and females. Barn swallows arriving earlier, and as a consequence starting to breed earlier, were in better physiological condition than the late breeders, which was reflected in the low number of different leukocytes in peripheral blood stream. Furthermore, I found a significant difference between sexes since the number of heterophiles, lymphocytes and total white blood cell numbers were lower for males than for females. The se results support the previous findings on the condition dependent breeding time of barn swallows, namely birds with superior condition breed earlier, and have an overall higher fitness than late breeders. The difference in condition indices between early and late breeding birds disappeared during the brood-rearing period, which was a result of an increase in leukocytes of early breeding males, and a decrease in leukocytes of late breeding females. The change in health status of female barn swallows was in line with the increased T-cell immunocompetence of late breeding females related to the early ones. The change in condition indices of breeding birds was indicated also by the variation in the plasma carotenoids concentration, since for both sexes birds captured later in the breeding season had an elevated carotenoids level in the peripheral blood related to the birds captured in the egg-laying period. My findings point out the importance of the physiological condition of barn swallows in determining the breeding time. However, in order to understand the role of different condition indices, experimental manipulation is needed, for which my results could serve as a good background.

4.1.2. Health status, T-cell immunocompetence and reproductive effort (Paper II)

In this study I tested the supposed negative relationship between the reproductive effort and condition in a brood size manipulation. In the experiment I demonstrated that the increased brood size is costly not only for the female parent, but also for the nestlings, because for both the T-cell immunocompetence was lower in enlarged broods as related to the reduced ones. This result supports the cost of reproduction in terms of immunocompetence of the birds. Contrary with the previous findings, there was no difference in the number of different leukocytes, nor between heterophile/lymphocyte ratio between experimental groups for both adult females and nestlings. This seems to support the assumption that the reduced immunocompetence of the birds is not related to the increased stress, and it is most probably due to energy limitation in case of adults, or to the insufficient food supply in case of nestlings. This last is supported by the observation that adults do not compensate for the increased food demand due to the increased number of nestlings, and as a consequence the per capita feeding rate was lower in case of enlarged brood related to reduced group. Furthermore my results support the assumption that under mild stress birds first reduce the energy consumed acquiring immunity, and then under enduring and heavy stress they reduce the innate immunity. I consider these results important in understanding the trade-off between reproduction and immunocompetence, since as revealed the immune system cannot be considered as a whole trait, and accordingly different branches could be responsible for different traits.

4.2. Heritability of morphological and physiological traits (Paper III)

A prerequisite to the spread of an adaptive trait in a population is its heritability. Considering the importance of morphological and physiological traits in determining the fitness of the birds, and the role of immune system in defense against parasites, I calculated the heritability of several condition indices in a partial cross-fostering experiment. In this study I showed that the morphological and physiological traits of the barn swallow are significant but low, and the latest have lower heritability than the morphological variables. The low heritability was due to the high environmental variance and the low genetic variance. My results seems to support the assumption that traits under strong natural selection (e.g. health status and immunocompetence, which is important in defense against parasites) realize a lower heritability than traits exposed to less intense selection. This result is in accordance with the assumption that the physiological traits studied in the first two studies have an important role in the evolution of life history traits.

4.3. The origin of feather holes and their role in the fitness of the barn swallow (Paper IV)

In order to elucidate the origin of feather holes, I contribute several pieces of indirect data to the previous findings, which support that holes are produced by lice. The abundance and distribution of feather holes among birds comply with the data for lice of this species. Furthermore, I demonstrated the significant effect of feather holes on the breeding time of the birds. Namely, I found a strong negative relationship between breeding time and the number of feather holes for both sexes. The effect of feather holes on the survival of the birds was suggested by the significant difference in the number of feather holes between survived and non-survived female barn swallows. Assuming that the feather holes are produced by lice, based on this correlative study I suppose that contrary to the previous assumptions (e.g. Clayton and Tompkins 1995), these parasites have an important role in determining the breeding success and survival of the birds.

4.4. Host-feather mite relationship (Papers V, VI)

Based on a correlative study between the number of feather mites, condition indices and breeding performance of breeding and post-breeding barn swallows, I did not find any evidence supporting the parasitic life of mites. The commensal life of feather mites was supported by the experiment, in which similarly I did not find any effect of these symbiontes on the fitness of the birds, despite the significant difference in the intensity of infestation one year later after the fumigation between the experimental and control groups. I consider these results important, because this represents a further step in understanding the role of feather mites in the life history of the birds. In the following, I showed that one-year-old birds have less mites than birds two-years-old and up, after this age the intensity of infestation remains unchanged. I hypothesize that the difference in the abundance of mites between age classes can be explained by the low reproductive potential of the mites, which is reflected in their inability to populate the exploitable space until the second year of life of the host. Alternatively, differences between age classes in the resources provided by the host could explain the variation in the number of mites, since young birds could have less supply for mites than old birds. The present study does not allow for the testing of these two alternative explanations, but it gives a background for further experimental study.

Lastly, I studied the distribution of feather mites on the wing feathers and their escape behavior during molting and following an experiment in which I simulated molting. In the case of non-molting birds, feather mites showed consistent preference for the second outermost primary, with a steady decrease in proximal distance and avoiding the outermost primary. Several explanations are suggested to explain this unusual distribution. Analyzing the escape behavior of feather mites on molting primaries, I showed that mites avoid the feathers destined to fall next on molting barn swallows, and based on the experiment we showed that mites have the capacity to sense either the vibrating feather or the altered airflow produced by the gap of the fallen feather. My study demonstrates that feather mites have a developed capacity to avoid the molting feathers, contradicting the previous assumption on molting as an effective defending behavior of the birds against ectoparasites.

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