



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

Hirundo rustica

PhD thesis

**A füsti fecske *Hirundo rustica* immunkompetenciájának,
egészségi állapotának és parazitáltságának hatása
rátermettségére**

Pap Péter László

Supervisors: Dr. Szép Tibor, Prof. Dr. Varga Zoltán

University of Debrecen

Debrecen 2005



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

Hirundo rustica

PhD thesis

**A füsti fecske *Hirundo rustica* immunkompetenciájának,
egészségi állapotának és parazitáltságának hatása
rátermettségére**

Pap Péter László

Supervisors: Dr. Szép Tibor, Prof. Dr. Varga Zoltán

University of Debrecen

Debrecen 2005

Ezen értekezést a Debreceni Egyetem TTK Biológia Doktori Iskola Biodiverzitás programja keretében készítettem a Debreceni Egyetem TTK doktori (PhD) fokozatának elnyerése céljából.

Debrecen, 2005. január 20.

Pap Péter László

Tanúsítom, hogy Pap Péter László doktorjelölt 1998- 2001 között a fent megnevezett Doktori Iskola Biodiverzitás programjának keretében irányításommal végezte munkáját. Az értekezésben foglalt eredményekhez a jelölt önálló alkotó tevékenységével meghatározóan hozzájárult. Az értekezés elfogadását javaslom.

Debrecen, 2005. január 20.

Prof. Dr. Varga Zoltán

Tanúsítom, hogy Pap Péter László doktorjelölt 1998- 2001 között a fent megnevezett Doktori Iskola Biodiverzitás programjának keretében irányításommal végezte munkáját. Az értekezésben foglalt eredményekhez a jelölt önálló alkotó tevékenységével meghatározóan hozzájárult. Az értekezés elfogadását javaslom.

Debrecen, 2005. január 20.

Dr. Szép Tibor

CONTENTS

ACKNOWLEDGEMENTS	2
LIST OF ORIGINAL PAPERS	3
1. INTRODUCTION	4
2. OBJECTIVES, QUESTIONS AND HYPOTHESES	5
2.1. The role of health status and immunocompetence in the reproductive success of the Barn swallows	5
2.2. Heritability of morphological and physiological traits	6
2.3. The origin and the role of feather holes in the fitness of the barn swallows	6
2.4. The relationship between feather mites and the barn swallow	6
3. THE STUDY SPECIES, AREA AND METHODS	7
3.1. The barn swallows	7
3.2. The study site	8
3.3. General methods	8
3.4. Measures of the health state indices and immunocompetence	8
3.5. Brood size manipulation	10
3.6. Counting the number of feather holes and feather mites	10
3.7. Manipulation of the number of feather mites	10
3.8. Testing the avoidance of molting feathers by feather mites and the simulation of molt	10
4. RESULTS AND CONCLUSION	11
4.1. The health status, immunocompetence and breeding performance of the barn swallows	11
4.1.1. The variation in condition indices during the breeding season ...	11
4.1.2. Health status, T-cell immunocompetence and reproductive effort	12
4.2. Heritability of morphological and physiological traits	12
4.3. The origin of feather holes and their role in the fitness of the barn swallow	13
4.4. Host-feather mite relationship	13
5. REFERENCES	14
LIST OF PUBLICATION	18
RESEARCH PAPERS	20

ACKNOWLEDGEMENTS

My supervisor Dr. Tibor Szép provided valuable help during the study for which I would like to thank him, especially for giving me the possibility to participate in a research trip to South Africa. Without the support of him and Prof. Zoltán Varga at the beginning, I could have not started my studies. My research was financially supported by a three year PhD grant offered by the Hungarian Government. Prof. Anders Pape Møller selflessly helped me, and with his help I have had the opportunity to work several times in the Laboratoire de Parasitologie Évolutive in Paris. Jácint Tökölyi and Eszter Ruprecht have assisted me in my fieldwork. I also want to thank to many people from Hortobágy who supported my work, especially to the Koroknai family, Sándor Veress and Ildikó Pinczés. I am grateful to the administration of the Hortobágy National Park for permission to carry out the investigations. The VÖCS community of the Debrecen University provided me with great support during my summer visits, Krisztián Szabó, Miklós Bán and Orsolya Feró accepted me all the time in their home during my stay in Debrecen. The support of Dr. Zoltán Barta was a valuable encourage for me, and our discussions during my work meant the importance to continue this work. I especially want to thank Eszter Ruprecht for her patience in reading and correcting the manuscripts, and my parents for their support. Several other people helped me a lot during my six year study: Géza Szabó, Róbert Márkus, Claudie Haussy, Dr. John Ewen, Andrea Virginás, Dr. Lajos Rózsa, Csongor I. Vágási, István Kovács, the Milvus group, the workers from the Kossuth cow-farm, Margit Papp, Dr. Ronald Schmäscke, Kyle Kenyon, Prof. Steven Piper, Malcolm Gemmel, Rick Nuttal and Prof. Les Underhill. My study was supported by the Domus Hungarica Foundation, the Arany János Foundation and by the Sapientia Hungariae Foundation, OTKA by the grants T29853 and T42879, the European Science Foundation and the Romanian Ministry of Education by travel grants, as well as by the Iskola Foundation by several small research grants.

LIST OF ORIGINAL PAPERS

1. Pap, P.L. (2002). Breeding time and sex-specific health status in the Barn Swallow (*Hirundo rustica*). *Canadian Journal of Zoology* **80**, pp. 2090-2099.
2. Pap P.L. and Márkus, R. (2003). Cost of reproduction, T-lymphocyte mediated immunocompetence and health status in female and nestling Barn Swallows *Hirundo rustica*. *Journal of Avian Biology* **34**, pp. 428-434.
3. Pap, P.L. and Márkus, R. Genetic and environmental components of morphology, health status and immune response in nestling Barn Swallows (*Hirundo rustica*). Manuscript.
4. Pap, P.L., Tökölyi, J. and Szép, T. (2004). Frequency and consequences of feather holes in Barn Swallows *Hirundo rustica*. *Ibis* **146**, pp. xxx-xxx.
5. Pap, P.L., Tökölyi, J. and Szép, T. (in review). Host-symbiote relationship and abundance of feather mites in relation to age and body condition of the barn swallow, *Hirundo rustica*: an experimental study. Submitted in *Canadian Journal of Zoology*.
6. Pap, P.L., Szép, T., Tökölyi, J. and Piper, S.E. (in review). Microhabitat preference, escape behavior and cues used by feather mites to avoid molting wing feathers: an experimental test. Submitted in *Behavioral Ecology*.

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, Møller 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 Møller and his co-workers (Barbosa et al. 2002, Kose and Møller 1999, Kose et al. 1999, Møller 1994, Møller et al. 2004a,b, Saino et al. 1995), feather holes are considered to be produced by lice. However, except for a correlational study (Møller 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, but see 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. Alternatively, 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 (Møller 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 µl 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 heterophils because of the low proportion in the blood of other leukocytes (eosinophiles, monocytes and basophiles). 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 (Møller 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% permethrin and 0.07% bioresmethrin (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, $Z = 3.89$, $n_1 = 10$, $n_2 = 17$, $P = 0.0001$), and among birds which survived the following year this difference remained significant ($F_{1,59} = 34.84$, $P < 0.0001$).

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 6th 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 groups.

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. These 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. Alternatively, if feather holes are an indicator of quality, those birds with a high intensity of feather holes may have been of poor quality. These birds may have been less able to cope with the environmental conditions, resulting in the lower survival and later arrival of the birds to the breeding grounds.

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.

6. REFERENCES

- Alonso-Alvarez, C. and Tella, J.L. (2001). Effects of experimental food restriction and body-mass changes on the avian T-cell-mediated immune response. *Can. J. Zool.* 79, pp. 101–105.
- Barbosa, A., Merino, S., de Lope, F. and Møller, A.P. (2002). Effects of feather lice on flight behavior of male Barn Swallows (*Hirundo rustica*). *Auk* 119, pp. 213-216.
- Bull, J.J., Molineux, I.J. and Rice, W.R. (1991). Selection of benevolence in a host-parasite system. *Evolution* 45, pp. 875-882.
- Bush, A.O., Fernández, J.C., Esch, G.W. and Seed, J.R. (2001). *Parasitism. The Diversity and Ecology of Animal Parasites*. Cambridge University Press, Cambridge.
- Clayton, D.H. and Moore, J. (1997) (eds). *Host-parasite evolution: general principles and avian models*. Oxford University Press, Oxford.
- Clayton, D.H. and Tompkins, D.M. (1995). Comparative effects of mites and lice on the reproductive success of rock doves (*Columba livia*). *Parasitology* 110, pp. 195-206.

- Cramp, S. (1988). The birds of western Palearctic, vol. 5. Oxford University Press, Oxford.
- Davis, P.J. (1981). Immunity to Coccidia. In: Rose, M.E., Payne, L.N. and Freeman, B.M. (eds) Avian Immunology. British Poultry Science, Edinburgh, pp. 361–385.
- Dawkins, R. and Krebs, J.R. (1979). Arms races between and within species. Proc. R. Soc. London B 205, pp. 489–511.
- Ewald, P.W. (1991). Transmission modes and the evolution of virulence. Hum. Nat. 2, pp. 1–30.
- Ewald, P.W. (1994). Evolution of infectious disease. Oxford University Press, New York.
- Figuerola, J., Domènech, J. and Senar, J.C. (2003). Plumage colour is related to ectosymbiont load during moult in the serin, *Serinus serinus*: an experimental study. Anim. Behav. 65, pp. 551–557.
- Fudge, A.M. (1989). Avian hematology: identification and interpretation. Proc. Assoc. Avian. Vet. Ann. Meet., pp. 284–292.
- Goto, N., Kodama, H., Okada, K. and Fujimoto, Y. (1978). Suppression of phytohemagglutinin skin response in thymectomized chickens. Poultry Sci. 62, pp. 1889–1893.
- Gustafsson, L., Nordling, D., Andersson, M.S., Sheldon, B.C. and Qvarnström, A. (1994). Infectious diseases, reproductive effort and the cost of reproduction in birds. Phil. Trans. R. Soc. Lond. B 346, pp. 323–331.
- Hasselquist, D., Wasson, M.F. and Winkler, D.W. (2001). Humoral immunocompetence correlates with date of egg-laying and reflects work load in female tree swallows. Behav. Ecol. 12, pp. 93–97.
- Herre, E.A. (1993). Population structure and the evolution of virulence in nematode parasites of fig wasps. Science 259, pp. 1442–1445.
- Johnson, K.P. and Clayton, D.H. (2003). The biology, ecology, and evolution of chewing lice. In: Price, R.D., Hellenthal, R.A., Palma, R.L., Johnson, K.P. and Clayton, D.H. (eds) The chewing lice: World checklist and biological overview. Illinois Natural History Survey Special Publication 24, pp. 501
- Jovani, R. and Serrano, D. (2001). Feather mites (Acarina) avoid moulting wing feathers of passerine birds. Anim. Behav. 62, pp. 723–727.
- Kose, M. and Møller, A.P. (1999). Sexual selection, feather breakage and parasites: the importance of white spots in the tail of the barn swallow (*Hirundo rustica*). Behav. Ecol. Sociobiol. 45, pp. 430–436.
- Kose, M., Mand, R. and Møller, A.P. (1999). Sexual selection for white tail spots in the barn swallow in relation to habitat choice by feather lice. Anim. Behav. 58, pp. 1201–1205.
- Lehman, T. (1993). Ectoparasites: direct impact on host fitness. Parasitol. Today 9, pp. 8–13.

- Lochmiller, R.L., Vestey, M.R. and Boren, J.C. (1993). Relationship between protein nutritional status and immunocompetence in Northern bobwhite chicks. *Auk* 110, pp. 503-510.
- Maxwell, M.H. (1993). Avian blood leukocyte responses to stress. *World's Poultry Sci. J.* 49, pp. 34-43.
- Møller, A.P. (1991). Parasites, sexual ornaments and mate choice in the barn swallow *Hirundo rustica*. In: Loye, J.E. and Zuk, M. (eds) *Ecology, Behavior, and Evolution of Bird-parasite Interactions*. Oxford University Press, Oxford, pp. 328-343.
- Møller, A.P. (1994). *Sexual Selection and the Barn Swallow*. Oxford University Press, Oxford.
- Møller, A.P. (1997). Parasitism and the evolution of host life history. Host-parasite evolution. In: Clayton, D.H. and Moore, J. (eds) *Host-parasite evolution: general principles and avian models*. Oxford University Press, Oxford, pp. 105-127.
- Møller, A.P., Biard, C., Blount, J.D., Houston, D.C., Ninni, P., Saino, N. and Surai, P.F. (2000). Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian Poultry Biol. Rev.* 11, pp. 137-159.
- Møller, A.P., deLope, F. and Saino, N. (2004a). Parasitism, immunity and arrival date in a migratory bird. *Ecology* 85, pp. 206-219.
- Møller, A.P., Martinelli, R. and Saino, N. (2004b). Genetic variation in infestation with a directly transmitted ectoparasite. *J. Evol. Biol.* 17, pp. 41-47.
- Moreno, J., de León, A., Fargallo, J.A., and Moreno, E. (1998). Breeding time, health and immune response in the chinstrap penguin (*Pygoscelis antarctica*). *Oecologia* 115, pp. 312-319.
- Moyer, B.R., Gardiner, D.W. and Clayton, D.H. (2002). Impact of feather molt on ectoparasites: looks can be deceiving. *Oecologia* 131, pp. 203-210.
- Ots, I. (1999). Health state indices of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. PhD thesis, Tartu University Press, Tartu.
- Poulin, R. (1998). *Evolutionary ecology of parasites*. Chapman & Hall.
- Proctor, H. and Owens, I. (2000). Mites and birds: diversity, parasitism and coevolution. *Trends Ecol. Evol.* 15, pp. 358-364.
- Proctor, H.C. (2003). Feather mites (Acari: Astigmata): ecology, behavior, and evolution. *Annu. Rev. Entomol.* 48, pp. 185-209.
- Saino, N., Møller, A.P. and Bolzern, A.M. (1995). Testosterone effects on the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. *Behav. Ecol.* 6, pp. 397-404.

- vonSchantz, T., Staffan, B., Mats, G., Dennis, H. and Wittzell, H. (1999). Good genes, oxidative stress and condition-dependent sexual signals. *Proc. R. Soc. Lond. B* 266, pp. 1-12.
- Stearns, S. (1992). *The evolution of life histories*. Oxford University Press, Oxford.
- Wakelin, D. (1996). *Immunity to Parasites*. Cambridge University Press, Cambridge.

LIST OF PUBLICATIONS

Related publications and manuscripts

- Pap, P.L.**, Ambrus, L. and Szabó, D.Z. (1997). The study of the breeding biology of the Barn Swallow (*Hirundo rustica*) in a county in the Transsylvanian Plateau. Múzeumi Füzetek 6, pp. 169-171. (*in Hungarian with English abstract*)
- Pap, P.L.** and Szabó, D.Z. (1998). Clutch- and egg size variation in the Barn Swallow (*Hirundo rustica*) during the breeding season. Collegium Biologicum 2, pp. 75-89. (*in Hungarian with English abstract*)
- Pap, P.L.**, Szabó, D.Z. and Ambrus, L. (1998). The study of food composition of the Barn Swallow (*Hirundo rustica*): the question of load size. Collegium Biologicum 1, pp. 49-54. (*in Hungarian with English abstract*)
- Pap, P.L.** and Szabó, D.Z. (1999). The influence of weather on the nestling growth of the Barn Swallow (*Hirundo rustica*). Múzeumi Füzetek 8, pp. 122-130. (*in Hungarian with English abstract*)
- Pap, P.L.** and Møller, A.P. Plasma carotenoid variation in relation to the breeding cycle in the barn swallow (*Hirundo rustica*). Manuscript

Talks and posters

- Pap, P.L.** (1999). The effect of endoparasites on the breeding success of the barn swallow (*Hirundo rustica*) – preliminary results. (poster) 5th Conference of the Hungarian Ornithological Society, Budapest (*in Hungarian*)
- Pap, P.L.** and Szabó, D.Z. (1999). Clutch- and egg size variation in the Barn Swallow (*Hirundo rustica*) during the breeding season. (poster) 2nd Meeting of the European Ornithologist Union, Gdansk
- Pap, P.L.**, Szép, T. and Møller, A.P. (2000). The cost of reproduction in the female barn swallow (*Hirundo rustica*). (talk) 5th Hungarian Ecological Congress, Debrecen, Hungary (*in Hungarian*)
- Pap, P.L.** (2002). The cost of reproduction, T-cell immunity and the health status of the female barn swallow (*Hirundo rustica*). (talk) Models in Behavioural Ecology, Debrecen, Hungary (*in Hungarian*)
- Pap, P.L.** (2002). Cost of reproduction and immune defense in female barn swallows (*Hirundo rustica*). (talk) Journal scientifique Lab. d'Ecologie, Paris
- Szép, T., Szabó, D.Z. and **Pap, P.L.** (2003). Parasites and swallows – effects and responses. (talk) “Behavioural Ecology Studies in Hungary”,

Symposium of the Hungarian Academy of Science, Budapest (*in Hungarian*)

Pap, P.L., Tökölyi, J. and Szép, T. (2004). The origin and role of feather holes in the life of the barn swallow (*Hirundo rustica*). (talk) 6th Conference of the Hungarian Ornithological Society, Debrecen (*in Hungarian*)

Pap, P.L., Szép, T., Tökölyi, J. and Piper, S. (2004). The escape behaviour of feather mites on moulting barn swallows. (talk) “The evolution and ecology of parasites and pathogens”, Symposium of the Hungarian Natural History Museum, Budapest (*in Hungarian*)

RESEARCH PAPERS

Paper 1

In: *Canadian Journal of Zoology* **80**, pp. 2090-2099

Breeding time and sex-specific health status in the barn swallow
(Hirundo rustica)

by
Péter László Pap

Breeding time and sex-specific health status in the barn swallow (*Hirundo rustica*)

Péter László Pap

Abstract: Health status has been hypothesized to be an important factor associated with individual reproductive performance in birds. Sex-specific health status of early- and late-breeding barn swallows (*Hirundo rustica*) was studied during the prelaying and brood-rearing periods to test the prediction that early-arriving birds are in better health status than late-arriving individuals. During prelaying, early breeders (of both sexes) had lower leukocyte counts than late breeders, and males had fewer lymphocytes, heterophils, and total white blood cells than females. Sex differences in health status at arrival disappeared during brood rearing, owing to an increase in leukocytes of early-breeding males and a decrease in leukocytes of late-breeding females. Late-breeding males had a higher plasma lutein concentration than early breeders during brood rearing, and the lutein concentration increased significantly for both sexes between prelaying and brood rearing. T-cell immune response of late-breeding females was stronger than for early breeders. These results are consistent with the prediction that health status of breeding barn swallows affects individual performance in terms of breeding time. Individuals differing in quality experience different costs and benefits of early breeding, and these costs and benefits are sex specific.

Résumé : Une hypothèse veut que l'état de santé influence fortement la performance reproductive individuelle chez les oiseaux. Nous avons examiné l'état de santé d'hirondelles rustiques (*Hirundo rustica*) à reproduction hâtive ou tardive durant la période précédant la ponte et durant l'élevage de la nichée afin de vérifier la prédiction selon laquelle les oiseaux qui arrivent tôt sont en meilleure santé que ceux qui arrivent tard. Durant la période précédant la ponte, les oiseaux à reproduction hâtive (des deux sexes) ont moins de leucocytes que les reproducteurs tardifs et les mâles ont moins de lymphocytes, d'hétérocytes et de globules blancs totaux que les femelles. Les différences sexuelles dans l'état de santé à l'arrivée disparaissent durant la période d'élevage des petits à cause d'une augmentation du nombre de leucocytes chez les mâles à reproduction hâtive et d'une baisse du nombre de leucocytes chez les femelles à reproduction tardive. Les mâles à reproduction tardive ont de plus fortes concentrations de lutéine plasmatique durant la période d'élevage et la concentration de lutéine augmente de façon significative chez les deux sexes entre la période qui précède la ponte et celle de l'élevage des oisillons. La réponse immunitaire des cellules T est plus marquée chez les femelles à reproduction tardive que chez celles qui se reproduisent tôt. Ces résultats corroborent l'hypothèse selon laquelle l'état de santé des hirondelles rustiques reproductrices affecte leur performance individuelle en ce qui a trait au moment de la reproduction. Les coûts et bénéfices d'une reproduction hâtive ne sont pas les mêmes chez les individus de qualité différente et ils varient d'un sexe à l'autre.

[Traduit par la Rédaction]

Introduction

Timing of breeding is one of the most important life-history traits for passerine birds breeding in temperate areas. Many studies have shown that reproductive success declines as the breeding season advances (e.g., Perrins 1970; Hatchwell 1991; Brinkhof et al. 1993, 1997; Wiggins et al. 1994; Winkler and Allen 1996). Factors affecting arrival date of migratory species and breeding time are caused by both extrin-

sic (e.g., food supply, parasite burden) and intrinsic (e.g., phenotype and genotype) effects, as previously shown (Perrins 1970; van Noordwijk et al. 1981; Møller 1994a; Oppliger et al. 1994; Møller 2001). Differences in breeding time between individuals of a population can be understood on the basis of individual optimization models (Price et al. 1988; Rowe et al. 1994; but see Kokko 1999 for confounding factors). These models state that differences in timing of reproduction among individuals are due to individual differences in quality. Such optimization was demonstrated by Brinkhof et al. (1993, 1997), who showed by a series of experiments that both delayed and advanced breeding date resulted in a fitness loss in the precocial coot (*Fulica atra*).

The condition of individuals is reflected by morphological and physiological variables. For example, Møller (1994a) has shown that male barn swallows (*Hirundo rustica*) with long tails (a condition-dependent sexual character) arrive earlier in the breeding season and have higher fitness than late-arriving male barn swallows with shorter tails. However, the proximate factors determining arrival date of barn swallows,

Received 5 March 2002. Accepted 25 October 2002.
Published on the NRC Research Press Web site at
<http://cjz.nrc.ca> on 16 January 2003.

P.L. Pap,¹ Behavioural Ecology Research Group, Department of Evolutionary Zoology and Human Biology, University of Debrecen (formerly Kossuth Lajos University), H-4010 Debrecen, Hungary.

¹Present address: Department of Zoology, Babeş-Bolyai University, R-3400 Cluj Napoca, Clinicilor Street No. 5-7, Romania (e-mail: peterpl@hasdeu.ubbcluj.ro).

like health status and immunocompetence (the ability to raise an immune response to a challenge to the immune system), are unknown. Moreover, a growing number of studies indicate that the physiology of birds can be a reliable indicator of condition (Folstad and Karter 1992) and plays an important role in mediating the trade-offs among different fitness components (Norris and Evans 2000). Health status and immunocompetence have been shown to affect reproductive effort (e.g., Ots and Hörak 1996; Deerenberg et al. 1997; Moreno et al. 1999), survival (Saino et al. 1997a), parasite-mediated sexual selection (Saino and Møller 1996; Zuk and Johnsen 1998), and the evolution of life-history traits such as clutch size (Martin et al. 2001).

Most recently, health status and immunocompetence of birds have been suggested to be possible factors affecting timing of breeding. In female tree swallows (*Tachycineta bicolor*), humoral immunocompetence was strongly negatively correlated with laying date, suggesting that early-breeding females were in better physiological condition than late-breeding females (Hasselquist et al. 2001). Similarly, early-breeding female collared flycatchers (*Ficedula albicollis*) also appeared to be in better condition than late breeders, since early breeders had lower total white blood cell (WBC) counts, sedimentation rate, and immunoglobulins for which elevated values could indicate stress and infection (Gustafsson et al. 1994). Chinstrap penguins (*Pygoscelis antarctica*) that started to breed early during the season were in better physiological health status than late breeders, as indicated by lower total WBC, heterophile, and lymphocyte counts and a stronger T-cell immune response to an antigen compared with late breeders (Moreno et al. 1998). Finally, Sorci et al. (1997) showed in an experimental study that low immunocompetence of magpie (*Pica pica*) nestlings hatched late during the breeding season selected for early breeding. Thus, there is a growing body of evidence suggesting that the physiological health status of birds can be an important proximal factor determining the optimal timing of breeding, although there is still a great need to explore the generality of this suggestion.

The health status of males and females can be constrained by their sex-specific physiological and morphological condition, both before and during breeding. Immunocompetence of males is constrained by a negative effect of elevated testosterone concentration on the immune system during the mating period (Folstad and Karter 1992; Møller et al. 1999) and by aerodynamically costly morphological secondary sexual characters in sexually dimorphic birds like the barn swallow (Møller 1994b). During the prelaying period, females are constrained mostly by energetically demanding egg production. Thus, sex-specific differences in health status during the breeding season should be expected in dimorphic bird species such as the barn swallow.

The aim of the present study was to examine health status and immunocompetence of male and female barn swallows during the prelaying and the brood-rearing period to assess the sex-specific difference in condition between early- and late-breeding birds. Tail length in both male and female barn swallows is a reliable indicator of condition and hence arrival date and fitness in both males (Møller 1994a, 1994b) and females (Møller 1993). Therefore, individuals in better

physiological condition and with higher immunocompetence should enjoy elevated reproductive success. Firstly, I examined the difference in health status of early- and late-breeding barn swallows during the prelaying period. I predicted that early-arriving birds would be in better condition than late-breeding birds. Secondly, I examined the change in health status between early and late breeders between the prelaying and brood-rearing periods. Finally, I examined the sex difference in health status during prelaying and brood rearing.

These predictions were investigated by examining total and differential WBC counts of early- and late-breeding barn swallows both during prelaying and brood rearing. Since carotenoids have been hypothesized to play an important role in immune function (see review in Møller et al. 2000), I also investigated patterns of plasma carotenoids in adult male and female barn swallows during these two periods. Finally, I investigated a measure of T-cell-dependent immunocompetence in breeding females by injecting them with mitogenic phytohemagglutinin during brood rearing.

Methods

General

The study was conducted during spring and summer 2000 in three colonies near Balmazújváros (47°37'N, 21°21'E), eastern Hungary. Adult barn swallows were captured daily during the arrival period between 06:00 and 20:00 from 26 April by intensive mist netting. The capture date relative to the laying of the first egg was 7.4 ± 1.3 days (mean \pm SE) ($n = 50$) before laying. Breeding birds were also captured while feeding 12-day-old nestlings. At capture, birds were measured using standard techniques and weighed with a Pesola spring balance with an accuracy of 0.1 g. Since most breeding barn swallows were captured the previous year, birds could be classified as old (more than 2 years), whereas unmarked birds were classified as young (less than 2 years). This assumption was based on previous observations of high breeding philopatry of this species (Møller 1994b) and on my own capture–recapture data. None of the breeding birds from 1999 had moved to a different breeding site in 2000.

Health-status indices and measure of a component of immunocompetence

To assess the physiological status and a component of immunocompetence of barn swallows, several serological condition indices (Ots et al. 1998) and the T-lymphocyte response to an antigen were recorded for breeding birds. Blood samples (80 μ L) for analyses were collected from the brachial vein in a capillary tube, and a drop of blood was smeared on a slide, air-dried, fixed in absolute methanol, and stained with May-Grünwald and Giemsa. All smears were examined by the same person at 1000 \times magnification and the proportion of different types of leukocytes was assessed on the basis of examination of 100 leukocytes. The number of different WBC was expressed per approximately 10 000 erythrocytes. The repeatability of leukocyte counts estimated by rescanning 16 smears was significantly high ($r = 0.86$, $F_{[15]} = 13.5$ (heterophile counts); $r = 0.74$, $F_{[15]} = 6.8$ (lymphocyte counts); and $r = 0.86$, $F_{[15]} = 11.7$ (total WBC counts), $p < 0.001$), whereas monocytes, eosinophils, and

basophils did not show significant repeatability. In the subsequent analyses, I excluded monocytes, eosinophils, and basophils. Total leukocyte counts and the number and level of different leukocyte types are considered to be indicators of health status, parasitic infestation, and stress (Davis 1981; Ots et al. 1998). Leukocytosis (increase in the number of leukocytes) is most commonly due to infectious disease (Davis 1981; Fudge 1989). Heterophils are nonspecific phagocytizing immune cells, whereas lymphocytes are highly specific immune cells that can be classified into two main types: T-lymphocytes and B-lymphocytes. T-lymphocytes are considered to be the main part of cell-mediated immunity. Lymphocyte concentration in peripheral blood can be an indirect indicator of cell-mediated immunity, whereas its response to an antigen assesses immunocompetence of the individuals (see below).

Haematocrit is the relative amount of red blood cells of the total blood volume. Low values (anemia) indicate different infections, and haematocrit values can increase as a result of high workload. The haematocrit value was obtained by centrifugation of heparinized capillary tubes for 10 min at 10 000 rpm. After the haematocrit was determined, plasma was separated from blood cells and stored at -20°C until analysis.

Carotenoids are known to play an important role in immunoregulation and immunostimulation (see review in Møller et al. 2000). Lutein, which is the most important carotenoid component in the barn swallow, enhances the effectiveness of T-lymphocytes in mice and reduces the cytotoxic effects of toxins in chickens (Jyonouchi et al. 1995; Leal et al. 1998). Plasma lutein concentration was determined by extracting carotenoids from 20 μL of plasma by dilution in 180 μL of ethanol. After mixing and centrifugation (7000 rpm for 7 min), the supernatant was analysed for absorbance at 450-nm wavelength, the lutein absorbance peak (Stradi et al. 1995), by a photometer (model 550 microplate reader). Plasma lutein concentration was calculated from the absorbance data and expressed as micrograms per millilitre.

Adult female birds were assessed for their capacity to produce a T-cell-mediated immune response using the phytohemagglutinin test. Female barn swallows were captured on their nests when nestlings were 12 days old and their T-lymphocyte response assessed by injection of 0.2 mg of phytohemagglutinin (Sigma, L-8754) in 0.04 mL of phosphate-buffered saline in the right-wing web. An estimate of the response to the immune-challenge test was obtained by measuring the increase in thickness of the right-wing web (Lochmiller et al. 1993) 12 h after injection with a pressure-sensitive caliper with an accuracy of 0.01 mm. The birds were captured shortly before sunset, measured, blood sampled, and injected. Subsequently, the birds were put into a cloth bag until the next morning when their wing web was remeasured for the immune response. I used this procedure for measuring the wing-web index following Smits et al. (1999). The repeatability of wing-web measurements calculated by measuring the same bird twice was highly significant ($r = 0.86$, $F_{[27]} = 13.28$, $p < 0.0001$). Capture and manipulation had no effect on the behavior and condition of adults or nestlings, as no differences were shown between the condition of birds among the treated group and a control

group. None of the birds deserted their nests as a result of the manipulation.

Statistics

The effects of breeding date, sex, and age on health-status variables were analysed by analysis of covariance (ANCOVA) while controlling for potentially confounding effects. Repeated samples collected during the two sampling periods from the same individuals were analysed by repeated-measures analysis of variance (ANOVA) and paired t tests. Age was included in the analysis as a factor because of differences in tail length (young versus old: 98.15 ± 10.85 (mean \pm SD) versus 101.75 ± 10.22 mm, $t = -1.95$, $\text{df} = 130$, $p = 0.05$) and laying date (60.33 ± 13.79 (where 1 is 1 April) versus 45.56 ± 12.33 , $t = 6.41$, $\text{df} = 128$, $p < 0.0001$ for young and old birds, respectively). Because the laying date was clearly bimodally distributed, all birds were categorized as early or late breeders based on the mean laying date (21 May). Dates were transformed into relative dates, with 1 April being 1.

Because a significant difference was shown between early- and late-breeding males and females for capture date during the prelaying period (early versus late breeders: males: 34.31 ± 8.25 versus 53.44 ± 23.67 , $t = -4.08$, $p < 0.001$, $\text{df} = 52$; females: 34.67 ± 6.5 versus 58.7 ± 20.3 , $t = -6.05$, $p < 0.0001$, $\text{df} = 48$), this variable was included as a covariate in ANCOVA analyses. Relative capture date (capture date relative to the laying date of the first egg, which was set to 1 so that earlier dates have negative values) during the prelaying period did not differ significantly for males and females between early and late breeders. The effect of breeding time during the brood-rearing period was controlled by including laying date as a covariate. The effect of tail length on health-status differences between males and females and early and late breeders was tested by including tail length as a covariate in an ANCOVA. Because leukocyte concentration may vary diurnally (Ots et al. 1998), I first checked the effect of capture time on different health-state indices. After controlling for capture date during the first sampling, WBC and heterophile number increased significantly during the day for males (partial correlation, WBC: $r = 0.36$, $\text{df} = 1,45$, $p = 0.01$; heterophils: $r = 0.29$, $\text{df} = 1,45$, $p = 0.05$), whereas none of the variables analysed changed for females. Owing to the difference in capture time between the first and second sampling periods (on average 13.00 versus 18.00, respectively), I tested for the confounding effect of capture time for the change in health-status variables between the two capture sessions. However, none of the results changed as expected from the minor change in leukocytes during daytime in relation to time in the breeding season (see Results). Heterophile, lymphocyte, and WBC counts were log transformed when it was necessary to normalize their distributions. Untransformed data are shown in Table 4 for the presentation of the real values. Values reported are the mean \pm SD.

Results

Breeding performance of early and late breeders

Comparing the breeding performance of early and late breeders revealed a significantly higher reproductive success for early breeders, as shown by clutch size, brood size, and probability of producing a second clutch during the breeding

Table 1. Difference in breeding parameters and tail length (mean \pm SD) between early- and late-breeding barn swallows (*Hirundo rustica*) (t test for laying date, clutch size, brood size, and tail length and χ^2 test for the probability of second clutch).

	Early breeders	Late breeders	Test statistic	p	df
Breeding data					
Laying date	42.0 \pm 6.8	66.2 \pm 10.4	-11.66	<0.0001	68
Clutch size	5.0 \pm 0.7	4.6 \pm 0.5	2.44	0.0174	68
Brood size at fledging	4.6 \pm 1.2	3.8 \pm 0.9	3.02	0.0038	57
Second clutch (%)	63.9	4	19.82	<0.0001	60
Tail length (mm)					
Male	110.48 \pm 8.65	106.04 \pm 7.99	2.08	0.0423	59
Female	94.58 \pm 5.01	89.91 \pm 5.36	3.74	0.0004	67

season (Table 1). There was also a significant difference in tail length between early and late breeders for both sexes, with early breeders having significantly longer tails.

Leukocytes

Breeding date and sex were significantly related to the health status of barn swallows during prelaying, since early breeders and males generally had lower leukocyte counts than later breeders and females, respectively (Fig. 1, Table 2). Male and female barn swallows that started to breed late had significantly larger lymphocyte, heterophile, and WBC counts than early breeders. After controlling for tail length, the sex differences in WBC count disappeared, indicating an effect of tail length on sex-specific health status.

During brood rearing, none of the leukocyte types showed a significant difference between early and late breeders or between sexes (Table 2). Early-breeding males experienced a general increase in differential leukocyte count during the nestling period than during the prelaying period (first versus second sampling: heterophils: 8.72 \pm 5.08 versus 12.96 \pm 8.45, t = -2.22, df = 53, p = 0.03; lymphocytes: 17.72 \pm 8.03 versus 24.74 \pm 12.43, t = -2.46, df = 53, p = 0.02; WBC: 28.30 \pm 13.17 versus 39.23 \pm 17.03, t = -2.64, df = 53, p = 0.01). In contrast, late-breeding females experienced a decrease in heterophile and WBC number and an increase in lymphocyte number between the prelaying and brood-rearing periods (first versus second sampling: heterophils: 18.72 \pm 15.65 versus 12.14 \pm 7.74, t = 2.54, df = 40, p = 0.02; lymphocytes: 22.57 \pm 9.15 versus 35.32 \pm 20.45, t = 2.98, df = 40, p < 0.01; WBC: 56.96 \pm 35.03 versus 34.78 \pm 12.57, t = 2.83, df = 40, p < 0.01). After controlling for tail length, none of these conclusions changed.

When analysing the effects of breeding time and sampling period on males and females sampled twice, the previous findings were partially confirmed. I did not find any significant difference in differential count for males during brood-rearing than during the egg-laying period, whereas lymphocyte and WBC concentrations were lower for females during brood-rearing than during the prelaying period (Table 3), indicating an increase in physiological condition of females between the two sampling periods. The significant interaction between breeding date and sampling period suggests that the lymphocyte concentration for late-breeding females decreased significantly (a posteriori Scheffé test, p < 0.05), whereas there was no significant trend for early breeders. None of the tests changed after controlling for tail length.

The decrease in leukocyte count for late-breeding females was also evident when I compared multiple samples collected from the same individuals by paired t tests (Table 4).

Haematocrit

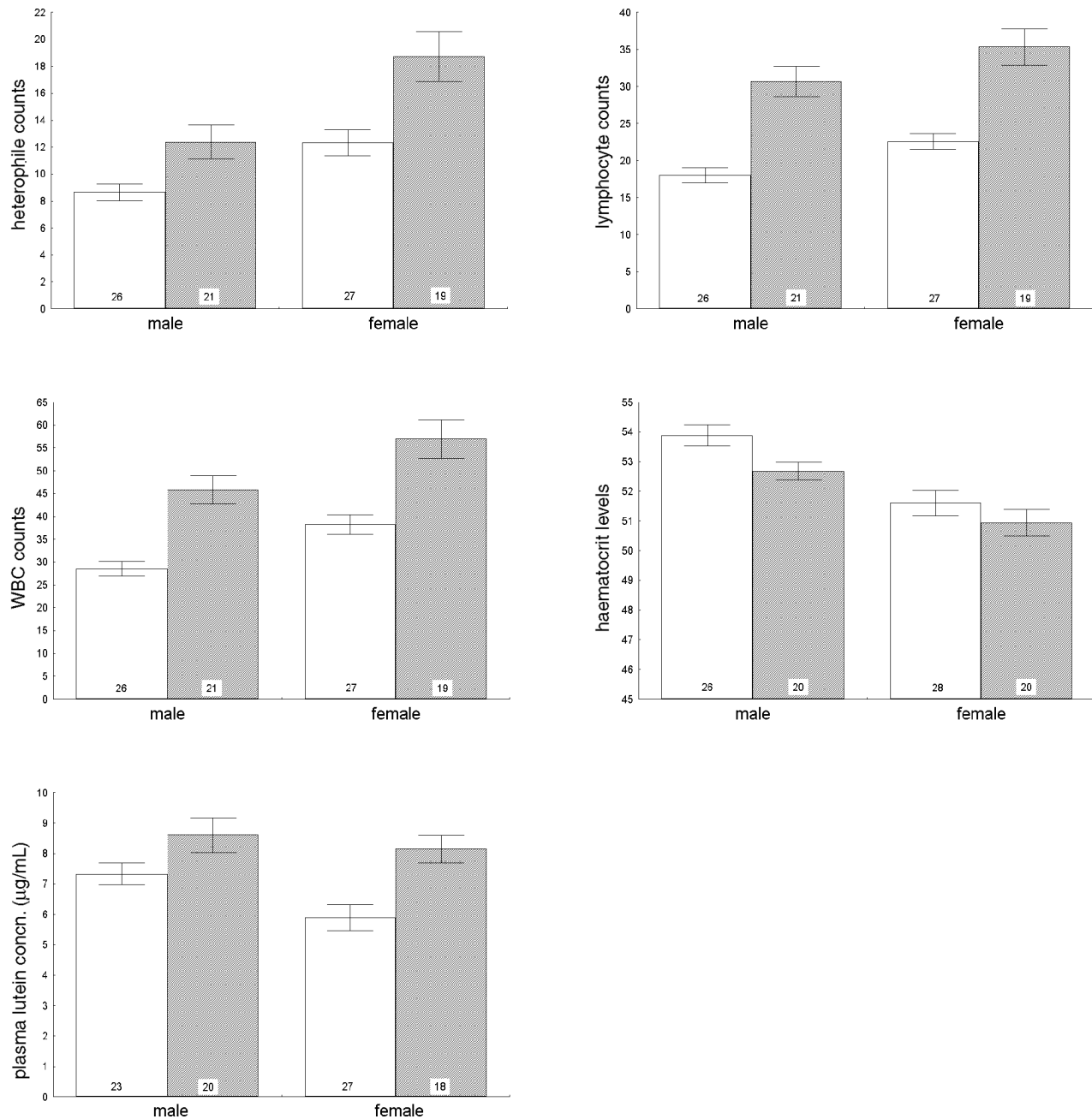
Haematocrit levels did not differ between early- and late-breeding males or females for the prelaying and nestling periods (Fig. 1, Table 2). Haematocrit was significantly higher for males than for females during both sampling periods. However, after introducing tail length as a covariate in an ANCOVA, the difference between sexes disappeared during the prelaying period ($F_{[1,85]} = 1.91$, p = 0.17), indicating the effect of tail length in the difference observed in haematocrit between males and females. There was a significant decrease in haematocrit between egg laying and brood rearing for both early- (first versus second sampling: 54.10 \pm 2.81 versus 49.80 \pm 2.61, t = 5.77, df = 52, p < 0.0001) and late-breeding males (first versus second sampling: 52.85 \pm 2.39 versus 49.57 \pm 2.62, t = 4.13, df = 38, p < 0.001) but not for females (data not shown). Haematocrit change between prelaying and brood rearing was confirmed by comparing the haematocrit of the same individuals sampled during both periods (Tables 3 and 4).

Plasma lutein concentration

During the prelaying period, there was no significant difference between early and late breeders and between sexes in plasma lutein concentration (Fig. 1, Table 2). The ANCOVA revealed a nearly significant effect of breeding date on plasma lutein concentration during brood rearing. This difference was caused by a larger increase in plasma lutein concentration between the egg-laying and brood-rearing periods for late-breeding males (8.53 \pm 4.65 versus 15.84 \pm 2.83, t = -4.61, df = 31, p < 0.0001) than for early breeders (7.33 \pm 2.77 versus 10.82 \pm 4.01, t = -3.41, df = 43, p < 0.005). This increase in plasma lutein concentration between early- and late-breeding males was also indicated by a significant interaction between breeding date and sampling (Table 3). However, this significant interaction disappeared after table-wide Bonferroni correction. During the breeding season, for both early- and late-breeding females, there was a significant increase in plasma lutein concentration between the prelaying and nestling periods (early breeders: 5.88 \pm 3.72 versus 11.23 \pm 3.51, t = -5.52, df = 52, p < 0.0001; late breeders: 8.15 \pm 3.84 versus 12.96 \pm 3.81, t = -3.66, df = 32, p < 0.001).

When comparing the plasma lutein concentration of the same individuals in the two sampling periods, I again found

Fig. 1. Absolute and differential leukocyte counts per 10 000 erythrocytes, haematocrit level, and plasma lutein concentration ($\mu\text{g/mL}$) of barn swallows (*Hirundo rustica*) in relation to sex and breeding date during the prelaying period. Open bars indicate early breeders and shaded bars indicate late breeders. Numbers within bars denote sample size. Data are presented as the mean \pm SE.



a significant increase between prelaying and brood rearing for both sexes (Tables 3 and 4). None of the significance levels changed after controlling for tail length.

Because the plasma lutein concentration has an immuno-moderator effect, I tested whether the increase in plasma lutein concentration is related to the change in leukocyte number between the prelaying and brood-rearing periods. This trade-off was confirmed by a negative relationship between increase in plasma lutein of males and change in heterophile and WBC counts between egg laying and brood rearing (heterophils: $r = -0.46$, $n = 22$, $p = 0.03$; WBC: $r =$

-0.56 , $n = 22$, $p < 0.01$), whereas no significant relationship was shown for females ($p > 0.1$).

T-cell-mediated immune response

T-cell-mediated immunocompetence of female barn swallows was tested during the nestling period. When comparing early- and late-breeding birds, I found no significant difference in immune response (Table 2). Because the immune response was positively related to body mass ($r = 0.36$, $n = 56$, $p < 0.01$), and this latter variable varied with breeding time, body mass was included in a multiple-regression analysis as

Table 2. Results of ANCOVA on differential and total leukocyte counts, haematocrit, and plasma lutein concentration of barn swallows in relation to breeding date, sex, and age after controlling for capture date in the prelaying and laying date in the brood-rearing period.

Effect	Heterophils			Lymphocytes			WBC			Haematocrit			Plasma lutein			T-cell response		
	$F_{[df]}$	p		$F_{[df]}$	p		$F_{[df]}$	p		$F_{[df]}$	p		$F_{[df]}$	p		$F_{[df]}$	p	
Prelaying period																		
Breeding date	7.90 _[1,85]	0.0061		15.52 _[1,85]	0.0002		13.59 _[1,85]	0.0004		0.80 _[1,86]	0.38		4.96 _[1,80]	0.0288*				
Sex	8.24 _[1,85]	0.0052		4.71 _[1,85]	0.0329*		6.95 _[1,85]	0.0100		6.07 _[1,86]	0.0158		1.06 _[1,80]	0.31				
Age	0.61 _[1,85]	0.44		0.30 _[1,85]	0.59		0.33 _[1,85]	0.57		1.41 _[1,86]	0.24		0.39 _[1,80]	0.53				
Breeding date × sex	0.46 _[1,85]	0.50		0.46 _[1,85]	0.50		0.05 _[1,85]	0.83		0.00 _[1,86]	0.98		0.36 _[1,80]	0.55				
Capture date (covariate)	0.30 _[1,85]	0.59		1.94 _[1,85]	0.17		3.17 _[1,85]	0.08		3.16 _[1,86]	0.08		0.00 _[1,80]	0.99				
Brood-rearing period																		
Breeding date	0.22 _[1,92]	0.64		0.54 _[1,92]	0.47		0.00 _[1,92]	0.97		3.23 _[1,95]	0.08		3.85 _[1,68]	0.0537		1.08 _[1,55]	0.30	
Sex	0.06 _[1,92]	0.81		4.95 _[1,92]	0.0285*		1.93 _[1,92]	0.17		8.54 _[1,95]	0.0043		2.40 _[1,68]	0.13				
Age	1.03 _[1,92]	0.31		5.82 _[1,92]	0.0179*		1.87 _[1,92]	0.18		0.06 _[1,95]	0.81		1.66 _[1,68]	0.20				
Breeding date × sex	0.34 _[1,92]	0.56		1.40 _[1,92]	0.24		1.33 _[1,92]	0.25		0.00 _[1,95]	0.95		5.96 _[1,68]	0.0173*				
Laying date (covariate)	0.55 _[1,92]	0.46		0.26 _[1,92]	0.61		0.05 _[1,92]	0.81		7.01 _[1,95]	0.0095		0.38 _[1,68]	0.53		2.96 _[1,55]	0.09	

Note: Asterisks indicate a change from significant to nonsignificant relationships after Bonferroni correction for multiple statistical tests (Rice 1989). Values in boldfaced type are significant after Bonferroni correction. WBC, white blood cells.

Table 3. Results of repeated-measures ANOVA on the effects of breeding date, sampling period (egg laying versus brood rearing), and age on differential and total leukocyte counts, haematocrit, and plasma lutein concentration of male and female barn swallows captured twice in both periods.

Effect	Heterophils			Lymphocytes			WBC			Haematocrit			Plasma lutein		
	$F_{[df]}$	p		$F_{[df]}$	p		$F_{[df]}$	p		$F_{[df]}$	p		$F_{[df]}$	p	
Males															
Breeding date	0.57 _[1,31]	0.46		1.64 _[1,31]	0.21		1.21 _[1,31]	0.28		0.70 _[1,31]	0.41		8.08 _[1,18]	0.0108*	
Sampling period	1.29 _[1,31]	0.27		2.72 _[1,31]	0.11		4.86 _[1,31]	0.0351*		58.07 _[1,31]	<0.0001		30.86 _[1,18]	<0.0001	
Age	0.05 _[1,31]	0.82		4.60 _[1,31]	0.0400*		1.28 _[1,31]	0.27		2.71 _[1,31]	0.11		3.47 _[1,18]	0.08	
Breeding date × sampling	0.09 _[1,31]	0.75		1.65 _[1,31]	0.21		0.75 _[1,31]	0.39		1.44 _[1,31]	0.24		6.74 _[1,18]	0.0183*	
Sampling × age	0.02 _[1,31]	0.88		0.49 _[1,31]	0.49		0.05 _[1,31]	0.82		6.09 _[1,31]	0.0193*		2.69 _[1,18]	0.12	
Females															
Breeding date	1.82 _[1,30]	0.19		3.39 _[1,30]	0.08		1.37 _[1,30]	0.25		0.27 _[1,33]	0.60		2.11 _[1,24]	0.16	
Sampling period	4.48 _[1,30]	0.0427*		18.99 _[1,30]	0.0001		7.63 _[1,30]	0.0097		1.31 _[1,33]	0.26		18.80 _[1,24]	0.0002	
Age	1.21 _[1,30]	0.28		0.94 _[1,30]	0.34		0.03 _[1,30]	0.87		1.14 _[1,33]	0.29		0.06 _[1,24]	0.82	
Breeding date × sampling	4.13 _[1,30]	0.0512		14.20 _[1,30]	0.0007		5.49 _[1,30]	0.0260*		0.46 _[1,33]	0.50		0.07 _[1,24]	0.80	
Sampling × age	0.35 _[1,30]	0.56		6.40 _[1,30]	0.0169*		1.38 _[1,30]	0.25		0.01 _[1,33]	0.93		0.43 _[1,24]	0.52	

Note: Asterisks indicate change from significant to nonsignificant relationships after Bonferroni correction for multiple statistical tests (Rice 1989). Values in boldfaced type are significant after Bonferroni correction.

Table 4. Comparison of different and total leukocyte counts, haematocrit, and plasma lutein concentration ($\mu\text{g/mL}$) (mean \pm SD) of initial and final sampling of the same individuals for early- and late-breeding barn swallows.

	Males				Females					
	Egg laying	Brood rearing	<i>t</i>	<i>n</i>	<i>p</i>	Egg laying	Brood rearing	<i>t</i>	<i>n</i>	<i>p</i>
Early breeders										
Heterophils	8.98±5.19	13.28±9.14	-2.49	24	0.0203*	11.71±7.51	13.90±8.67	-0.19	23	0.85
Lymphocytes	18.20±8.10	23.73±12.59	-2.10	24	0.0466*	22.51±9.91	21.47±12.70	1.05	23	0.30
WBC	29.16±13.25	38.52±17.68	-2.32	24	0.0298*	37.46±19.45	37.07±17.41	0.13	23	0.90
Haematocrit	53.88±2.90	49.81±2.56	5.93	23	<0.0001	51.48±3.64	51.41±2.87	0.08	26	0.93
Plasma lutein	6.73±2.67	11.55±4.37	-3.00	15	0.0095	5.84±3.60	10.83±3.98	-4.00	19	<0.0001
Late breeders										
Heterophils	11.38±9.66	12.37±4.23	-0.59	11	0.57	21.68±19.93	12.73±8.91	2.08	11	0.06
Lymphocytes	29.46±21.15	27.91±11.13	0.27	11	0.79	35.06±25.02	20.28±9.33	3.11	11	0.0111
WBC	42.39±28.59	41.75±13.35	-0.54	11	0.60	58.42±44.48	34.77±14.99	2.53	11	0.0296*
Haematocrit	53.07±1.95	48.57±2.35	4.46	12	0.0010	51.92±3.30	50.84±4.25	1.05	11	0.32
Plasma lutein	6.46±4.33	16.44±2.58	-7.88	7	0.0002	8.07±4.37	13.12±3.65	-4.62	9	0.0017

Note: Asterisks indicate change from significant to nonsignificant relationships after Bonferroni correction for multiple statistical tests (Rice 1989). Values in boldface type are significant after Bonferroni correction.

an independent variable. After controlling for body mass, late-breeding females showed a significantly higher immune response than early breeders ($F_{[2,53]} = 7.16$, $p < 0.005$, slope \pm SE = 0.29 ± 0.13).

Correlation between health status and reproductive success

I tested the predicted negative correlation between clutch size and health status for males and females. In this analysis, I included only the subset of birds sampled before the start of laying. Because egg laying and relative capture date can affect the health status of birds, I used residual health-status indices obtained after entering laying date and relative capture date in a multiple regression as independent variables. Residual health-status indices did not significantly predict clutch size for males ($F_{[1,34]} = 0.93$, $p = 0.49$) or for females ($F_{[1,33]} = 0.59$, $p = 0.74$). These results indicate that the health status of birds prior to egg laying had no effect on the number of eggs laid.

Discussion

Health status, arrival date, and start of breeding

I found a significant negative relationship between health-status indices and breeding date for both male and female barn swallows. During the prelaying period, birds that started to breed early had lower lymphocyte, heterophile, and WBC counts than late breeders, indicating superior physiological condition for early breeders. Haematocrit was not significantly related to breeding date. There was no difference in plasma lutein concentration between birds breeding early or late.

Many studies have investigated the effect of breeding time on reproductive success and fitness (e.g., Perrins 1970; Hatchwell 1991; Brinkhof et al. 1993; Wiggins et al. 1994; Winkler and Allen 1996). They have generally shown that late breeders have lower reproductive success than early breeders owing to lower rates of recruitment and lower survival rates of adults. The lower reproductive success of late breeders was also confirmed in this population of barn swallows. Several hypotheses have been suggested to explain this seasonal decrease in fitness. The date hypothesis suggests that gradual deterioration of food supply is the principal factor affecting brood reduction, whereas the parental-quality hypothesis suggests that the difference in quality observed between early- and late-breeding birds is the principal factor affecting breeding time and hence reproductive success.

There is evidence supporting the mechanistic basis of the latter hypothesis because individuals of higher phenotypic quality arrive early and breed first (e.g., Lundberg and Alatalo 1992; Møller 1994a, 1994b). In the barn swallow, males with longer tails (a condition-dependent secondary sexual character) arrived earlier at the breeding site, had a shorter interval between arrival and egg laying, and generally had higher fitness as measured by reproductive success and survival (Møller 1994b) (see also Table 1). Health status of birds could be very important during the breeding season because of the high risk of infestation by parasites and because low immunocompetence can affect reproductive success and survival to the next breeding season (Møller 1990; Saino et al. 1997a; Sorci et al. 1997; Moreno et al. 1999).

Thus, parent birds should invest in maintenance of physiological condition.

Considering the energetic or physiological cost of the immune system (Lochmiller and Deerenberg 2000; Møller et al. 2000; Moret and Schmid-Hempel 2000), we should expect that low-quality individuals have a trade-off between health status and life-history traits different from that of high-quality individuals. In this context, we should expect individuals with lower condition to postpone breeding to recover their condition after depletion, owing to migration. Barn swallows that started to breed earlier seemed to be in better condition than late breeders, as also reported in other studies on breeding date and physiological health status of birds (Gustafsson et al. 1994; Moreno et al. 1998; Hasselquist et al. 2001). Hence, my data support previous findings on the barn swallow, namely that early-arriving males are of superior quality as reflected by their longer tail (as was also the case in this population), better health status, and higher fitness (Møller 1994a, 1994b; Saino and Møller 1996; Saino et al. 1997a). Because of the problems in interpreting results of correlational studies of breeding date and condition (van Noordwijk and de Jong 1986), I cannot be sure of the direction of causality between the investigated factors. However, the observations are consistent with the hypothesis that both phenotypic and physiological condition affect breeding time.

I also found differences in health status and haematocrit between sexes during the prelaying period, with males having generally lower leukocyte counts and higher haematocrit than females. The higher leukocyte counts in females than in males may indicate a higher level of stress or alternatively an elevated exposure to parasites. However, this latter explanation seems unlikely, since I did not find any significant sex difference in infestation rate and prevalence for any parasites (ectoparasites (mites, *Acari* spp., and louseflies, Hippoboscidae spp.), blood parasites (the average prevalence of *Haemoproteus* sp., *Trypanosoma* sp., and *Microfilaria* sp.), and intestinal parasites (P.L. Pap, A.O. Benkö, and R. Márkus, unpublished data). There was no significant sex difference in plasma lutein concentration during prelaying. The higher haematocrit level in males is not surprising, given the aerodynamic cost of a long tail in male barn swallows. This handicap may be even more pronounced during migration, when the benefits to males of early arrival are greater than for females. The effect of tail length on elevated haematocrit of males during the prelaying period was confirmed after controlling for tail length, revealing a significant difference in the relationship between tail length and haematocrit in the two sexes. The observation of a better health status in males than in females is surprising, considering the cost of a long tail for males during the energetically demanding migration period (the birds were captured shortly after arrival at the breeding grounds). Furthermore, immunosuppressive effects of testosterone observed in barn swallows during prelaying may even further reduce the condition of males (Saino et al. 1995). However, in a correlational study, males in prime condition may be able to have long tail feathers, arrive early, and still be in good health status because of the overriding effects of condition (van Noordwijk and de Jong 1986). An experimental study would be required to reveal the trade-offs between condition, sexual ornamentation, and arrival. Hōrak et al. (1998) found for the great tit (*Parus major*) that males at

the beginning of the breeding cycle generally were in worse condition than females. However, comparisons between migrants and resident birds may not be straightforward. The relationship between tail length and WBC in the two sexes indicates a possible sex difference in health status during prelaying directly related to tail length.

Health status during the brood-rearing period

Differences in health status between early- and late-breeding barn swallows disappeared during the brood-rearing period owing to a general increase in leukocytes by early-breeding males and a recovery in condition by late-breeding females. A component of immunocompetence of brood-rearing females, assessed by the T-lymphocyte response to phytohemagglutinin, was also higher for barn swallows that started breeding late compared with early breeders. Deterioration of condition of early-breeding birds has previously been shown for the chinstrap penguin, although there was no sex difference (Moreno et al. 1998).

Haematocrit was higher for males than for females, and there was a decrease between prelaying and brood rearing for males but not for females. These results support the previous findings of Saino et al. (1997b) showing that males with longer tails generally have higher haematocrits, particularly when arriving from migration. The sex difference in haematocrit and the sex difference in seasonal change in haematocrit can be explained by the elevated oxygen uptake in males, owing to the energetic cost of a long tail during migration and to sexual display early during the breeding season.

Plasma lutein concentration increased significantly between prelaying and brood rearing for both males and females and for both early and late breeders. However, the rate of increase was higher for late- than for early-breeding males, whereas this was not the case for females. The greater increase in leukocyte counts for early-breeding males between prelaying and brood rearing and the smaller increase in plasma lutein concentration between prelaying and brood rearing for early-breeding males than for late breeders may suggest a trade-off between health status and circulating lutein, as suggested in previous studies of this species (Saino et al. 1999). This trade-off was confirmed by a negative relationship between increase in plasma lutein of males and change in leukocyte count between egg laying and brood rearing.

In conclusion, my results support the prediction that delayed breeding is associated with lower health status in the barn swallow, with early breeders being in superior condition. This finding suggests that health status is an important determinant of breeding date. There is a seasonal sex-specific change in condition of breeding barn swallows. The condition of early-breeding males deteriorated between prelaying and brood rearing, whereas late-breeding females showed the opposite trend. Thus, individuals of different quality seem to pay different physiological costs of reproduction during the course of the breeding season, and this cost is sex specific.

Acknowledgements

I am very grateful to Anders Pape Møller for his assistance and valuable comments on the manuscript. I thank

Tibor Szép and Eszter Ruprecht for their help during my work and Róbert Márkus for analyzing the smears. Claudie Haussy helped with the carotenoid analyses. John Ewen kindly corrected my English in an earlier version of the manuscript. This work was supported by a travel grant from the European Science Foundation. My work was also supported by the Romanian Ministry of Education, Hungarian Ministry of Education, Arany János Foundation, and Sapientia Hungariae Foundation.

References

- Brinkhof, M.W.G., Cavé, A.J., Hage, F.J., and Vehulst, S. 1993. Timing of reproduction and fledging success in the coot (*Fulica atra*): evidence for a causal relationship. *J. Anim. Ecol.* **62**: 577–587.
- Brinkhof, M.W.G., Cavé, A.J., and Perdeck, A.C. 1997. The seasonal decline in the first-year survival of juvenile coots: an experimental approach. *J. Anim. Ecol.* **66**: 73–82.
- Davis, P.J. 1981. Immunity to Coccidia. In *Avian immunology*. Edited by M.E. Rose, L.N. Payne, and B.M. Freeman. British Poultry Science, Edinburgh. pp. 361–385.
- Deerenberg, C., Apanius, V., Daan, S., and Bos, N. 1997. Reproductive effort decreases antibody responsiveness. *Proc. R. Soc. Lond. B Biol. Sci.* **264**: 1021–1029.
- Folstad, I., and Karter, A.J. 1992. Parasites, bright males, and the immunocompetence handicap. *Am. Nat.* **139**: 603–622.
- Fudge, A.M. 1989. Avian hematology: identification and interpretation. In *Proceedings of the Association of Avian Veterinarians*, Seattle, Wash., 1989. pp. 284–292.
- Gustafsson, L., Nordling, D., Andersson, M.S., Sheldon, B.C., and Qvarnström, A. 1994. Infectious diseases, reproductive effort and the cost of reproduction in birds. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* No. 346, pp. 323–331.
- Hasselquist, D., Wasson, M.F., and Winkler, D.W. 2001. Humoral immunocompetence correlates with date of egg-laying and reflects work load in female tree swallows. *Behav. Ecol.* **12**: 93–97.
- Hatchwell, B.J. 1991. An experimental study of the effects of timing of breeding on the reproductive success of common guillemots (*Uria aalge*). *J. Anim. Ecol.* **60**: 721–736.
- Hörak, P., Jenni-Eirmann, S., Ots, I., and Tegelmann, L. 1998. Health and reproduction: sex-specific clinical profile of great tits (*Parus major*) in relation to breeding. *Can. J. Zool.* **76**: 2235–2244.
- Jyonouchi, H., Sun, S., and Gross, M. 1995. Effects of carotenoids on in vitro immunoglobulins production by human peripheral blood mononuclear cells: astaxanthin, a carotenoid without vitamin A activity, enhances in vitro immunoglobulin production in response to a T-dependent simulant and antigen. *Nutr. Cancer*, **23**: 171–183.
- Kokko, H. 1999. Competition for early arrival in migratory birds. *J. Anim. Ecol.* **68**: 940–950.
- Leal, M., de Mejia, E.G., Ruiz, F., and Shimada, A. 1998. Effects of carotenoids on cytotoxicity of T-2 toxin on chicken hepatocytes *in vitro*. *Toxicol. In Vitro*, **12**: 133–139.
- Lochmiller, R.L., and Deerenberg, C. 2000. Trade-off in evolutionary immunology: just what is the cost of immunity? *Oikos*, **88**: 87–98.
- Lochmiller, R.L., Vestey, M.R., and Boren, J.C. 1993. Relationship between protein nutritional status and immunocompetence in northern bobwhite chicks. *Auk*, **110**: 503–510.
- Lundberg, A., and Alatalo, R.V. 1992. The pied flycatcher. Academic Press, San Diego.
- Martin, T.E., Møller, A.P., Merino, S., and Clobert, J. 2001. Does clutch size evolve in response to parasites and immunocompetence? *Proc. Natl. Acad. Sci. U.S.A.* **98**: 2071–2076.
- Moreno, J., de León, A., Fargallo, J.A., and Moreno, E. 1998. Breeding time, health and immune response in the chinstrap penguin (*Pygoscelis antarctica*). *Oecologia*, **115**: 312–319.
- Moreno, J., Sanz, J.J., and Arriero, E. 1999. Reproductive effort and T-lymphocyte cell-mediated immunocompetence in female pied flycatchers (*Ficedula hypoleuca*). *Proc. R. Soc. Lond. B Biol. Sci.* **266**: 1105–1109.
- Moret, Y., and Schmid-Hempel, P. 2000. Survival for immunity: the price of immune system activation for bumblebee workers. *Science* (Washington, D.C.), **290**: 1166–1168.
- Møller, A.P. 1990. Effects of an haematophagous mite on the barn swallow (*Hirundo rustica*): test of the Hamilton and Zuk hypothesis. *Evolution*, **44**: 771–784.
- Møller, A.P. 1993. Sexual selection in the barn swallow (*Hirundo rustica*). III. Female tail ornaments. *Evolution*, **47**: 417–431.
- Møller, A.P. 1994a. Phenotype-dependent arrival time and its consequences in a migratory bird. *Behav. Ecol. Sociobiol.* **35**: 115–122.
- Møller, A.P. 1994b. Sexual selection and the barn swallow. Oxford University Press, Oxford.
- Møller, A.P. 2001. Heritability of arrival date in a migratory bird. *Proc. R. Soc. Lond. B Biol. Sci.* **268**: 203–206.
- Møller, A.P., Christe, P., and Lux, E. 1999. Parasite-mediated sexual selection: effects of parasites and host immune function. *Q. Rev. Biol.* **74**: 3–20.
- Møller, A.P., Biard, C., Blount, J.D., Houston, D.C., Ninni, P., Saino, N., and Surai, P.F. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability? *Avian Poult. Biol. Rev.* **11**: 137–159.
- Norris, K., and Evans, M.R. 2000. Ecological immunology: life history trade-offs and immune defense in birds. *Behav. Ecol.* **11**: 19–26.
- Oppliger, A., Richner, H., and Christe, P. 1994. Effect of an ectoparasite on lay date, nest site choice, desertion, and hatching success in the great tit (*Parus major*). *Behav. Ecol.* **5**: 130–134.
- Ots, I., and Hörak, P. 1996. Great tits (*Parus major*) trade health for reproduction. *Proc. R. Soc. Lond. B Biol. Sci.* **263**: 1443–1447.
- Ots, I., Murumägi, A., and Hörak, P. 1998. Haematological health state indices of reproducing Great Tits: methodology and sources of natural variation. *Funct. Ecol.* **12**: 700–707.
- Perrins, C.M. 1970. The timing of birds' breeding seasons. *Ibis*, **112**: 242–255.
- Price, T., Kirkpatrick, M., and Arnold, S.J. 1988. Directional selection and the evolution of breeding date in birds. *Science* (Washington, D.C.), **240**: 798–799.
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution*, **43**: 223–225.
- Rowe, L., Ludwig, D., and Schluter, D. 1994. Time, condition, and the seasonal decline of avian clutch size. *Am. Nat.* **143**: 698–722.
- Saino, N., and Møller, A.P. 1996. Sexual ornamentation and immunocompetence in the barn swallow. *Behav. Ecol.* **7**: 227–232.
- Saino, N., Møller, A.P., and Bolzern, A.M. 1995. Testosterone effects on the immune system and parasite infestations in the barn swallow (*Hirundo rustica*): an experimental test of the immunocompetence hypothesis. *Behav. Ecol.* **6**: 397–404.
- Saino, N., Bolzern, A.M., and Møller, A.P. 1997a. Immunocompetence, ornamentation, and viability of male barn swallows (*Hirundo rustica*). *Proc. Natl. Acad. Sci. U.S.A.* **94**: 549–552.
- Saino, N., Cuervo, J.J., Krivacek, M., de Lope, F., and Møller, A.P. 1997b. Experimental manipulation of tail ornament size affects the

- hematocrit of male barn swallows (*Hirundo rustica*). *Oecologia*, **110**: 186–190.
- Saino, N., Stradi, R., Ninni, P., Pini, E., and Møller, A.P. 1999. Carotenoid plasma concentration, immune profile, and plumage ornamentation of male barn swallows (*Hirundo rustica*). *Am. Nat.* **154**: 441–448.
- Smits, J.E., Bortolotti, G.R., and Tella, J.L. 1999. Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Funct. Ecol.* **13**: 567–572.
- Sorci, G., Soler, J.J., and Møller, A.P. 1997. Reduced immunocompetence of nestlings in the replacement clutches of the European magpie (*Pica pica*). *Proc. R. Soc. Lond. B Biol. Sci.* **264**: 1593–1598.
- Stradi, R., Celentano, G., Rossi, E., Rovati, G., and Pastore, M. 1995. Carotenoids in bird plumage. I. The carotenoid pattern in a series of Palearctic Carduelinae. *Comp. Biochem. Physiol. B Comp. Biochem.* **110**: 131–143.
- van Noordwijk, A.J., and de Jong, G. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.* **128**: 137–142.
- van Noordwijk, A.J., van Balen, J.H., and Scharloo, W. 1981. Genetic variation in the timing of reproduction in the great tit. *Oecologia*, **49**: 158–166.
- Wiggins, D.A., Part, T., and Gustafsson, L. 1994. Seasonal decline in collared flycatcher (*Ficedula albicollis*) reproductive success: an experimental approach. *Oikos*, **70**: 359–364.
- Winkler, D.W., and Allen, P.E. 1996. The seasonal decline in tree swallow clutch size: physiological constraint or strategic adjustment? *Ecology*, **77**: 922–932.
- Zuk, M., and Johnsen, T.S. 1998. Seasonal changes in the relationship between ornamentation and immune response in red jungle fowl. *Proc. R. Soc. Lond. B Biol. Sci.* **265**: 1631–163.

Paper 2

In: *Journal of Avian Biology* **34**, pp. 428-434

Cost of reproduction, T-lymphocyte mediated immunocompetence and health status in female and nestling Barn Swallows *Hirundo rustica*

by
Péter László Pap and Róbert Márkus

Cost of reproduction, T-lymphocyte mediated immunocompetence and health status in female and nestling barn swallows *Hirundo rustica*

Péter L. Pap and Róbert Márkus

Pap, P. L. and Márkus, R. 2003. Cost of reproduction, T-lymphocyte mediated immunocompetence and health status in female and nestling barn swallows *Hirundo rustica*. – J. Avian Biol. 34: 428–434.

We investigate the trade-off between reproductive effort, health status and T-lymphocyte acquired immunity in female and nestling barn swallows *Hirundo rustica* using a brood size manipulation experiment. Maternal and total feeding effort increased with experimental brood size. Parents did not fully compensate for the increased food demand of the enlarged broods and as a consequence the per capita feeding rate of nestlings decreased with increasing experimental brood size. Body mass and a measure of T-cell mediated immunity in 12 days old nestlings also decreased with increasing experimental brood size. Different leucocyte concentrations and the heterophile/lymphocyte ratio – an index of stress – of nestlings did not change in relation to experimental brood size, suggesting that within brood competition did not affect stress to nestlings. The brood size manipulation had a significant effect on maternal T-cell mediated immunity, measured by the phytohemagglutinin skin test, but not on maternal body mass, haematocrit or differential or total white blood cell counts. Our results seem to support the prediction that under mild work stress females respond first by reducing the energetically expensive acquired immunity. Different leucocyte types and the heterophile/lymphocyte ratio appear less sensitive to parental workload.

P. L. Pap (correspondence), Behavioural Ecology Research Group, Department of Evolutionary Zoology, University of Debrecen, H-4010 Debrecen, Hungary, and Department of Zoology, Babes-Bolyai University, R-3400 Cluj Napoca, Clinicilor street no. 5-7, Romania. E-mail: peterpl@hasdeu.ubbcluj.ro. R. Márkus, Biological Research Center of the Hungarian Academy of Sciences, Institute of Genetics, Lab. of Molecular Immunology, H-6726 Szeged, Temesvári street no. 62, Hungary.

An increase in parental activity has been shown to increase parasite infestation, probably due to a reduction in immune function and health status of breeding individuals (e.g. Richner et al. 1995, Nordling et al. 1998). The immune system consists of two parts, the innate and the acquired immune system. Innate immunity acts as a primary defense mechanism during the initial stages of infestation. Heterophils, the most numerous granulocytes in peripheral blood, and monocytes are known as a primary means of controlling extracellular bacterial infestations and macro-parasites (Gergely and Erdei 1998). Natural killer cells act primarily during intracellular bacterial and viral infections. The more specific acquired immunity acts more

effectively against various pathogens, including viruses, bacteria and ectoparasites (Wakelin 1996, Gergely and Erdei 1998), but with a high metabolic cost due to rapid cell proliferation (Apanius 1998).

Previous studies of the effects of parental effort on parental health status have been of two kinds. First, a study of the great tit *Parus major* showed an increase in the number of heterophils (derived from the innate immune system) and a decrease in lymphocyte numbers (derived from both the innate and the acquired immune system) in peripheral blood of the great tits, when the broods were experimentally increased (Ots et al. 1998). The elevated heterophile concentration and a reduction of the lymphocyte number in the blood due to altered

parental effort could indicate either elevated stress, or it may express a depression of innate immunity. Second, other studies, based on responses to challenges of the acquired immune system by a novel antigen, have also investigated the effects of altered parental effort on the health status of reproducing birds. These studies show that cell mediated and humoral immunity can be suppressed by increased parental activity (Deerenberg et al. 1997, Nordling et al. 1998, Moreno et al. 1999). However, our knowledge of the effects of experimentally altered reproductive efforts on the immunocompetence of free living birds is limited (e.g. Nordling et al. 1998, Moreno et al. 1999).

The physiological background of the trade-off between parental effort and immunocompetence is two-fold. First, due to the cost of the immunological defence, both traits compete for the same resources, and as a consequence an increase in reproductive effort should result in suppression of the immune system. Second, an increased locomotory activity, like parental effort, results in increased work stress, which through neurological and endocrine linkage inhibits the immune system (Besedovsky and del Rey 1996, Wingfield et al. 1997, Apanius 1998).

In this study we used several health-state and immunological tests to investigate the physiological background responsible for the trade-off between reproductive effort and immunocompetence in female barn swallows *Hirundo rustica*. We did this by manipulating parental effort by increasing or decreasing the brood size with one nestling. To assess the stress level of females we determined the differential leucocyte number and the heterophile/lymphocyte (H:L) ratio in the peripheral blood based on blood smears. Furthermore, a change in differential leucocyte types could indicate the effect of our brood size manipulation on the innate immunity of the birds. The activity of the acquired immune system (henceforth immunocompetence) of the birds was assessed based on the activity of the T-cells towards to an antigen injected in the left wing web. Based on studies on poultry and wild birds (reviewed in Goto et al. 1978, Alonso-Alvarez and Tella 2001), we assumed that a strong response (inflammation) of the bird to an antigen reflects the activity of the T-cells and according to this, the stronger the response, the more immunocompetent the individual.

Just as in the adults, the health status and immunocompetence of the nestlings in increased broods can be suppressed by food shortage (Lochmiller et al. 1993, Saino et al. 1997a). Furthermore, in experimentally enlarged broods, due to within-brood competition, stress can render nestlings even more susceptible to parasites via the negative effect of the stress hormones on the immune system. Accordingly, we investigated the effect of our brood-size manipulation on the condition, health state and immunocompetence of the nestlings (see review by Alonso-Alvarez and Tella 2001).

Study area and methods

General

The study was conducted in 2000 and 2001 in three colonies near Balmazújváros (47°37'N, 21°21'E), eastern Hungary. The barn swallow breeds in colonies of up to 15 pairs, inside buildings. Nests were checked systematically from 20 April, and date of clutch initiation, clutch size and number of fledged young was noted. Nestlings were weighed 12 days after hatching (hatching day = 0), and their tarsus and wing length were measured. Adult birds were captured with mist-nets during the pre-laying period and while feeding 12-day old nestlings.

Manipulation of reproductive effort

We performed a brood size manipulation experiment by transferring nestlings between broods that hatched on the same day and with no more than a two nestlings difference in brood size among pairs of broods. We exchanged the nestlings between broods on the day after hatching (for a detailed description of the method, see Saino et al. 1997a). By this manipulation we created two experimental groups, where the nests were either randomly increased or decreased by one nestling. In the third group (control), half of the nestlings were taken out the day after the nestlings hatched for a period of 10 minutes (the average time needed for the exchange of nestlings between manipulated broods), in order to control for the effect of the manipulation. There was no difference in original clutch size (two-way ANOVA with year and groups as factors, $F_{2,75} = 0.42$, $P = 0.66$) or laying date ($F_{2,75} = 0.16$, $P = 0.85$) among groups after controlling for the effect of year. To control for the effect of year, we included year as a factor in the subsequent analyses. There was no significant difference among experimental groups in body mass, tarsus length, wing length and tail length of females ($P > 0.1$).

We recorded provisioning rates of males and females on days 10–12 after hatching for 1 h between 7.00 and 12.00, the time of day that feeding rate is highest. We recorded feeding rate only on sunny days and whenever possible on the same day for the pairs in a group of three manipulated nests.

Health status and immunocompetence

We collected blood samples (80 µl) from the brachial vein of breeding female barn swallows, 12 days after the hatching of nestlings, in heparinized capillary tubes in order to assess haematocrit. After collection, the blood samples were stored in a cooling box until centrifugation for 10 min at 10,000 r.p.m. Haematocrit is a serological variable that can be regarded as an indicator

of metabolic activity for the period preceding the date of blood sampling, due to the effect of dehydration and increased erythropoiesis on haematocrit (Carpenter 1975). Birds raise their individual haematocrit levels in response to intense locomotory activity (Palomeque and Planas 1978).

For estimating the number and proportion of different leucocyte types, a drop of blood was smeared on a microscope slide, air-dried, fixed in methanol and stained with May-Grünwald and Giemsa. All smears were examined by the same person (RM) at $\times 1000$ magnification and the proportion of different types of leucocytes was assessed on the basis of the examination of 100 leucocytes. The number of different white blood cells was expressed per approximately 10,000 erythrocytes. The repeatability of leucocyte counts estimated by rescanning 16 smears was significantly high ($r = 0.86$, $F = 13.5$; $r = 0.74$, $F = 6.8$ and $r = 0.86$, $F = 11.7$ for heterophile, lymphocyte and total white blood cell (WBC) counts, respectively, $df = 1,15$, $P < 0.001$) or intermediate ($r = 0.51$, $F = 3.1$ and $r = 0.54$, $F = 3.3$ for monocytes and H:L ratio, respectively, $df = 1,15$, $P < 0.05$), while eosinophils and basophils did not show significant repeatability. In the subsequent analyses we excluded eosinophils, basophils and monocytes due to their low repeatability and low proportion in the blood. Total leucocyte counts and the number and level of different leucocyte types are considered to be indicators of health status, parasitic infestation and stress (Davis 1981, Ots et al. 1998). 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. They act in the primary defense against parasites, during stress and various inflammations. The H:L ratio is widely used as an indicator of stress in poultry (Maxwell 1993), and it is known to increase under various stressful conditions. The adaptive immune system is comprised of the lymphocytes, highly specific immune cells 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 response to an antigen measure the cell mediated immune response of an individual (see below).

We assessed the cell-mediated immunity of breeding female barn swallows in response to brood size manipulation by T-lymphocyte activity to an antigen (phytohemagglutinin, Sigma, L-8754) injected intradermally in the wing web on day 12 after hatching (Saino et al. 1997a). The phytohemagglutinin skin test is considered a useful method for the evaluation of the thymus-de-

pendent function (Goto et al. 1978) and has been used as an estimate of T-lymphocyte cell mediated immune activity (Goto et al. 1978, Cheng and Lamont 1988, Alonso-Alvarez and Tella 2001). The immunization protocol was as follows: female birds were captured immediately before sunset at the nest, and after the measurements and blood samples had been taken, one of us (PLP) injected 0.2 mg PHA diluted in 0.04 ml of phosphate buffered saline (PBS) in the right wing web. The activity of T-lymphocytes was estimated by measuring the increase in inflammation of the wing web between injection and 12 hours after immunization with a pressure sensitive spessimeter (accuracy 0.01 mm). After the immunization protocol, the birds were put in a cloth bag until the next morning when the birds were re-measured for the immune response. None of the birds deserted their nests due to the manipulation. The nestlings were injected and measured too, but the immunization protocol differed from that of the adults by measuring the increase of the wing web 24 hours after immunization. We consider the 12 h immunization protocol on adult birds reliable in measuring the immunocompetence of the birds, since in a study on domestic chickens *Gallus gallus* the T-cell immunocompetence of birds was highly repeatable within individual birds, between measurements at 12 and 24 hours after immunization (Goto et al. 1978). We used the simplified protocol proposed by Smits et al. (1999) which avoids the injection of PBS in the opposite wing-web as a control. The advantage of this method is a decrease in handling time and a reduction in the probability of errors, by decreasing the coefficient of variation due to the difficulties in measuring accurately the thin wing web and the correct injection of the antigen. Because previous knowledge of the manipulation can influence the wing web measurements, we tried to avoid this bias as follows. First, on days when more than one experimental pair of birds was measured (31% of females), we measured the birds blindly without any previous knowledge about the group to which they belonged. Second, in order to reduce measurement errors as much as possible, we measured the wing web of each bird three times. The repeatability of wing web measurements was high ($r = 0.86$, $F = 13.28$, $df = 2,27$, $P < 0.0001$). Third, an indirect way which might indicate the reliability of our measurements is the positive correlation found between wing web index and body mass (see Results), a relationship generally found in other studies as well (Alonso-Alvarez and Tella 2001). Although we cannot exclude completely that our results on immunocompetence were influenced by our knowledge on the experimental status of the birds, we consider this effect to be minor. Because the PHA response was stronger in 2001 than in 2000 ($t = 4.72$, $df = 93$, $P < 0.0001$), we included year as a factor in multivariate analyses (two-way ANOVA).

Table 1. Effect of brood size manipulation on parental provisioning rate. The confounding effects of year was controlled for in a two-way ANOVA, where year was entered as a factor. Values are means \pm SD (n).

	Treatment			F	P
	Increased	Control	Reduced		
Female feeding rate	22.14 \pm 6.07 (22)	19.43 \pm 5.52 (30)	17.62 \pm 6.53 (26)	3.26	0.0442
Total feeding rate	42.41 \pm 11.73 (22)	37.33 \pm 10.99 (30)	32.35 \pm 9.82 (26)	4.82	0.0108
Per capita feeding rate	7.94 \pm 2.37 (22)	8.35 \pm 2.36 (30)	10.11 \pm 3.66 (26)	4.26	0.0178

Table 2. The mean brood size at 12 days old nestlings, and the effect of brood size manipulation on offspring body mass, T-cell response and health status. The confounding effects of year was controlled for in a two-way ANOVA, where year was entered as a factor. Values are means \pm SD (n).

	Treatment			F	P
	Increased	Control	Reduced		
Brood size at day 12	5.46 \pm 0.86 (22)	4.55 \pm 1.03 (33)	3.42 \pm 0.95 (26)	25.93	<0.0001
Body mass (g)	20.99 \pm 1.19 (22)	21.77 \pm 1.95 (33)	22.4 \pm 1.47 (26)	4.16	0.0194
PHA response	83.92 \pm 23.21 (22)	98.08 \pm 32.45 (31)	111.46 \pm 24.32 (26)	9.67	0.0002
Heterophils	20.21 \pm 7.12 (21)	24.64 \pm 16.34 (33)	19.39 \pm 9.58 (26)	1.86	0.16
Lymphocytes	40.96 \pm 9.93 (21)	37.96 \pm 17.36 (33)	41.15 \pm 18.25 (26)	0.87	0.43
Total White Blood Cell Counts	67.43 \pm 14.52 (21)	66.81 \pm 30.03 (33)	66.19 \pm 25.53 (26)	0.01	0.99
H:L	0.52 \pm 0.25 (21)	0.67 \pm 0.35 (33)	0.52 \pm 0.27 (26)	2.90	0.06

All variables were normally distributed except for the H:L ratio for females and lymphocyte count for nestlings, which were log-transformed to obtain a normal distribution. Because some individuals were sampled and assessed for immunocompetence in both years, we randomly excluded one item from the data in order to avoid pseudo-replication. All tests are two-tailed and the values reported are means \pm SD.

Results

The brood size manipulation resulted in a significant difference in provisioning rate when nestlings were 12 days old (Table 1). Female barn swallows responded to the brood size manipulation experiment by increasing their feeding rate in enlarged broods, and significantly reducing the feeding effort in reduced broods. Total feeding rate also differed significantly among experimental groups, since parents fed their nestlings more frequently in increased than in decreased broods. Nevertheless, the per capita feeding rate of nestlings was significantly higher in reduced than in the control and the enlarged groups (Table 1, Tukey's post hoc test, $P < 0.05$). The difference among treatments in nestling body mass and T-cell mediated immune response was significant (Table 2), while differential and total leucocyte counts were similar between experimental groups.

The T-cell immune response of female barn swallows captured for the first time in 2001 did not differ from that of birds with a previous immunization in 2000 (immunized in 2000 vs. unmanipulated: 27.2 ± 12.5 , $n = 15$ vs. 25.3 ± 8.5 , $n = 26$, $t = 0.58$, $P = 0.57$), even

after controlling for the potentially confounding effects of age, laying date and manipulation. Thus the difference in the cell mediated immune response of females in the two years could not be accounted for by differences in previous contact with the antigen. T-cell immune response of female barn swallows when nestlings were 12 days old was significantly and positively correlated with their body mass in 2000 (Fig. 1). Brood size manipulation significantly affected T-cell mediated immune response after controlling for differences among years (Table 3, Fig. 2). The effect of brood size manipulation on the T-cell response of females remained significant after controlling for body mass on day 12 of the nestling period (two-way ANCOVA, $F_{2,68} = 5.67$, $P = 0.005$). The effect of manipulation did not change

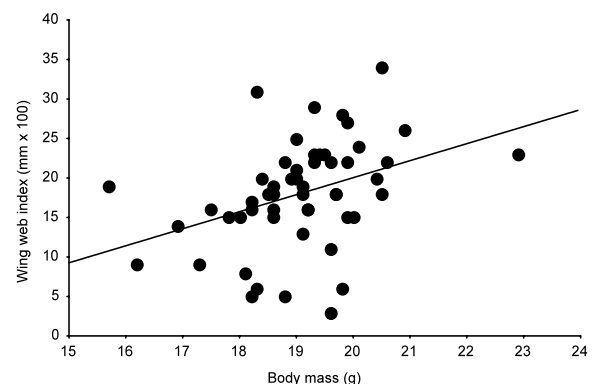


Fig. 1. T-cell response of breeding female barn swallows at 12 day old nestlings in relation to the body mass (g). The data are from 2000, $r = 0.38$, $n = 53$, $P = 0.005$, wing web index = $2.16 \times$ body mass $- 23.06$.

Table 3. Effect of brood size manipulation on T-cell response, body mass, haematocrit and health status of adult female barn swallows when nestlings were 12 days old. The confounding effect of year was controlled for in a two-way ANOVA, where year was entered as a factor. Values are mean \pm SD (n).

	Treatment			F	P
	Increased	Control	Reduced		
PHA response	18.55 \pm 8.39 (22)	19.87 \pm 7.40 (30)	24.42 \pm 8.00 (24)	5.60	0.0039
Female body mass (g)	18.8 \pm 1.11 (22)	18.97 \pm 1.23 (32)	19.13 \pm 0.87 (26)	0.57	0.57
Haematocrit	51.56 \pm 1.95 (22)	51.42 \pm 3.22 (33)	51.61 \pm 3.19 (26)	0.06	0.95
Heterophils	10.73 \pm 8.34 (19)	12.97 \pm 8.41 (32)	11.09 \pm 6.95 (26)	0.24	0.79
Lymphocytes	29.09 \pm 22.97 (19)	28.04 \pm 14.77 (32)	25.53 \pm 18.44 (26)	0.10	0.91
Total White Blood Cell Counts	45.02 \pm 33.75 (19)	45.10 \pm 19.64 (32)	39.83 \pm 25.47 (26)	0.20	0.82
H:L	0.45 \pm 0.35 (19)	0.68 \pm 0.83 (32)	0.52 \pm 0.40 (26)	0.19	0.83

after controlling for other variables such as tarsus, wing and tail length of the females ($P < 0.05$). Finally, we did not find any significant effects of brood size manipulation on adult body mass, haematocrit level, absolute lymphocyte count, heterophile count, total white blood cell count and H:L ratio, respectively.

Discussion

Our brood size manipulation had a significant effect on the feeding activity of female barn swallows, and adult females caring for enlarged broods provisioned their nestlings more frequently than females provisioning control or reduced broods. As a consequence, females that decreased parental activity had a stronger T-cell immune response than birds in the control and increased groups, while none of the condition indices, like body mass, haematocrit and differential and total leucocyte counts differed between experimental groups. This was also the case for the H:L ratio, an index of stress in birds.

In recent years several studies have indicated that the health status and the immunocompetence of breeding birds may play a role in mediating trade-offs between reproductive effort, parasitism and survival prospects,

and hence in the evolution of optimal parental decisions (Deerenberg et al. 1997, Saino et al. 1997b, 1999, Christe et al. 1998, Ots et al. 1998, Tella et al. 2000). Mounting an effective immune response against parasites is crucial due to the deleterious effects of parasites on mate choice, breeding success and survival of hosts (Møller 1997). An increase in parasite infestation during the breeding season can be linked directly to the depression of the immune system, due to the negative effects of sex hormones on the immunocompetence of breeding birds and the immunosuppressive effect of stress hormones on the immune system (see Besedovsky and del Rey 1996, Wingfield et al. 1997 and Apanius 1998 for reviews). Alternatively, the suppression of the immune system during intense exercise can be explained by the "nutrition limitation" hypothesis (Råberg et al. 1998). This hypothesis states that the down-regulation of the immune system during increased activity is a result of the resource limitation for the immune system and other fundamental processes, like parental effort. In this case individuals should suppress their immune system in order to save nutrients for life history traits which promote fitness more directly. Our brood size manipulation resulted in a significant reduction in the acquired immunity, supporting the previous findings on wild birds, namely a trade-off between acquired immunity and reproductive effort (Nordling et al. 1998, Moreno et al. 1999). The absence of a difference among experimental groups in H:L ratio of the female barn swallows indicates that birds were not strongly affected by stress under our experimental design, leading us to suppose that the principal mechanistic base of the immunosuppression of females is nutrition limitation, while stress probably had a minor effect in the down-regulation of T-cell activities. However, the use of H:L ratio cannot be accepted in all situations as an accurate measure of stress, since it depends much on the duration and form of stress (Maxwell 1993). Until now, we are aware of only one study series on wild birds where the H:L ratio was tested in response to parental activity (Ots et al. 1998). Thus, in order to elucidate the mechanistic background of the trade-off between the immune system and reproductive effort, further research is

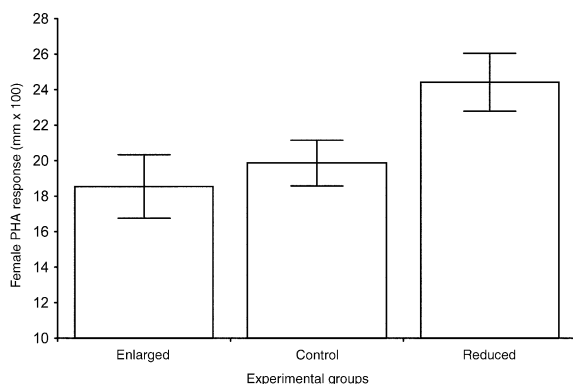


Fig. 2. T-cell mediated immune response of female barn swallows in enlarged, control and reduced broods at 12 day old nestlings (mean \pm SE (n)).

needed, with more exact stress measurements and with more than one component of the immune system measured simultaneously and under different experimental designs.

Studies on poultry have shown that during mild stress only the energetically costly acquired immunity is down-regulated, while phagocytes, the principal components of innate immunity in the blood, respond only during acute stress or during infestation with ectoparasites (review in Apanius 1998). We manipulated the brood size of the barn swallow by only a single nestling, which caused a significant, but not a dramatic increase in the provisioning rate of females. The lack of effect of the experimental treatment on body mass and haematocrit-level of adult females also indicates that the brood size manipulation experiment only had weak effects on parental condition. We suggest that this mild increase in stress due to increased reproductive effort could explain why the experiment only caused an effect on the energetically costly T-cell immunity. Another explanation of the moderate effect of the manipulation on females could be that we manipulated parental effort only during the nestling period, and not during the entire breeding cycle. However, when analyzing the cost of reproduction in birds, we should also take into consideration the energetic costs of egg laying for adults and the cost of incubation for both adults and nestlings (Monaghan and Nager 1997). Thus, we consider that the effects recorded in our study will be further increased if these additional components of parental effort would also be manipulated.

Components of immunocompetence in birds are strongly related to nutritional and physiological status, as indicated by a relationship between body mass and T-cell immune response in adult and nestling birds (review in Alonso-Alvarez and Tella 2001). Furthermore, T-cell mediated immune response can predict survival probability of breeding birds (González et al. 1999, Soler et al. 1999, Tella et al. 2000). Moreover, adult barn swallows trade their survival against the T-cell mediated immune response of their offspring (Saino et al. 1999). Saino et al. (1999) in their study of the barn swallow showed that brood size manipulation affected the survival probability of adults, reducing it in enlarged broods and increasing it in reduced broods. The survival prospects of parent barn swallows were negatively correlated with nestling T-cell response and body size. However, the proximate mechanisms affecting the survival of breeding barn swallows were not determined. In the present study, we used the same manipulation protocol as used by Saino et al. (1999), and we showed that female birds caring for enlarged broods had a lower T-cell mediated immune response than females in the control and reduced groups. Thus, we can infer that the T-cell mediated immune response of adult birds is mediating the trade-off between self-maintenance and reproduction. The crucial assumption

that a stronger immune response of adults is beneficial in terms of adult survival but detrimental in terms of reproduction has yet to be tested in a single experiment.

Parents did not fully compensate for the increased food demand of the nestlings. Thus per capita provisioning rate decreased significantly with experimental brood size. Mounting an effective immune response (e.g. T-lymphocyte response) to an antigen is limited by nutrition (Lochmiller et al. 1993, Saino et al. 1997a). Further, the reduced cell mediated immune response of nestlings from enlarged broods with a lower per capita provisioning rate than nestlings from reduced broods is in line with previous findings in the barn swallow (Saino et al. 1997a). The absence of a difference in differential and total leucocyte counts of nestlings between experimental groups could indicate – as in the case of adult females – an absence of stress caused by within-brood competition.

In conclusion, we have shown that T-cell mediated immunity is a good measure of the condition of birds as indicated by a strong positive correlation with body mass. At the same time T-cell mediated immunity is a sensitive indicator of one of the components of the immunocompetence as demonstrated by the effect of brood size manipulation on T-lymphocyte activity of adult females and nestlings. Our brood size manipulation had no significant effect on haematocrit-level, differential and total leucocyte numbers of females and nestlings, nor on the body mass of female barn swallows. These results support previous findings in wild birds, showing that mounting an effective immune response against an antigen is costly, and that it can result in a trade-off between parental effort and parental immunity. We consider these findings important in understanding the physiological background of the trade-off between the immune system and reproductive effort in birds.

Acknowledgements – We are grateful to Anders Pape Møller for his continuous support during our work and for constructive comments on an earlier version of the manuscript. We thank Eszter Ruprecht and Jácint Tökölyi for their help in the field, and Tibor Szép for his help. We are also grateful to the members of the Behavioural Ecology Research Group from Debrecen for their continuous support during our study. The administration of the Hortobágy National Park gave permission to work in the study area. Andrea Virginás kindly corrected our English of a former version of the manuscript. The work of PLP was supported financially by a travel grant from the Romanian Ministry of Education, the Hungarian Ministry of Education through a PhD grant and research grants offered by the Arany János Foundation and Sapientia Hungariae Foundation. We are grateful for the constructive criticism and suggestions provided by M. Andersson and an anonymous referee.

References

Alonso-Alvarez, C. and Tella, J. L. 2001. Effects of experimental food restriction and body-mass changes on the avian

- T-cell-mediated immune response. – *Can. J. Zool.* 79: 101–105.
- Apanius, V. 1998. Stress and immune response. – In: Møller, A. P., Milinski, M. and Slater, P. J. B. (eds). *Stress and behavior*. Academic Press, San Diego, California, pp. 133–154.
- Besedovsky, H. O. and del Rey, A. 1996. Immune-neuro-endocrine interactions: facts and hypotheses. – *Endocr. Rev.* 17: 64–102.
- Carpenter, F. L. 1975. Bird haematocrit: Effects of high altitude and strength of flight. – *Comp. Biochem. Physiol.* 50A: 415–417.
- Cheng, S. and Lamont, S. J. 1988. Genetic analysis of immunocompetence measures in a white leghorn chicken line. – *Poultry Sci.* 67: 989–995.
- Christe, P., Møller, A. P. and de Lope, F. 1998. Immunocompetence and nestling survival in the house martin: the tasty chick hypothesis. – *Oikos* 83: 175–179.
- Davis, P. J. 1981. Immunity to Coccidia. – In: Rose, M. E., Payne, L. N. and Freeman, B. M. (eds). *Avian Immunology*. British Poultry Science, Edinburgh, pp. 361–385.
- Deerenberg, C., Apanius, V., Daan, S. and Bos, N. 1997. Reproductive effort decreases antibody responsiveness. – *Proc. R. Soc. Lond. B* 264: 1021–1029.
- Fudge, A.M. 1989. Avian hematology: identification and interpretation. – *Proc. Assoc. Avian. Vet. Ann. Meet.* pp. 284–292.
- Gergely, J. and Erdei, A. 1998. *Immunobiológia*. – Medicina Press, Budapest.
- González, G., Sorci, G., Møller, A. P., Ninni, P., Haussy, C. and de Lope, F. 1999. Immunocompetence and condition-dependent sexual advertisement in male house sparrows (*Passer domesticus*). – *J. Anim. Ecol.* 68: 1225–1234.
- Goto, N., Kodama, H., Okada, K. and Fujimoto, Y. 1978. Suppression of phytohemagglutinin skin response in thymectomized chickens. – *Poultry Sci.* 62: 1889–1893.
- Lochmiller, R. L., Vestey, M. R. and Boren, J. C. 1993. Relationship between protein nutritional status and immunocompetence in Northern bobwhite chicks. – *Auk* 110: 503–510.
- Maxwell, M. H. 1993. Avian blood leukocyte responses to stress. – *World's Poultry Sci. J.* 49: 34–43.
- Møller, A. P. 1997. Parasitism and the evolution of host life history. – In: Clayton, D. H. and Moore, J. (eds). *Host-Parasite Evolution. General Principles and Avian Models*. Oxford University Press, Oxford, pp. 105–127.
- Monaghan, P. and Nager, R. G. 1997. Why don't birds lay more eggs? – *Trends. Ecol. Evol.* 12: 270–274.
- Moreno, J., Sanz, J. J. and Arriero, E. 1999. Reproductive effort and T-lymphocyte cell-mediated immunocompetence in female pied flycatchers *Ficedula hypoleuca*. – *Proc. R. Soc. Lond. B* 266: 1105–1109.
- Nordling, D., Andersson, M., Zohari, S. and Gustafsson, L. 1998. Reproductive effort reduces specific immune response and parasite resistance. – *Proc. R. Soc. Lond. B* 265: 1291–1298.
- Ots, I., Murumägi, A. and Hõrak, P. 1998. Hematological health state indices of reproducing great tits. *Methodology and sources of natural variation*. – *Funct. Ecol.* 12: 700–707.
- Palomeque, J. and Planas, J. 1978. Blood volume in domestic pigeons. – *Comp. Biochem. Physiol.* 59A: 413–417.
- Richner, H., Christe, P. and Oppliger, A. 1995. Paternal investment affects prevalence of malaria. – *Proc. Natl. Acad. Sci. USA* 92: 1192–1194.
- Råberg, L., Grahm, M., Hasselquist, D. and Svensson, E. 1998. On the adaptive significance of stress-induced immunosuppression. – *Proc. R. Soc. Lond. B* 265: 1637–1641.
- Saino, N., Calza, S. and Møller, A. P. 1997a. Immunocompetence of nestling barn swallows in relation to brood size and parental effort. – *J. Anim. Ecol.* 66: 728–836.
- Saino, N., Bolzern, A. M. and Møller, A. P. 1997b. Immunocompetence, ornamentation and viability of male barn swallows (*Hirundo rustica*). – *Proc. Natl. Acad. Sci. USA* 94: 579–585.
- Saino, N., Calza, S., Ninni, P. and Møller, A. P. 1999. Barn swallows trade survival against offspring condition and immunocompetence. – *J. Anim. Ecol.* 68: 999–1009.
- Smits, J. E., Bortolotti, G. R. and Tella, J. L. 1999. Simplifying the phytohemagglutinin skin testing technique in studies of avian immunocompetence. – *Funct. Ecol.* 13: 567–572.
- Soler, M., Martín-Vivaldi, M., Marín, J. M. and Møller, A. P. 1999. Weight lifting and health status in the black wheatear. – *Behav. Ecol.* 10: 281–286.
- Tella, J. L., Bortolotti, G. R., Dawson, R. D. and Forero, M. G. 2000. The T-cell-mediated immune response and return rate of fledging American kestrels are positively correlated with parental clutch size. – *Proc. R. Soc. Lond. B* 268: 891–895.
- Wakelin, D. 1996. *Immunity to Parasites*. – Cambridge University Press, Cambridge.
- Wingfield, J. C., Hunt, K., Breuner, C., Dunlap, K., Fowler, G. S., Freed, L. and Lepson, J. 1997. Environmental stress, field endocrinology, and conservation biology. – In: Clemmons, J. R. and Bucholz, R. (eds). *Behavioral Approaches to Conservation in the Wild*. Cambridge University Press, Cambridge, pp. 95–131.

(Received 5 February 2002, revised 17 February 2003, accepted 26 February 2003.)

Paper 3

Manuscript

**Environmental and genetic variation in body size, health state
indices and T-cell immunocompetence of fledgling barn swallows
(*Hirundo rustica*)**

by
Péter László Pap¹ and Róbert Márkus

**Environmental and genetic variation in body size, health state
indices and T-cell immunocompetence of fledgling barn swallows
(*Hirundo rustica*)**

Péter László Pap¹ and Róbert Márkus²

¹Behavioural Ecology Research Group, Department of Evolutionary Zoology,
University of Debrecen (formerly Kossuth Lajos University), H-4010 Debrecen,
Hungary

²Biological Research Center, Institute of Genetics, Lab. of Molecular
Immunology, H-6726 Szeged, Temesvári street 62, Hungary

ABSTRACT

We studied genetic and environmental variation in several morphological traits, health status indices and immune response of barn swallow nestlings in a partial cross-fostering experiment with simultaneous brood size manipulation in two consecutive years. The brood size manipulation had a significant effect on nestlings condition, as the body mass, T-cell immune response, haematocrit and plasma lutein concentration was lower in the experimentally increased group related to the reduced group. We found a significant additive genetic variance for tarsus length, and low genetic component for haematocrit, T-cell immunocompetence and white blood cell number. No significant additive genetic component was detected for body mass, wing length and plasma lutein concentration. Heritability estimates of traits were generally low, with a median value of 0.16 for morphological measures and 0.08 for physiological traits. Environmental variance was greater than additive genetic variance, and the low heritability of phenotypic traits was due to the large environmental variance and small genetic variance. Our results support previous findings on high environmental variance and low heritability of physiological traits on wild birds.

INTRODUCTION

The immune system is one of the most effective defence that hosts have evolved to counter infestation (Wakelin, 1996). Parasites impose a strong natural selection on hosts, leading to an increased investment in immune system of species under high risk of infestation (e.g. Møller & Erritzøe, 1996). Environmental conditions experienced during the developmental period, such as food supply, parental investment and parasitism have a strong impact on offspring body condition and immunocompetence at fledging (e.g. Saino et al., 1997a, 1998). Phenotypic traits are known to be genetically influenced in poultry and in wild birds (e.g. Boag & van Noordwijk, 1987; Brinkhof et al., 1999; Christe et al., 2000; Saino et al., 1997a; review in Merilä & Sheldon, 2000; Mousseau & Roff, 1987). Data on heritability of resistance to parasites indicate indirectly the genetic component of immunocompetence (review by Sorci et al., 1997a). The ability of hosts to raise a defence against parasites can evolve only if the trait is determined partially by additive genetic variation and if it is under selection. Due to the importance of immunocompetence in the defence against parasites, we expect a strong directional selection on host's immune response caused by parasites, which can be reflected in polymorphism at the genetic level (Bernatchez & Landry, 2003). Results of studies on the relationship between immunocompetence, parasitism and fitness of birds comply with this assumption (Sorci et al. 1997b; Christe et al., 1998; Nordling et al., 1998; Hõrak et al., 1999; Szép & Møller, 1999; Saino et al., 1997b, 2002).

Quantitative genetic theories on natural selection suggests that strong directional selection tends to deplete additive genetic variation, resulting in low heritability of phenotypic traits closely related to fitness (Fisher 1930). Consistent with this idea, Mousseau & Roff (1987) in a review of 1120 heritability estimates have found that life history traits possess lower heritability than morphological traits, and the physiological traits fall in the middle. Similar results were derived from an analysis of a large amount of samples collected from collared flycatcher (*Ficedula albicollis*) on lifetime reproductive success and phenotypic traits (Merilä & Sheldon, 2000). While heritability is defined as the ratio of additive genetic variance to total phenotypic variance (Falconer & Mackay, 1996), the low heritability of fitness related traits may arise from a low additive genetic variance, a high environmental variance, or a combination of them. Hence, the negative relationship between the degree of association of traits with fitness and heritability does not preclude the existence of low additive genetic variation of the trait. Complying with this idea, data on heritability of fitness related traits indicate that low heritability is not due to lower levels of additive genetic variation but to their high residual variance (Houle, 1992; Pomiankowski & Møller, 1995; Merilä & Sheldon, 2000).

Consequently, fitness traits do not necessarily have low levels of genetic variability.

Barn swallow (*Hirundo rustica*) is a semicolonial species breeding often in high density, at which high rates of horizontal transmission and multiple infection of parasites occur frequently (Møller, 1990). As a consequence, birds are under strong natural selection due to intensive parasitism by haematophagous mites and louse flies. Health status and immunocompetence, measured as the T-cell immune response of the birds to an antigen, immunoglobulin concentration in the blood, plasma protein concentration and leukocyte activity play an important role in the defence mechanism against parasites, and ultimately determine the fitness of the birds (Saino et al., 1998, 2002). Hence, considering the role of immunocompetence of birds to cope with the harmful effect of parasites and as a consequence the strong directional selection on immune system, we expect a lower heritability of phenotypic traits related to parasite defence than of morphological traits. Contrary to the substantial evidence that morphological measures are heritable in wild birds, there is little information about the heritability of health status indices and immunocompetence (Merila & Sheldon, 2001). Thus, heritability analyses of physiological traits are required.

The present study addressed the following point. First, through a brood size manipulation we investigated the effect of different quality environment on morphological traits and several physiological indices of the barn swallow nestlings. Second, we estimated the environmental and genetic component of variation of morphological and physiological traits. Furthermore, we examined whether the performance of nestlings from different families differs in experimentally created environments, in order to test whether there is an indication for the degree of plasticity of traits being genetically determined. Finally, we present phenotypic and additive genetic coefficients of variation for traits under study in order to examine the assumption that traits closely associated with fitness have low heritability due to high phenotypic variance rather than to low additive genetic variance.

METHODS

The study area and data collection

The experiment was carried out during the field season of 2000 and 2001 in a colony of barn swallow situated in the Hortobágy National Park, near Balmazújváros (47°37'N, 21°21'E), eastern Hungary. Barn swallow breeds solitary and in three colonies up to 15 pairs. Each nest was inspected systematically, and date of clutch initiation, clutch size, hatching date and number of hatched and fledged nestlings was recorded. Nestlings were weighed and measured for wing length and tarsus length at 12 days old. All

measurements were performed by the same person (PLP), including T-cell immune response of nestlings (see below) in order to reduce measurement errors.

Cross-fostering and brood size manipulation

To separate genetic and environmental causes of resemblance, we performed cross-fostering experiments, creating broods that consisted in an approximately equal number of nestlings from two different families. Nests hatched on the same day were paired and used in the cross-fostering experiment with simultaneous brood size manipulation. Within a pair, broods were randomly reduced or increased by one nestling on the day after the nestlings hatched, when approximately half of the nestlings were exchanged between the broods. Using this procedure, the competition between the resident and foster nestlings should be minimized. Pairs of broods were selected, as the difference in the brood size was less than two nestlings on the day of hatching. Due to the differences observed on the hatching date within a nest between siblings (generally not exceeding 1 day), the initial difference in size between nestlings raised. We controlled this effect as follows: individual nestlings were ranked according to body mass within their brood of origin, afterwards half of the nestlings were cross-fostered with nestlings of similar rank in their original brood. Nestlings were marked by painting their claw with a nail polish until they were ringed at 6 days old. In order to control the effect of manipulation of the fostered nestlings, we took out their foster siblings raised at home and we put them in a warmed box for approximately the same time period as their foster siblings were on their way to another nest. No apparent effect of the experiment and transport on the condition and mortality of the nestlings were found. In 2000 and 2001 16 and 13 pairs of broods were manipulated, respectively.

Measuring health status and immunocompetence of the birds

The procedure of sampling, analysis of haematocrit, plasma lutein and differential white blood cell countings were already described in detail (Pap, 2002). We want to add here briefly that after measuring the nestlings, we collected a blood sample from the brachial vein in a capillary tube, and a drop of blood was smeared on a slide for leukocyte counts (see below). The blood samples collected in capillaries were centrifuged for 10 min at 10,000 r.p.m., the haematocrit was assessed, and the plasma was separated for the lutein extraction. Carotenoids are known to play an important role in defence mechanisms against parasites, as they are essential for immune functions because of their immuno-stimulative and immuno-regulative effects (review in Møller et al., 2000). Plasma lutein, which is an abundant carotenoid in the

peripheral blood of barn swallow (Saino et al., 1999), was determined by extracting carotenoids from 20 µl of plasma by dilution in 180 µl ethanol. After mixing and centrifugating (7,000 r.p.m. for 7 min), the supernatant was analysed for absorbance at 450 nm wavelength – the lutein absorbance peak (Stradi et. al., 1995) – with a spectrophotometer (Model 550 Microplate Reader). Plasma lutein concentration was calculated from the absorbance data, and was expressed in µg/ml.

For analysing the leukocyte number in the peripheral blood, a drop was smeared on a slide, fixed in absolute methanol and stained with May-Grünwald and Giemsa. The total white blood cell number was counted under x 1000 magnification by the same person (RM), and was expressed per approximately 10,000 erythrocytes. The leukocyte counts were highly repeatable ($r = 0.86$, $F = 11.7$, $df = 1,15$, $p < 0.001$). T-cell mediated immune response was measured shortly after measuring the nestlings and after blood sampling, by injecting in the left wing web 0.1 mg of phytohemagglutinin (PHA)(Sigma, L-8754) dissolved in 0.02 ml phosphate buffered saline (PBS). The thickness of the wing web was measured before injecting and 24 hours later with a pressure sensitive pessimeter. The difference in the swelling between the two measurements in the wing web was taken as a measure of the T-cell mediated immune response (see Smits et al., 1999). In order to reduce the measurement error, the wing web was measured three times, and average values were used in the subsequent analyses.

Statistical analysis

Genetic and environmental effects on nestling body condition and physiological variables were estimated using a mixed model nested-ANOVA using type III sum-of-squares (SAS Institute, 1996), method proposed and widely used for heritability analyses (Merilä, 1996,1997; Merilä & Fry, 1998). Factors affecting nestlings' morphology and physiology were investigated by performing a four-factorial mixed-model nested ANOVA. The effect of pairs of broods (nested within the year) and origin (nested within pairs of broods and within the year) were entered as random effects, and the experiment was considered as fixed effect. Experiment-origin and experiment-dyad interactions were considered random effects. The year effects indicate the consequences of year and manipulation effects indicate the effect of brood size manipulation on variables under consideration. The pair effects estimate heterogeneity between pairs of nests, and indicate differences between pairs of nests due to the temporal environmental variation. The origin effect estimate half of the additive genetic variance ($\frac{1}{2} V_A$) and $\frac{1}{4} V_D + \frac{1}{2} V_M$, if present. The dominance effect (V_D) and maternal effect (V_M) by this experimental design can not be estimated, and if they are present, our heritability estimates should be overestimated (see Discussion). The environmental component of variance is the sum of the

variance due to the experiment, pair, error and interactions among these variables. Phenotypic variance is composed by $V_A + V_E + V_{GE}$. The genetic-environmental interaction (GE) estimated by the Origin x Experiment interaction would indicate that nestlings with common origin, placed in different environments have different performance. Variance components were obtained using the Restricted Maximum Likelihood option (REML) in the VARCOMP procedure in SAS (SAS, Institute 1996). Heritability (h^2) estimates were calculated by dividing the variance due to the nest of origin with the total phenotypic variance and multiplying this value by two (Falconer & Mackay, 1996). We calculated phenotypic (CV_P) and additive genetic (CV_A) coefficients of variation using formula $CV = \sqrt{V/X}$, where V is the phenotypic or additive variation and the X is the average value of the character under consideration (Charlesworth, 1984). All variables were normally distributed, except the white blood cell counts for which we used logarithmic values in order to obtain data with normal distribution. All P-values reported are two-tailed.

RESULTS

Effect of brood size manipulation on nestling body condition and physiological traits

The brood size manipulation had a significant effect on nestlings condition, as the body mass, T-cell immunocompetence, haematocrit and plasma lutein concentration were lower in experimentally increased group compared to reduced group (Table 1,2). The experiment had no significant effect on tarsus length, wing length (in 2000 and in the combined data) and WBC of the nestlings. Overall nestlings in the reduced broods were in better condition than their full-sibs in the enlarged broods.

Phenotypic and genetic components of nestling measures

Dyads showed significant differences for all measures (Table 2), indicating the temporal variation of traits during the breeding season. There was a significant difference in nestlings' body condition between the two years, as the wing length and body mass were higher, whereas the plasma lutein concentration and T-cell immunocompetence were lower in 2000 comparing to 2001. The mixed-model ANOVA revealed a significant effect of the nest of origin on offspring tarsus length, T-cell immune response and a weak, but still significant effect on haematocrit and WBC, indicating the presence of additive genetic variation for these traits (Table 2). However, except for tarsus length and WBC, for which the nest of origin and environmental variance explained approximately the same proportion of phenotypic variance, the nest of origin did not explain the variance of T-cell immunocompetence and haematocrit (1.6 and 6%). Origin-

environment interaction was significant only for T-cell immunocompetence, indicating that offsprings from different families had different response in the experimentally created environments, i.e. some siblings performed better in reduced broods, whereas others performed better in increased broods. The significant dyad x experiment interaction for body mass, haematocrit, plasma lutein concentration, immunocompetence and WBC indicate that there were differences between pairs of birds in nestlings' response to different experimental conditions.

Heritability and genetic variation of traits

The additive genetic coefficient of variation for morphological traits was lower than for physiological traits (Table 3), as the median values were 0.026 and 0.055, respectively. Phenotypic coefficients of variation for morphological traits were also low related to the physiological traits, the median values were 0.083 and 0.233, respectively. Heritability estimates of traits were generally low, with the highest value of 0.37 for tarsus length (Table 3). The disproportionally high phenotypic variation of traits resulted in low heritability for both morphological and physiological measures with a median value of 0.16 and 0.08, respectively.

DISCUSSION

Heritability estimates of traits

The high heritability for tarsus length complies with the data in literature on birds (review in Boag & van Noordwijk, 1987; Merilä & Sheldon, 2000). Heritability in full-sib analyses is defined in some studies as twice the origin variance divided by the sum of the variance components for the random effects, whereas in other studies the heritability is defined as twice the origin variance divided by total phenotypic variance, which mean the sum of the additive genetic variance (twice the origin variance), environmental variance and the variance of origin-environment interaction. Furthermore, environmental variance in some of the studies is defined as the sum of the variance component due to manipulation, pair of broods, error and the manipulation-pair interaction, whereas in others the variance component of interaction effect is not considered. Finally, in some studies the origin-environment interaction was taken into consideration while in others not. The difference in estimating environmental and phenotypic variance components of traits lead to a discrepancy in heritability estimates even within the same species. Our relatively low heritability for tarsus length (0.37) related to the median value of 0.59 reported for wild birds (Boag & van Noordwijk, 1987) could be related at least partially to the method used. If we recalculate the heritability for tarsus length following Merilä & Fry (1998), the estimate rises to 0.50.

Full-sib heritability analysis rests on the assumptions that there is no extra-pair paternity (EPP), no maternal effects, no dominance variation, no assortative mating and no competitive differences between nestlings. Extra pair paternity reduces the resemblance between sibs in different nests, thus the heritability estimates are potentially underestimated. We have no information on the EPP, but other population studies in barn swallow with similar social pattern (e.g. semicolonial breeding association of the birds) have shown that nestlings sired by foster fathers is high (28%; Møller & Tegelström, 1997). Thus, our heritability estimates are probably underestimated and this renders our conclusions rather conservative. Maternal effects and size differences of nestlings between families are known to increase the resemblance of siblings fostered. Some studies indicate that maternal effects and size differences between sibs at hatching persist until the late developmental period (e.g. Schifferli, 1973; Forbes, 2002), whereas others suggest that these effects are small. We tried to reduce the maternal effects and the competitive difference between nestlings by exchanging the nestlings after hatching as early as possible (e.g. generally at one day old) and by randomly assigning them based on their size hierarchy between different groups. However, early maternal effects (e.g. egg size, nutrition, antioxidants and hormones within the egg) could not be eliminated by this method, so we cannot exclude the possibility that our heritability estimates are biased upwards. Assortative mating of birds could bias also the heritability estimates upward (Falconer & Mackay, 1996). We found assortative mating between pairs of birds for several morphological traits, that is significant relationship between several morphological characters of males and females (tarsus: $r = 0.06$, $n = 92$, $p = \text{ns}$, wing length: $r = 0.32$, $n = 93$, $p < 0.01$, tail length: $r = 0.30$, $n = 93$, $p < 0.01$), suggesting that our heritability estimates are probably affected by this factor. Finally, we did not take into consideration the dominance effect, which is known to be high especially in fitness traits (Merilä & Sheldon, 1999). Considering these shortfalls of the experimental design, the results should be viewed with restraint.

Saino et al.'s (1997a) partial cross-foster study is considered to provide evidence for significant origin-related variation in T-cell immunocompetence of nestling barn swallows (e.g. Brinkhof et al., 1999). However, in that study the environmental and origin related variation components of phenotypic traits were not calculated and hence the heritability could not be estimated. Our results of nested analysis of variance, based on the same experimental design as Saino et al.'s study gave approximately similar results for the origin related effect of T-cell immunocompetence, but the calculated variance components indicate a low additive genetic variance for this trait ($V_A = 0.00007$). The low heritability of immunocompetence in our study is due to the low additive genetic variance and high environmental variance. Taking into consideration the modalities used to

estimate the genetic components of the traits, we advise more attention before a final conclusion is drawn.

Genetic and environmental variation in traits. Components of heritability in morphological and physiological measures

We found significant genetic variation of morphological measures only for tarsus length, whereas the partial cross-fostering provided clear evidence for strong environmental effects on nestlings' body mass and wing length. Our result is consistent with Cadée's (2000) study on barn swallow, where the variation of tarsus length, but not wing length, had a significant additive genetic component. Contrary to our results, Saino et al.'s (1997a) study on the same species show a significant heritability for body mass, whereas tarsus length was not explained by common origin. The significant origin effect of T-cell immunocompetence is consistent with a previous study on this species (but see above for biases in heritability estimates) and other two studies on altricial species (Saino et al., 1997a; Brinkhof et al., 1999; Christe et al., 2000). In addition, we found a weak, but significant additive genetic component variation for haematocrit and WBC. However, the variance component of immunocompetence and haematocrit was explained by the nest in which they were reared, meaning that there was a small resemblance in these traits of genetically related sibs when reared in different environments, while unrelated nestlings reared in the same nest had similar traits. A strong environmental effect on plasma carotenoids concentration of nestlings was demonstrated, adding further evidence to the previous findings about the high environmental variation in carotenoids concentration of birds (Bortolotti et al., 2000; Hörak et al., 2000; but see Fitze et al., 2003). To conclude, our results comply with previous findings on wild birds about high environmental component of the phenotypic variation of physiological traits (Potti et al., 1999; Bortolotti et al., 2000; Christe et al., 2000; Tella et al., 2000).

The low heritability of physiological traits support the previous reports on birds (see review Mousseau & Roff, 1987). An explanation for the low heritability is related to the high connection to fitness of physiological measures, as traits conferring higher fitness are expected to have low levels of additive genetic variance due to rapid fixation of alleles (Fisher 1930). The measured physiological traits are assumed to be closely related to fitness, since a number of studies indicate the role of immunocompetence and health status of the birds in determining the fitness, measured through the acquired immune response to antigen, haematocrit level, carotenoid concentration and leukocyte counts (review in Lochmiller & Deerenberg, 2000; Møller et al., 2000; Norris & Evans, 2000). For example, survival of male barn swallows was positively correlated with the sheep red blood cell antibody response in the previous

breeding season (Saino et al., 1997b) and house martin (*Delichon urbica*) nestlings with higher T-cell immune response have a greater chance for survival than nestlings with poor response (Christie et al., 1998). However, in order to draw a conclusion about the relationship between heritability of traits in relation with the connectivity to fitness, we have to determine the relative role in fitness of morphological and physiological traits to each other. Consequently, it is worthwhile to know how much the amount of the variation in lifetime reproductive success is determined by each trait under consideration (see Merilä & Sheldon, 2000). Analysing coefficients of additive genetic variance revealed that traits closely associated with fitness have higher additive genetic variance than traits more distantly associated with fitness, and the low heritability of physiological traits was not due to the low additive genetic variance, but to the increased phenotypic variance. Thus, our results do not support the assumption of the negative relationship between fitness connectivity and additive genetic variance. Our findings comply with Merilä & Sheldon (2000) results, where fitness traits showed high phenotypic and additive variance related to the non-fitness traits (see also Houle, 1992; Pomiankowski & Møller, 1995). The high phenotypic variation of fitness traits should be related to the fact that these traits are more susceptible to the environmental variation related to the non-fitness traits, since they cope with the environmental variation in the morphology and additional environmental variation directly on the trait (Price & Schluter, 1991). Factors affecting the genetic and phenotypic variance are beyond the scope of this study, they remain a challenge to be addressed by further studies.

Our heritability estimates except of tarsus length were low, rising the possibility of limited chance of evolutionary response of birds to selection. Christie et al.'s (2000) study on the house martin, a closely related species to the barn swallow, show similar heritability results for physiological measures. Hence our results support their assumption, namely that species which are highly exposed to parasitism and infection and have low heritability of traits which confer protection against infection have little opportunity to evolve a physiological defence. The plasticity of genetic variation of nestlings' immunocompetence was indicated by significant genotype-environment interaction that accounted for 11% of all phenotypic variance. This indicates a genetic variation in the sensitivity to environmental effects among different families. The adaptive plasticity in phenotypic response may be advantageous to species in variable environmental conditions.

In conclusion, our results suggest that among morphological measures tarsus length is genetically determined, whereas the variation of haematocrit, immunocompetence and WBC have low additive components. Morphological traits have lower additive genetic variance and phenotypic variance than physiological measures, and the small heritability of traits were mainly caused by the large environmental variance and small genetic variance. Our results

support the assumption that the physiological traits, which are closely related to fitness, have low heritability. Despite of the shortfalls in heritability estimates, we consider our results an important step to understand some aspects of condition, health status and immunocompetence of the barn swallow.

ACKNOWLEDGMENTS

We are grateful to Anders Pape Møller for his continuous support during our work. We thank Eszter Ruprecht, Jácint Tökölyi and Tibor Szép for their help. The administration of the Hortobágy National Park gave permission to work in the study area. The work of PLP was supported financially by a travel grant from the Romanian Ministry of Education, the Hungarian Ministry of Education through a PhD grant and research grants offered by the Arany János Foundation and Sapientia Hungariae Foundation.

REFERENCES

- Bernatchez, L. & Landry, C. 2003. MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years. *J. Evol. Biol.* **16**: 363-377.
- Boag, P.T. & Noordwijk, A.J. van 1987. Quantitative genetics. In: Avian Genetics (eds: Cooke, F. and Buckley, P.A.), pp. 45-78. Academic Press, London.
- Bortolotti, G.R., Tella, J.L., Forero, M.G., Dawson, R.D. & Negro, J.J. 2000. Genetics, local environment and health as factors influencing plasma carotenoids in wild American kestrels (*Falco sparverius*). *Proc. R. Soc. Lond. B* **267**: 1433-1438.
- Brinkhof, M.W.G., Heeb, P., Kölliker, M. & Richner, H. 1999. Immunocompetence of nestling great tits in relation to rearing environment and parentage. *Proc. R. Soc. Lond. B* **266**: 2315-2322.
- Cadée, N. 2000. Genetic and environmental effects on morphology and fluctuating asymmetry in nestling barn swallow. *J. Evol. Biol.* **13**: 359-370.
- Charlesworth, B. 1984. The evolutionary genetics of life histories. In: Evolutionary Ecology (ed: Shorrocks, B.), pp. 117-133. Blackwell Scientific Publications, Oxford.
- Christe, P., Møller, A.P. & Lope, F. de 1998. Immunocompetence and nestling survival in the house martin: the tasty chick hypothesis. *Oikos* **83**: 175-179.
- Christe, P., Møller, A.P., Saino, N. & Lope, F. de 2000. Genetic and environmental components of phenotypic variation in immune response and body size of a colonial bird, *Delichon urbica* (the house martin). *Heredity* **85**: 75-83.

- Falconer, D.S. & Mackay, T.F.C. 1996. Introduction to quantitative genetics. 4th edn. Longman, Harlow.
- Forbes, S., Grosshans, R. & Glassey, B. 2002. Multiple incentives for parental optimism and brood reduction in blackbirds. *Ecology* **83**: 2529-2541.
- Fisher, R.A. 1930. The Genetical Theory of Natural Selection. Clarendon Press, Oxford.
- Fitze, P.S., Kölliker, M. & Richner, H. 2003. Effects of common origin and common environment on nestling plumage coloration in the great tit (*Parus major*). *Evolution* **57**: 144-150.
- Houle, D. 1992. Comparing evolvability of quantitative traits. *Genetics* **130**: 195-204.
- Hörak, P., Tegelmann, L., Ots, I. & Møller, A.P. 1999. Immune function and survival of great tit nestlings in relation to growth conditions. *Oecologia* **121**: 316-322.
- Hörak, P., Vellau, H., Ots, I. & Møller, A.P. 2000. Growth conditions affect carotenoid-based plumage coloration of great tit nestlings. *Naturwissenschaften* **87**: 460-464.
- Lochmiller, R.L. & Deerenberg, C. 2000. Trade-off in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**: 87-98.
- Merilä, J. 1996. Genetic variation in offspring condition: an experiment. *Funct. Ecol.* **10**: 465-474.
- Merilä, J. 1997. Expression of genetic variation in body size of the collared flycatcher under different environmental conditions. *Evolution* **51**: 526-536.
- Merilä, J. & Fry, J.D. 1998. Genetic variation and causes of genotype-environment interaction in the body size of blue tit (*Parus caeruleus*). *Genetics* **148**: 1233-1244.
- Merilä, J. & Sheldon, B.C. 1999. Genetic architecture of fitness and non-fitness traits: empirical patterns and development of ideas. *Heredity* **83**: 103-109.
- Merilä, J. & Sheldon, B.C. 2000. Lifetime reproductive success and heritability in nature. *Am. Nat.* **155**: 301-310.
- Merilä, J. & Sheldon, B.C. 2001. Avian quantitative genetics. *Curr. Ornithol.* **16**: 179-255.
- Møller, A.P. 1990. Effects of a haematophagous mite on the barn swallow (*Hirundo rustica*): a test of the Hamilton and Zuk hypothesis. *Evolution* **44**: 771-784.
- Møller, A.P. & Erritzøe, J. 1996. Parasite virulence and host immune defense: host immune response is related to nest reuse in birds. *Evolution* **50**: 2066-2072.
- Møller, A.P. & Tegelström, H. 1997. Extra-pair paternity and tail ornamentation in the barn swallow *Hirundo rustica*. *Behav. Ecol. Sociobiol.* **41**: 353-360.
- Møller, A.P., Biard, C., Blount, J.D., Houston, D.C., Ninni, P., Saino, N. & Surai, P.F. 2000. Carotenoid-dependent signals: indicators of foraging

- efficiency, immunocompetence or detoxification ability? *Avian Poul. Biol. Rev.* **11**: 137-159.
- Mousseau, T.A. & Roff, D.A. 1987. Natural selection and the heritability of fitness components. *Heredity* **59**: 181-197.
- Nordling, D., Andersson, M., Zohari, S. & Gustafsson, L. 1998. Reproductive effort reduces specific immune response and parasite resistance. *Proc. R. Soc. Lond. B* **265**: 1291-1298.
- Norris, K. & Evans, M.R. 2000. Ecological immunology: life history trade-offs and immune defense in birds. *Behav. Ecol.* **11**: 19-26.
- Pap, P.L. 2002. Breeding time and sex-specific health status in the barn swallow (*Hirundo rustica*). *Can. J. Zool.* **80**: 2090-2099.
- Potti, J., Moreno, J., Frías, O. & Rodríguez, R. 1999. Environmental and genetic variation in the haematocrit of fledgling pied flycatcher *Ficedula hypoleuca*. *Oecologia* **120**: 1-8.
- Pomiankowski, A. & Møller, A.P. 1995. A resolution of the lek paradox. *Proc. R. Soc. Lond. B* **260**: 21-29.
- Price, T. & Schluter, D. 1991. On the low heritability of life history traits. *Evolution* **45**: 853-861.
- SAS Institute 1996. The SAS System for Windows 6.12. SAS Institute INC., Cary, N.C.
- Saino, N., Calza, S. & Møller, A.P. 1997a. Immunocompetence of nestling barn swallows in relation to brood size and parental effort. *J. Anim. Ecol.* **66**: 827-836.
- Saino, N., Bolzern, A.M. & Møller, A.P. 1997b. Immunocompetence, ornamentation, and viability of male barn swallows (*Hirundo rustica*). *Proc. Natl. Acad. Sci. USA* **94**: 549-552.
- Saino, N., Calza, S. & Møller, A.P. 1998. Effects of a dipteran ectoparasite on immune response and growth trade-offs in barn swallow, *Hirundo rustica*, nestlings. *Oikos* **81**: 217-228.
- Saino, N., Stradi, R., Ninni, P., Pini, E. & Møller, A.P. 1999. Carotenoid plasma concentration, immune profile, and plumage ornamentation of male barn swallows (*Hirundo rustica*). *Am. Nat.* **154**: 441-448.
- Saino, N., Ferrari, R.P., Romano, M., Ambrosini, R. & Møller, A.P. 2002. Ectoparasites and reproductive trade-offs in the barn swallow (*Hirundo rustica*). *Oecologia* **133**: 139-145.
- Schifferli, L. 1973. The effect of weight on the subsequent growth of nestling great tits *Parus major*. *Ibis* **115**: 549-558.
- Smits, J.E., Bortolotti, G.R. & Tella, J.L. 1999. Simplifying the phytohemagglutinin skin testing technique in studies of avian immunocompetence. *Funct. Ecol.* **13**: 567-572.
- Sorci, G., Møller, A.P. & Boulinier, T. 1997a. Genetics of host-parasite interactions. *Trends Ecol. Evol.* **12**: 196-199.

- Sorci, G., Soler, J.J. & Møller, A.P. 1997b. Reduced immunocompetence of nestlings in replacement clutches of the European magpie (*Pica pica*). *Proc. R. Soc. Lond. B* **264**: 1593-1598.
- Stradi, R., Celentano, G., Rossi, E., Rovati, G. & Pastore, M. 1995. Carotenoids in bird plumage. I. The carotenoid pattern in a series of Palearctic Carduelinae. *Comp. Biochem. Physiol.* **110 B**: 131-143.
- Szép, T. & Møller, A.P. 1999. Cost of parasitism and host immune defence in the sand martin *Riparia riparia*: a role for parent-offspring conflict? *Oecologia* **119**: 9-15.
- Tella, J.L., Bortolotti, G.R., Forero, M.G. & Dawson, R.D. 2000. Environmental and genetic variation in T-cell-mediated immune response of fledgling American kestrels. *Oecologia* **123**: 453-459.
- Wakelin, D. 1996. Immunology to Parasites, 2nd edn. Cambridge University Press, Cambridge.

Table 1. Mean condition index (\pm SE) of full-sibs reared in experimentally enlarged and decreased broods. The tarsus and wing length are in mm, the T-cell response in mm*10, the body mass in g, the haematocrit in %, the plasma lutein concentration in $\mu\text{g/ml}$ blood and the WBC for 10,000 erythrocytes. Paired sample t-test are used for years separately and one-way repeated ANOVA for the combined data, in which year is a factor, and full-sibs are repeated measures.

Trait	Reduced	Enlarged	Df	T
2000				
Tarsus length	11.34 \pm 0.08	11.24 \pm 0.07	31	1.82 ^{ns}
Body mass	22.61 \pm 0.33	21.43 \pm 0.34	31	3.37**
Wing length	62.29 \pm 0.70	62.43 \pm 0.55	31	0.24 ^{ns}
Haematocrit	45.85 \pm 0.78	43.75 \pm 0.71	31	2.49*
Plasma lutein	7.96 \pm 0.50	7.03 \pm 0.37	29	1.98 ^{ns}
T-cell response	0.92 \pm 0.06	0.76 \pm 0.05	29	2.65*
WBC	66.1 \pm 4.9	58.4 \pm 4.1	25	1.66 ^{ns}
2001				
Tarsus length	11.30 \pm 0.07	11.41 \pm 0.07	25	1.57 ^{ns}
Body mass	21.9 \pm 0.34	20.97 \pm 0.21	25	2.76*
Wing length	60.93 \pm 0.77	59.54 \pm 0.71	25	2.31*
Haematocrit	46.43 \pm 0.57	44.83 \pm 0.86	25	1.95 ^{ns}
Plasma lutein	10.65 \pm 0.62	9.28 \pm 0.64	23	1.76 ^{ns}
T-cell response	1.28 \pm 0.06	0.98 \pm 0.06	25	3.96**
WBC	80.8 \pm 12.7	64.6 \pm 5.5	21	-.90 ^{ns}
2000-2001			Df	F
Tarsus length	11.32 \pm 0.05	11.31 \pm 0.05	56	0.00 ^{ns}
Body mass	22.29 \pm 0.24	21.22 \pm 0.21	56	18.29***
Wing length	61.68 \pm 0.52	61.14 \pm 0.47	56	2.25 ^{ns}
Haematocrit	46.11 \pm 0.50	44.23 \pm 0.55	56	9.63**
Plasma lutein	9.15 \pm 0.43	8.03 \pm 0.38	52	6.97*
T-cell response	1.09 \pm 0.05	0.86 \pm 0.04	54	22.70***
WBC	72.8 \pm 6.42	61.2 \pm 3.38	46	2.72 ^{ns}

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 2. Analysis of variance of morphological and physiological traits of barn swallow nestlings from full-sib analyses from three-way mixed model ANOVA. Variance (%) gives variance as a percentage of the total.

a. morphological traits

	df	F	Var.	Var. (%)
Tarsus length				
Year	1	2.18	0.00	0.0
Dyad (Year)	27	5.35***	0.03770	19.7
Origin (Year, Dyad)	29	2.95***	0.04323	22.6
Experiment	1	0.00	0.00	0.0
Year x Experiment	1	6.82*	0.00650	3.4
Origin x Experiment	29	1.33	0.01318	6.9
Dyad x Experiment	27	1.19	0.00	0.0
Error	136		0.09083	47.4
Model	115	3.15***		
Body mass				
Year	1	8.10***	0.00161	0.0
Dyad (Year)	27	6.00***	1.24704	27.4
Origin (Year, Dyad)	29	0.98	0.00	0.0
Experiment	1	26.43***	0.51527	11.3
Year x Experiment	1	0.35	0.00	0.0
Origin x Experiment	29	1.18	0.16387	3.6
Dyad x Experiment	27	1.93**	0.47882	10.5
Error	136		2.15052	47.2
Model	115	2.98***		
Wing length				
Year	1	12.97***	1.02617	4.2
Dyad (Year)	27	3.47***	5.56272	22.6
Origin (Year, Dyad)	29	1.06	1.99223	8.1
Experiment	1	1.10	0.00	0.0
Year x Experiment	1	1.68	0.32335	1.3
Origin x Experiment	29	0.70	0.00	0.0
Dyad x Experiment	27	0.36	0.00	0.0
Error	136		15.66762	63.8
Model	115	1.69**		

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

b. physiological traits				
	df	F	Var.	Var. (%)
Haematocrit				
Year	1	3.41	0.00	
Dyad (Year)	27	6.21***	4.26099	19.6
Origin (Year, Dyad)	29	1.57*	1.30008	6.0
Experiment	1	16.94***	1.14523	5.3
Year x Experiment	1	0.30	0.00	0.0
Origin x Experiment	29	1.08	0.00	0.0
Dyad x Experiment	27	2.44***	4.61770	21.2
Error	133		10.46887	47.9
Model	115	3.22***		
Plasma lutein				
Year	1	52.27***	3.16118	23.7
Dyad (Year)	25	5.96***	3.03569	22.7
Origin (Year, Dyad)	27	0.87	0.00	0.0
Experiment	1	11.11**	0.37618	2.8
Year x Experiment	1	0.38	0.00	0.0
Origin x Experiment	27	1.16	0.00	0.0
Dyad x Experiment	25	2.28**	1.68802	12.7
Error	106		5.08599	38.1
Model	107	3.54***		
T-cell response				
Year	1	62.51***	0.00041	22.1
Dyad (Year)	26	4.94***	0.00028	15.1
Origin (Year, Dyad)	28	1.90**	0.00003	1.6
Experiment	1	38.18***	0.00021	11.4
Year x Experiment	1	2.86	0.00002	1.1
Origin x Experiment	28	1.74*	0.00020	10.8
Dyad x Experiment	26	1.66*	0.00	0.0
Error	131		0.00070	37.9
Model	111	3.68***		

(Table 2b continued)

WBC				
Year	1	0.51	0.00	0.0
Dyad (Year)	22	2.67***	0.00288	1.0
Origin (Year, Dyad)	24	1.86*	0.04538	15.4
Experiment	1	3.56	0.00	0.0
Year x Experiment	1	0.00	0.00	0.0
Origin x Experiment	24	0.84	0.00	0.0
Dyad x Experiment	22	1.82*	0.03829	13.0
Error			0.20889	70.6
Model	95	1.76**		

P < 0.05, ** P < 0.01, *** P < 0.001

Table 3. Phenotypic (V_P), environmental (V_E) and additive genetic (V_A) components of variation, the additive genetic coefficient of variation (CV_A), the phenotypic coefficient of variation (CV_P), and the heritability (h^2) for morphological and physiological variables in nestling barn swallows.

a. morphological traits

Trait	V_A	V_E	V_P	CV_A	CV_P	H^2
Tarsus length	0.08646	0.12853	0.22817	0.026	0.042	0.37
Body mass	0.00	4.39165	4.55552	0.00	0.099	0.00
Wing length	3.98446	21.23034	25.2148	0.033	0.083	0.16

b. physiological traits

Trait	V_A	V_E	V_P	CV_A	CV_P	h^2
Haematocrit	2.60016	20.49279	23.09295	0.037	0.109	0.11
Plasma lutein	0.00	10.18588	10.18588	0.00	0.325	0.00
T-cell response	0.00007	0.00118	0.00145	0.091	0.389	0.05
WBC	0.09076	0.25006	0.34082	0.073	0.141	0.27

Paper 4

In: *Ibis* **146**, pp. xxx-xxx

Frequency and consequences of feather holes in Barn Swallows
Hirundo rustica

by
Péter László Pap, Jácint Tökölyi and Tibor Szép

Frequency and consequences of feather holes in Barn Swallows
Hirundo rustica

Péter László Pap^{1,2*}, Jácint Tökölyi² and Tibor Szép³

¹Behavioural Ecology Research Group, Department of Evolutionary Zoology,
University of Debrecen, H-4010 Debrecen, Hungary

²Department of Taxonomy and Ecology, Babeş-Bolyai University, RO-3400
Cluj Napoca, Clinicilor street 5-7, Romania

³Department of Environmental Sciences, College of Nyíregyháza, P.O. Box
166, H-4401 Nyíregyháza, Hungary

ABSTRACT

The relationship between feather quality, estimated through the prevalence and intensity of feather holes, and the breeding performance and survival of the Barn Swallow *Hirundo rustica* was studied over a 5-year period. In addition, we present some indirect data on the role of chewing lice in producing the feather holes. The balanced distribution of feather holes (high prevalence, low aggregation) complies with the pattern of distribution of lice on colonial birds, and in the Barn Swallow. Feather holes were significantly and positively associated with the arrival dates of the birds, as both males and females with an increased number of feather holes started laying later. Females that survived the winter had significantly fewer holes than non-surviving females, whereas there was no difference in feather hole number between surviving and non-surviving males. Given that there was no association between the number of feather holes and body condition indices, except for the tail length of male Barn Swallows, we suggest that the negative effect of feather holes on the fitness of the birds is mainly apparent during periods of intensive locomotor activity, such as migration. Alternatively, if feather holes are an indicator of quality, those birds with a high intensity of feather holes may have been of poor quality. These birds may have been less able to cope with the environmental conditions, resulting in the lower survival and later arrival of the birds to the breeding grounds. The negative relationship between the length of the outermost tail feathers of males and the incidence of feather holes suggests that the tail is a condition-dependent secondary sexual characteristic.

INTRODUCTION

Feather length is a crucial factor determining the fitness of birds by altering flight and manoeuvre capacity (Swaddle et al. 1996). Feather damage is closely related to feather quality (Bonser 1995), which is determined by intrinsic (e.g. genetic, developmental) and extrinsic (e.g. habitat preference, locomotor activity) factors (Dawson et al. 2000, Merilä & Hemborg 2000). Feathers are damaged by mechanical abrasion (e.g. wear, feather breakage), photochemical processes and ectoparasites, such as chewing lice (Clayton 1990, Jenni & Winkler 1994).

As with any other feature of life history, reproduction represents a significant cost to the host, which can be expressed in various ways (Stearns 1992). Enhanced parental activity of breeding birds, which leads to an increased use of traits such as flight feathers, results in a trade-off between reproduction and self-maintenance. In birds, increased reproductive effort might increase feather wear due to the intensity of foraging trips, together with a loss of feather mass as well as increased feather damage caused by feather lice as the parents have insufficient time to preen adequately. Despite evidence of feather damage, few attempts have been made to investigate the sources of variation in feather quality and the effect of feather quality on the breeding performance and survival of birds (but see Dawson et al. 2000, Merilä & Hemborg 2000). An experimental study on the migratory Collared Flycatcher *Ficedula hypoleuca* has shown that parents trade-off parental activity with feather wear, and ultimately feather quality seems to affect the survival capacity of the birds (Merilä & Hemborg 2000). Similarly, feather quality estimated as the number of feather holes of the male Barn Swallow *Hirundo rustica* had been related to individual variation in breeding performance throughout mate selection (Kose & Møller 1999), although direct evidence of the effect of feather holes on fitness is still largely lacking. The reduced fitness of birds with more feather holes can be related partly to the detrimental effect of feather holes on the flight performance of the birds through the transmission of air through feathers. This could alter the aerodynamic efficiency, and increase the risk of feather breakage (Kose & Møller 1999, Barbosa et al. 2002). Thus, the quality of flight feathers should be crucial to individuals performing intensive locomotor activity, such as that undertaken by aerial insectivorous and migratory birds.

It had been thought that the feather holes of Barn Swallows were produced by chewing lice (*Mallophaga*; Møller 1991, Kose & Møller 1999). However, apart from a positive correlation between the numbers of feather holes and of individual lice (*Phthiraptera*, *Machaerilaemus malleus*, formerly *Hirundoecus malleus*), that most feather holes occurred in the white parts of the largely black tail feathers of Barn Swallows, and the white spots of feathers are preferred by

lice (Møller 1991, Kose & Møller 1999, Kose et al.1999), we can find no study in which the origin of feather holes has been tested.

The aim of this study is two-fold: first, we present some indirect evidence that chewing lice produced feather holes, by comparing indices of distribution and aggregation of feather holes with data on lice, and we describe factors that might affect the variation in the number of feather holes of individuals. Secondly, we examine the relationship between feather holes and several fitness measures of the Barn Swallow. We studied the possible short- and long-term effect of feather holes on breeding performance of the birds during a 5-year period by examining the relationship between feather holes, differential morphological variables and breeding parameters, and by analysing retrospectively the different number of feather holes in surviving and non-surviving birds.

METHODS

The study was carried out in Balmazújváros (47°37'N, 21°21'E), eastern Hungary, during the breeding seasons 1999–2003. The Swallows bred either in one of three colonies or solitarily on farms, and were studied from their arrival in April to the start of their second clutch at the end of July. During this time more than 95% of the breeding birds were captured and individually marked with a combination of colour rings and with a numbered metal ring. For each nest the breeding birds were identified from their colour ring combinations and by capturing them with a nest trap. While trapping, one of the authors (P.L.P.) used standard measurements including: wing length and outermost tail feather length (0.5 mm precision using a ruler), tarsus length (0.01 mm precision using a pair of calipers) and body mass (0.1 g precision using a spring balance) (Svensson 1984). Considering bilateral morphological variables, the left and right sides were measured and we used the average value of the two measurements in the analyses. The number of feather holes on left and right primaries and on tail feathers were counted separately. For the present study we used the pooled values for feather holes from the wing and tail feathers.

The breeding performance of the birds was assessed by inspecting the nests every second day, and determining the laying date of the first egg, the clutch size and the brood size when the nestlings were 12 days old. In three years (2000–02) we estimated the condition of 12-day-old nestlings by measuring the body mass, wing length and tarsus length, and we recorded the feeding rate of males and females on days 10–12 after hatching for 1 h between 07:00 and 12:00 h, the time of day when feeding rate is highest. We recorded feeding rates only on sunny days. We performed the same capture–recapture effort each year, and standardized the measurements and feather hole counting on the birds. Thus the results obtained during the 5-year study could be compared.

Previous observations on this species (Møller 1994) and our capture–recapture data indicate a high breeding philopatry. As the capture rate of the marked birds was high and constant over the study period, the survival of the birds between years can be assessed accurately, permitting the relationship between feather-hole number and fitness to be studied. Survival of the birds was assessed only for those captured as breeders in both capture and recapture years (1999–2002). For birds captured at least twice in different years, their data from a single year were included randomly in all analyses, except in the analysis of feather-hole repeatability. This was undertaken to avoid pseudoreplication, as the birds presented very similar values in different years (see Results for analyses of repeatability of feather holes). As feather holes showed a normal distribution, we used parametric statistics in the analysis.

To describe the distribution patterns of feather holes, we used several indices from the parasitological literature following the recommendations of Rózsa et al. (2000). The following measures were applied: prevalence (%) was estimated as the proportion of hosts with feather holes within the samples examined; mean intensity as the arithmetic mean of the number of feather holes per individual with feather holes; and indices of aggregation such as k of the negative binomial distribution and D the index of discrepancy. The value of k decreases and D increases with increasing levels of aggregation. Feather-hole quantification was carried out using the program ‘Quantitative Parasitology 2.0’ (Reiczigel & Rózsa 2001).

RESULTS

Distribution and repeatability of feather holes on males and females

Prevalence of feather holes was high for both sexes: 99% of both males and females (345/348 males and 321/325 females) had feather holes. The mean intensity of feather holes was 28.7 and 28.2 for males and females, respectively. Feather holes showed a normal distribution for both sexes (Kolmogorov–Smirnov test, males: $d = 0.05$, $P > 0.2$; females: $d = 0.06$, $P > 0.1$, Fig. 1), but the data tended to skew in accordance with a negative binomial model. The aggregation indices k and D were 2.71 and 0.312 for males and 2.20 and 0.339 for females. The total number of holes did not differ significantly between sexes (Student’s t -test, $t = 0.5$, $n = 671$, $P = 0.62$). This was the case also for the relative number of holes, because after controlling for tail length and wing length the difference between sexes remained non-significant ($F_{1,663} = 2.49$, $P = 0.06$). Repeatability between years of feather-hole number was low to moderate, but significant (males: $R = 0.27$, $F_{77,119} = 1.94$, $P < 0.001$; females: $R = 0.41$, $F_{80,116} = 2.70$, $P < 0.001$). Because a complete moult would have occurred

between observations, this indicates that the susceptibility of individuals to obtaining feather holes was consistent over time.

Breeding performance and survival

To test the relationship between laying date, feather hole number and condition indices we used a stepwise generalized linear model (GLM), in which first we introduced all possible explanatory variables (number of feather holes, year, wing length, tail length, tarsus length and body mass), and then, using a backwards procedure, we eliminated all continuous predictor variables lower than the critical value (see Zar 1984, p. 342). The potentially confounding variable (year) was included in the model as a categorical explanatory variable, as the number of feather holes and different morphological variables varied between years for males (one-way ANOVA, feather holes: $F_{4,343} = 4.89$, $P < 0.001$; wing length: $F_{4,361} = 4.71$, $P = 0.001$; tarsus length: $F_{4,355} = 3.54$, $P = 0.008$; body mass: $F_{4,352} = 3.55$, $P = 0.008$) and females (feather holes: $F_{4,320} = 5.38$, $P = 0.0003$; wing length: $F_{4,338} = 6.36$, $P < 0.0001$; body mass: $F_{4,332} = 3.85$, $P = 0.005$). The number of feather holes was related significantly to the female's laying date in both sexes (Table 1a, Fig. 2), whereas, with the exception of female tail length, morphological variables known as condition indices were far less important in explaining variation in the laying date.

Analysing the relationship between the number of feather holes and the breeding performance of males and females, while controlling for potentially confounding variables (laying date, wing length, tail length, tarsus length and body mass) in a stepwise GLM (see Table 1a), we found no significant effect on clutch size, brood size, breeding success or the condition of the nestlings. We found similar results for feeding activity of males and females, because there was no relationship between the number of feather holes and parental activity (males: $F_{3,124} = 0.41$, $P = 0.75$; females: $F_{3,116} = 2.1$, $P = 0.1$).

Over the 4 years we found that non-surviving females consistently had more feather holes than did surviving females (two-way ANOVA, year: $F_{3,142} = 1.79$, $P = 0.15$; survival: $F_{1,142} = 4.58$, $P = 0.04$, Fig. 3), suggesting a negative effect of feather holes on the survival prospects of female Barn Swallows. The difference between groups remained significant even after controlling for tail length ($F_{1,139} = 4.75$, $P = 0.03$), wing length ($F_{1,141} = 4.77$, $P = 0.03$) and tarsus length ($F_{1,141} = 4.44$, $P = 0.04$). We found no such difference for males ($F_{1,128} = 0.06$, $P = 0.87$, Fig. 3), even after controlling for tail length, wing length and tarsus length ($P > 0.1$). In contrast to females, the difference between surviving and non-surviving males with respect to the number of feather holes was not consistent between years ($F_{3,128} = 3.77$, $P = 0.013$): in some years the surviving birds had more feather holes, whereas in other years the non-surviving birds had more feather holes, although this was not statistically significant.

Relationship between body condition and number of feather holes

We analysed the relationship between feather holes (dependent variable) and different morphological variables separately for both sexes, in a stepwise GLM, while controlling for potentially confounding variables (see above). We found no significant relationship between feather-hole number and different morphological variables, except that for males the tail length declined with increasing feather-hole number (Table 1b).

Correlation of feather holes within pairs of birds

If feather holes are produced by lice as suggested above (see Introduction), given that lice are transmitted among hosts through direct contact (Clayton & Tompkins 1994, Hillgarth 1996), we should expect a positive correlation in the number of feather holes between birds within pairs. We tested the possibility that birds can be infected from their mates in two ways. First, testing the relationship across pairs of birds in year i and the number of holes counted in the same year (i) we found no significant correlation in feather-hole number ($r = 0.1$, $n = 235$, $P = 0.15$). Secondly, as the birds perform a complete moult during winter, the feather holes counted during the breeding period must have been produced since the last moult. As a consequence, if the birds are infested at least partially from their mates in year i , then a correlation between the infestation rate – based on the number of feather holes – and between birds in a pair might be revealed 1 year later. Analysing the relationship between feather holes of males and females that formed a breeding pair in the previous year ($i - 1$), but counted in year i , the correlation again showed no significant relationship ($r = 0.32$, $n = 28$, $P = 0.1$), indicating either that there is a low transmission rate of lice between mates during the breeding season, or that this effect is masked by the horizontal transmission of lice between individuals during the roosting period in the winter grounds.

DISCUSSION

Aggregation is a well-known property of parasite distributions, which is best described by the negative binomial model (Bush et al. 2001). This means that most individual hosts have few parasites, while a few individuals have many parasites. The distribution of lice depends on various extrinsic factors such as the host's social behaviour. Comparative studies on birds have shown that louse aggregation is reduced in colonial species (Rózsa et al. 1996, Rékási et al. 1997). In relation to territorial birds, the low aggregation of parasites on colonial hosts is reflected by an increased prevalence and a decreased level of

aggregation, which is reflected by an increase in k and a decrease in D . The ‘uniform’ distribution of lice on colonial birds is presumably due to an increased opportunity for horizontal transmission (Rózsa et al. 1996). If we consider feather holes as an accurate index of louse infestation (Møller 1991), the distribution values observed by us are lower than the average values observed for lice in other studies, but are still within the range of louse prevalence and aggregation observed in colonial birds (Rékási et al. 1997). Furthermore, the high prevalence of feather holes described here is close to the values for two louse species observed by Camplani et al. (1999) in three Barn Swallow populations (on average 84.2% and 97.1% for two louse species). Thus our data on feather holes agree with the general pattern observed for lice on colonial birds and specifically with that of the semi-colonial Barn Swallow. Despite this evidence, other possible sources of origin for the feather holes cannot be excluded. For example, developmental anomalies during the moulting period (Bortolotti et al. 2002) can produce feather abrasions similar to feather holes, resulting in an overestimate of their prevalence and a change in distribution pattern. Direct experiments are required to elucidate the importance of feather lice as the cause of feather holes. Furthermore, it is important to consider the relative importance of the two most common louse species found on the Barn Swallow with respect to the occurrence of feather holes. Whereas in some studies the feather holes are considered to be produced by the species *Machaerilaemus malleus* (Møller 1991, Kose & Møller 1999, Kose et al. 1999), others considered them to be produced by *Myrsidea rustica* (Barbosa et al. 2002), the latter a species also found in the earlier studies.

Assuming that feather holes are indeed produced by lice, the repeatability of feather-hole intensity between years indicates the persistence of lice, and as a consequence a long-term effect of lice on birds, especially on females (but for an alternative explanation of the relationship between feather holes and fitness see below). As a consequence it should be adaptive for females to avoid infested males during the breeding season. The feature of white patches in tail feathers (Kose & Møller 1999) and the negative relationship between feather-hole number and the length of the outermost tail feathers of male Barn Swallows could act as a signal for females allowing them to avoid infested individuals. The negative relationship between feather-hole number and tail length indicates that males may reliably signal their parasite load, and that the tail length remains an honest signal of phenotypic quality, because they are particularly demanding to grow for individuals of low quality (Møller 1994). Our results comply with Kose & Møller’s (1999) findings, because both studies found a significant negative relationship between the length of the outermost tail feathers and feather hole number of males, but not of females.

Our results suggest that for both male and female Barn Swallows, feather holes have a significant effect on fitness, as birds with more feather holes

delayed laying, and the survival rate of these females was significantly reduced. However, a drawback of this non-experimental study is that alternative explanations cannot be excluded. For example, the difference in the number of feather holes between surviving and non-surviving females, and the negative relationship between the number of feather holes and laying date might be explained as follows: poor quality birds with low survival prospects and delayed breeding are more susceptible to infection, and as a consequence they will show a high intensity of feather holes. Under this scenario there would be no causal effect of feather holes on the fitness of the birds. Our results seem not to support this alternative explanation, because we found no significant relationship between the number of feather holes and condition indices of females, except for tail length of males.

Our results on the negative relationship between feather holes and fitness are consistent with the most recent findings on the importance of feather holes on flight performance of the Barn Swallow (Barbosa et al. 2002). However, the mechanism behind this is still unclear. A possible explanation for the implications of feather holes should be their effect in altering the flight performance of the birds (Barbosa et al. 2002). Another implication of feather holes is that as the outermost tail feathers of the forked tail of Barn Swallow grow thin they are particularly predisposed to be damaged (Kose & Møller 1999) and lead to a fluctuating asymmetry. Finally, because the white spots in the tail of the Barn Swallow are melanin-free, they are preferred by chewing lice, resulting in a disproportional high number of feather holes. Ultimately, the white spots are more susceptible to feather breakage than the melanized parts of the feather (Kose & Møller 1999). Feather breakage seems to impose strong natural selection on the host, given the aerodynamic cost of the asymmetric tail feathers (Møller & Swaddle 1997). Our result on the negative relationship between tail length and feather hole intensity agrees with the negative association found by Møller (1994) between tail length and feather breakage of male Barn Swallows.

Assuming that feather holes are produced by feather lice, this raises some interesting questions. Previous studies on wild birds performed mostly during the breeding season demonstrated that chewing lice have only minor direct effects on the fitness of the host (Clayton & Tompkins 1995, Lee & Clayton 1995, Tompkins et al. 1996; but see Booth et al. 1993). This view is probably based on just a few influential papers, which support the benign effect of lice (see Clayton & Tompkins 1995, Tompkins et al. 1996). Feather lice cannot elicit an immune reaction from the host as can the relatively well-studied haematophagous mites, louse flies, fleas and ticks, which have serious effects on bird fitness. Consequently, the short-term effect of lice on the physiology and the breeding performance of the hosts should be low. However, by overexploiting the resources of birds they can damage the feathers significantly,

and may affect the homeostasis and flight capability of birds (Booth et al. 1993, Barbosa et al. 2002). In this case, we should expect a long-term effect of parasitism on fitness, especially during an intensive locomotor period, such as migration. Here we have shown that the number of feather holes correlates with laying date and survival of Barn Swallows, both features being strongly related to the migratory activity of birds generally. Thus our results raise the importance of studies on the fitness consequences of parasitism of feather lice during the whole life-cycle of birds. In comparing the fitness consequence of parasitism on migratory and non-migratory birds we should expect a more severe effect on the former group. This waits to be tested.

ACKNOWLEDGMENTS

We are grateful to Anders Pape Møller for his continuous help during the study period, to Lajos Rózsa for his constructive comments on an earlier version of the manuscript and Eszter Ruprecht for her help. The administration of the Hortobágy National Park gave us permission to work in the study area. P.L.P. was supported financially by the Hungarian Ministry of Education through a PhD grant and research grants offered by the Domus Hungarica Foundation, Arany János Foundation and Sapientia Hungariae Foundation. T.S. was supported by OTKA T29853 and T42879. We are grateful for to two anonymous referees for their constructive criticisms.

REFERENCES

- Barbosa, A., Merino, S., de Lope, F. & Møller, A.P.** 2002. Effects of feather lice on flight behavior of male Barn Swallows (*Hirundo rustica*). *Auk* **119**: 213-216.
- Booth, D.T., Clayton, D.H. & Block, B.A.** 1993. Experimental demonstration of the energetic cost of parasitism in free-ranging hosts. *Proc. R. Soc. Lond. B* **253**: 125-129.
- Bonsler, R.H.C.** 1995. Melanin and the abrasion resistance of feathers. *Condor* **97**: 590-591.
- Bortolotti, G.R., Dawson, R.D. & Murza, G.L.** 2002. Stress during feather development predicts fitness potential. *J. Anim Ecol.* **71**: 333-342.
- Bush, A.O., Fernández, J.C., Esch, G.W. & Seed, J.R.** 2001. *Parasitism. The Diversity and Ecology of Animal Parasites*. Cambridge University Press, Cambridge.
- Camplani, A., Saino, N. & Møller, A.P.** 1999. Carotenoids, sexual signals and immune function in barn swallows from Chernobyl. *Proc. R. Soc. Lond. B* **266**: 1111-1116.

- Clayton, D.H.** 1990. Mate choice in experimentally parasitized Rock Doves: lousy males lose. *Amer. Zool.* **30**: 251-262.
- Clayton, D.H. & Tompkins, D.M.** 1994. Ectoparasite virulence is related to mode of transmission. *Proc. R. Soc. Lond. B* **256**: 211-217.
- Clayton, D.H. & Tompkins, D.M.** 1995. Comparative effects of mites and lice on the reproductive success of rock doves (*Columba livia*). *Parasitology* **110**: 195-206.
- Dawson, A., Hinsley, S.A., Ferns, P.N., Bonser, R.H.C. & Eccleston, L.** 2000. Rate of moult affects feather quality: a mechanism linking current reproductive effort to future survival. *Proc. R. Soc. Lond. B* **257**: 2093-2098.
- Hillgarth, N.** 1996. Ectoparasite transfer during mating in Ring-necked Pheasants (*Phasianus colchicus*). *J. Avian Biol.* **27**: 260-262.
- Jenni, L. & Winkler, R.** 1994. *Moult and Ageing of European Passerines*. Academic Press, San Diego.
- Kose, M. & Møller, A.P.** 1999. Sexual selection, feather breakage and parasites: the importance of white spots in the tail of the barn swallow (*Hirundo rustica*). *Behav. Ecol. Sociobiol.* **45**: 430-436.
- Kose, M., Mand, R. & Møller, A.P.** 1999. Sexual selection for white tail spots in the barn swallow in relation to habitat choice by feather lice. *Anim. Behav.* **58**: 1201-1205.
- Lee, P.L.M. & Clayton, D.H.** 1995. Population biology of swift (*Apus apus*) ectoparasites in relation to host reproductive success. *Ecol. Entom.* **20**: 43-50.
- Merilä, J. & Hemborg, C.** 2000. Fitness and feather wear in the Collared Flycatcher *Ficedula albicollis*. *J. Avian Biol.* **31**: 504-510.
- Møller, A.P.** 1991. Parasites, sexual ornaments and mate choice in the barn swallow *Hirundo rustica*. In: Loye, J.E. & Zuk, M. (eds) *Ecology, Behavior, and Evolution of Bird-parasite Interactions*: pp. 328-343. Oxford University Press, Oxford.
- Møller, A.P.** 1994. *Sexual Selection and the Barn Swallow*. Oxford University Press, Oxford.
- Møller, A.P. & Swaddle, J.P.** 1997. *Asymmetry, Developmental Stability and Evolution*. Oxford University Press, Oxford.
- Reiczigel, J. & Rózsa, L.** 2001. *Quantitative Parasitology 2.0*. Budapest. Available at <<http://bio.univet.hu/QP/>>
- Rékási, J., Rózsa, L. & Kiss, J.B.** 1997. Patterns in the distribution of avian lice (*Phthiraptera: Amblycera, Ischnocera*). *J. Avian Biol.* **28**: 150-156.
- Rózsa, L., Rékási, J. & Reiczigel, J.** 1996. Relationship of host coloniality to the population ecology of avian lice (*Insecta: Phthiraptera*). *J. Anim. Ecol.* **65**: 242-248.

- Rózsa, L., Reiczigel, J. & Majoros, G.** 2000. Quantifying parasites in samples of hosts. *J. Parasitol.* **82**: 228-232.
- Stearns, S.** 1992. *The evolution of life histories*. Oxford University Press, Oxford.
- Svensson, L.** 1984. *Identification Guide to European Passerines*. Naturhistoriska Riksmuseet, Stockholm.
- Swaddle, J.P., Witter, M.S., Cuthill, I.C., Budden, A. & McCowen, P.** 1996. Plumage condition affects flight performance in common starlings: implications for developmental homeostasis, abrasion and molt. *J. Avian Biol.* **27**: 103-111.
- Tompkins, D.M., Jones, T. & Clayton, D.H.** 1996. Effect of vertically transmitted ectoparasites on the reproductive success of swifts (*Apus apus*). *Funct. Ecol.* **10**: 733-740.
- Zar, J.H.** 1984. Biostatistical analysis. Prentice Hall, New Jersey

Table 1. Results of the stepwise GLMs of the relationship (a) between laying date and condition indices and the number of feather holes in males and females and (b) between number of feather holes and condition indices in male Barn Swallows. Tail length, wing length, tarsus length and body mass are considered as condition indices and we show only the selected variables. For females there was no effect of feather holes on any condition indices.

a. laying date				
Variable	MS	<i>Df</i>	F	<i>P</i>
Male				
Feather holes	1232.4	1	9.03	0.003
Year	345.2	4	2.53	0.04
Model	$F_{5,183} = 12.43, r^2 = 0.06, P < 0.001$			
Female				
Tail length	1014.5	1	7.4	0.007
Feather holes	3596.5	1	26.24	< 0.001
Model	$F_{2,200} = 19.29, r^2 = 0.16, P < 0.001$			
b. number of feather holes				
Variable	MS	<i>Df</i>	F	<i>P</i>
Male				
Tail length	945.6	1	4.04	0.045
Year	1091.1	4	4.66	0.001
Model	$F_{5,340} = 4.66, r^2 = 0.06, P < 0.001$			

Figure 1. Frequency distribution of feather holes on male (a) and female (b) Barn Swallows.

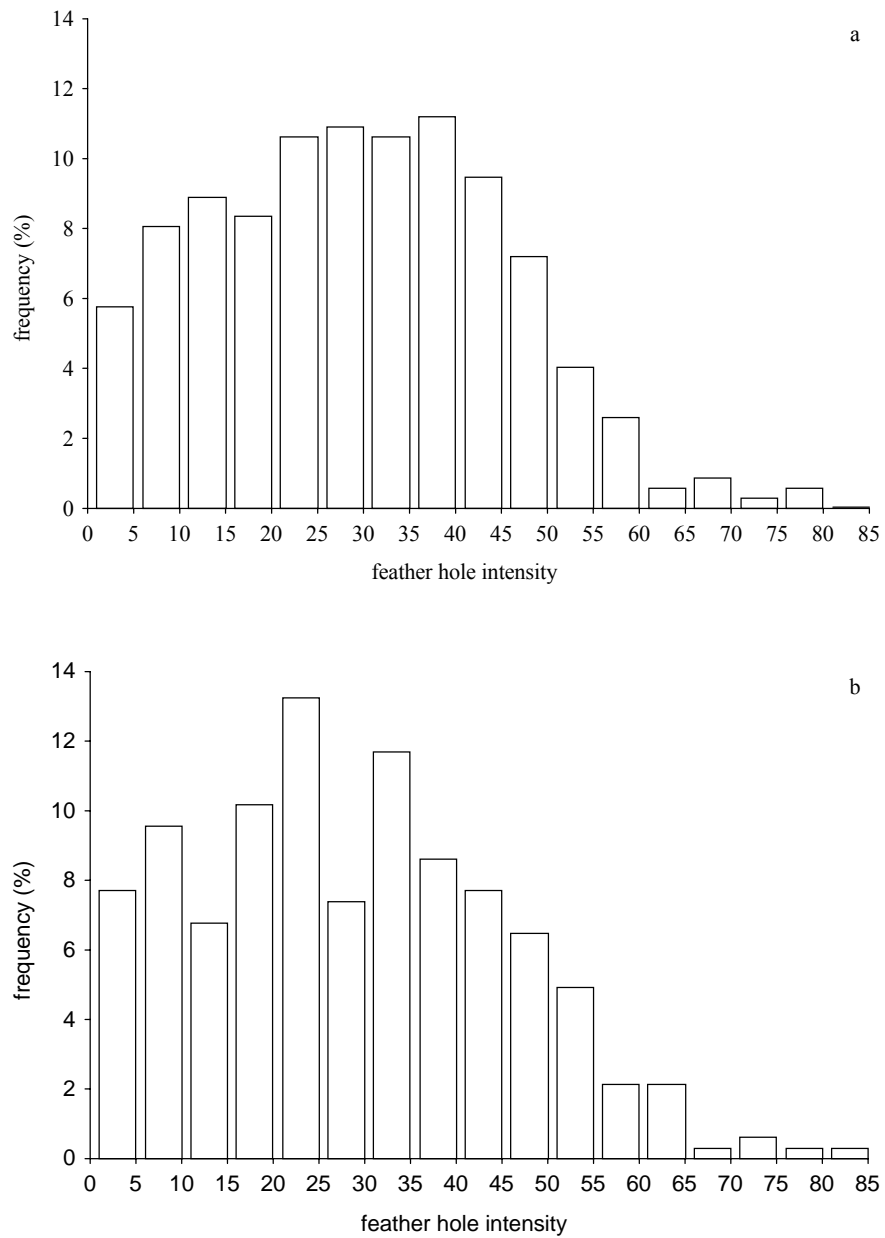


Figure 2. Feather hole number of male (a) and female (b) Barn Swallows in relation to laying date. male: $F_{1,183} = 12.43$, $P < 0.001$, slope (SE) = 0.252 (0.072); female: $F_{1,202} = 31.06$, $P < 0.001$, slope (SE) = 0.365 (0.066).

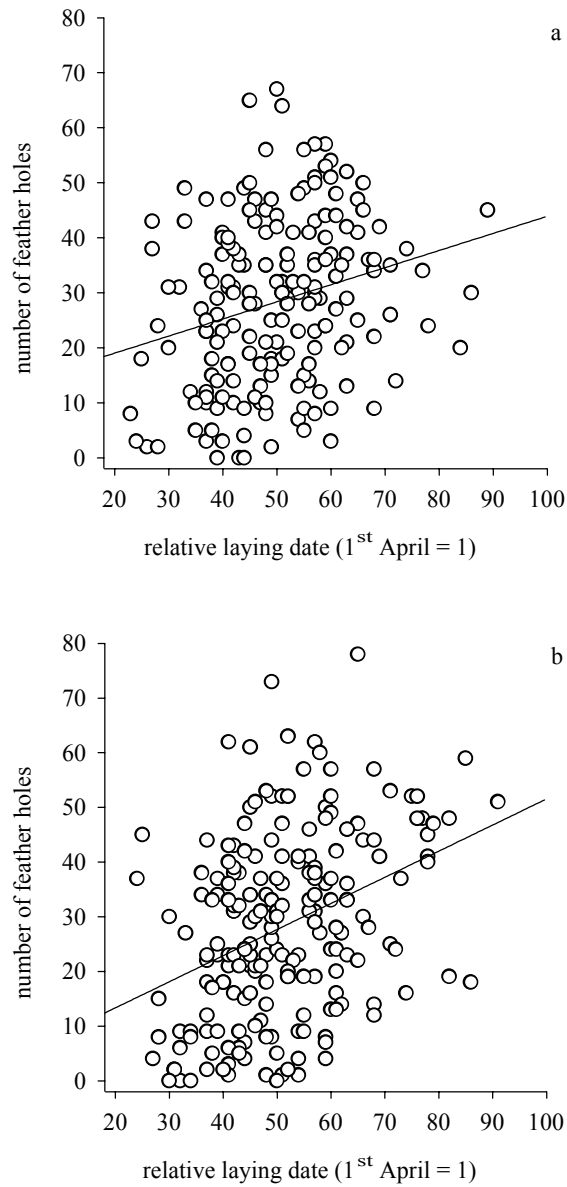
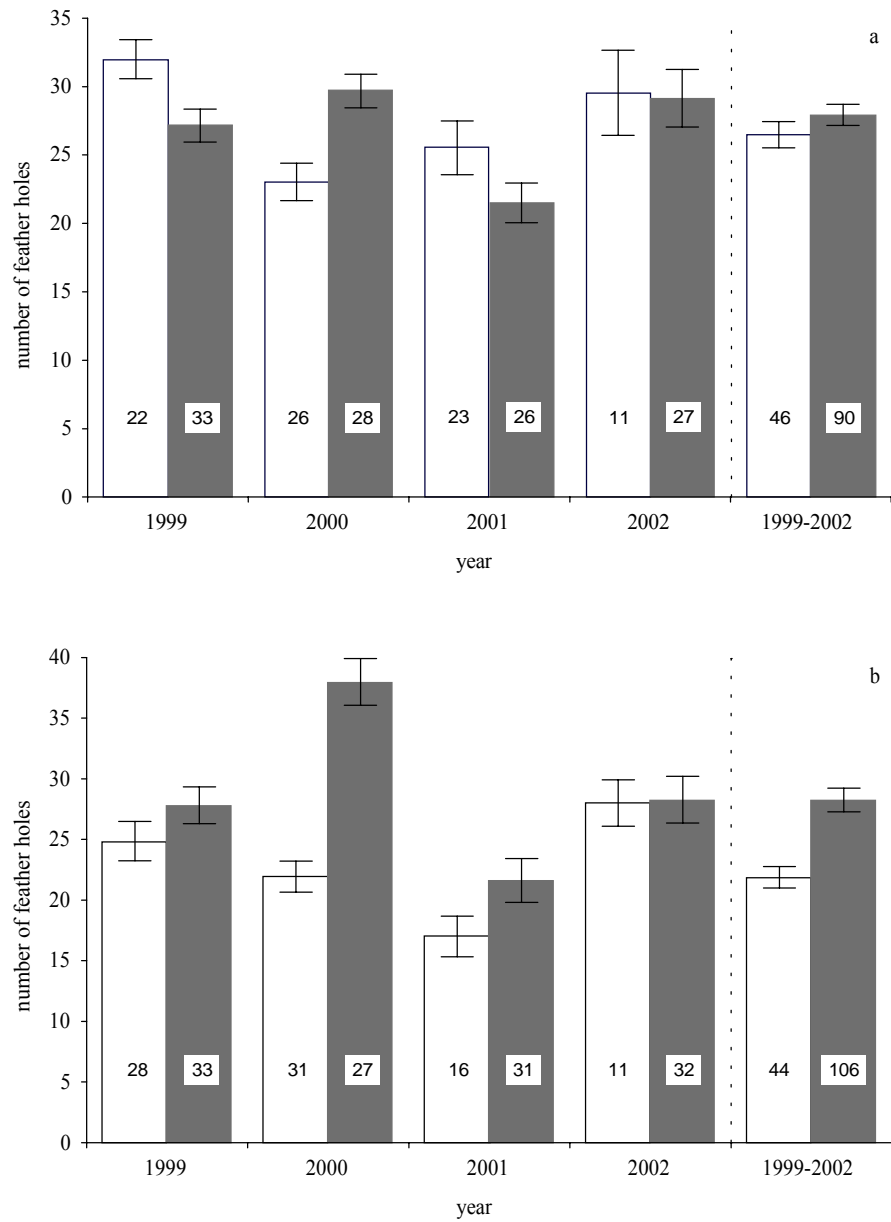


Figure 3. Difference in the number of feather holes between surviving and non-surviving male (a) and female (b) Barn Swallows between 1999-2002 and in the pooled data (mean \pm SE). Open - surviving, shaded – non-surviving.



Paper 5

Manuscript (Submitted in *Canadian Journal of Zoology*)

Host-symbiote relationship and abundance of feather mites in relation to age and body condition of the barn swallow, *Hirundo rustica*: an experimental study

by
Péter László Pap, Jácint Tökölyi and Tibor Szép

Host-symbiont relationship and abundance of feather mites in relation to age and body condition of the barn swallow (*Hirundo rustica*): an experimental study

Péter László Pap^{1,2*}, Jácint Tökölyi and Tibor Szép³

¹Behavioural Ecology Research Group, Department of Evolutionary Zoology, University of Debrecen, H-4010 Debrecen, Hungary

²Department of Taxonomy and Ecology, Babeş-Bolyai University, RO-3400 Cluj Napoca, Clinicilor street 5-7, Romania

³Department of Environmental Sciences, College of Nyíregyháza, P.O. Box 166, H-4401 Nyíregyháza, Hungary.

ABSTRACT

We analyzed the host-symbiont relationship and factors determining the abundance of feather mites between individual barn swallows (*Hirundo rustica*) in two different host populations during the breeding and post-breeding season. By experimentally removing the feather mites from the flight feathers of the birds with an insecticide, we show that these symbiotic organisms have neither harmful nor beneficial effects on the fitness of the host, supporting the commensal nature of mites. This was indicated by the absence of any difference in the change in condition indices, and the breeding performance and survival of the birds between the fumigated and control groups one year after the experiment. During the post-breeding season juveniles harbored fewer mites than adults, and the difference between age classes was also significant between the one-year-old birds and more than one-year-old birds of the breeding population. The number of mites did not change after the second year of life of the birds. We hypothesize that the difference in the abundance of mites between age classes can be explained by the low reproductive potential of the mites, and they are not able 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 have less supply for mites than old birds. The negative significant association between the number of mites and the laying date of female barn swallows seems to support the condition dependent abundance of mites. Because there was no relationship between other condition indices of males and females and the number of mites, it necessitates further research to strengthen this assumption.

INTRODUCTION

Host-symbiont relationships have attracted the attention of many ecological and behavioral researchers during the last two decades, due to the importance of symbionts (especially parasites) in shaping the evolution of life history of the host (e.g. Clayton and Moore 1997, Poulin 1998). Feather mites are one of the most common ectosymbionts of birds, with both high variance in prevalence and intensity of infestation among individuals (e.g. Figuerola 2000, Proctor 2003). Despite the high abundance of feather mites on birds (Proctor and Owens 2000), and contrary to other common groups of parasites (e.g. hematophagous mites, fleas, ticks and viruses, such as the avian pox virus), this group of obligate avian symbiont is not well studied, especially in relation to their role in the host's life history (Proctor and Owens 2000). The lack of studies on the host-feather mite relationship can be explained, at least partly, by the lack of interest in this group of symbiont. Since feather mites are considered to be commensals, mutualistic organisms or parasites with mild effect (Proctor and Owens 2000), their influence on the host is expected to be minimal, and as a consequence their role in evolutionary processes is probably extenuated. This lack of interest has led to the confusion we presently have in relation to the effect of feather mites on the fitness of the host, since in different publications from recent years they are mentioned as parasites, commensals or even mutuals (e.g. Pérez-Tris et al. 2002, Lindström et al. 2004, Shutler et al. 2004). These contradictory assumptions are reflected in the few correlational studies, and in our one best known experimental study (Figuerola et al. 2003), which found negative (e.g. Thompson et al. 1997, Rózsa 1997, Harper 1999), positive and no effect (Blanco et al. 1997, 1999, Dowling et al. 2001, Møller et al. 2004) of the mites on the host's measures. However, correlational studies have the drawback that they cannot overcome important caveats in regards to the functional relationship between feather traits and parasites. It is worth mentioning that the only experimental study mentioned above showed the negative effect of feather mites on the plumage color of the serin (*Serinus serinus*). In summary, there is a great need for experimental studies of mites (see Proctor and Owens 2000), in which their abundance is manipulated. In this way their impact on the host can be tested by excluding confounding factors (Proctor and Owens 2000) such as the effect of other co-occurring parasites (Pérez-Tris et al. 2002, Lindström 2004). Another seldom addressed problem in studies of the impact of feather mites on birds is the species identity of symbionts, even ignoring completely the genus or family in which mites belong (e.g. Thompson et al. 1997, Blanco and Frías 2001, Møller et al. 2004). Much of the past work was done on feather mites living on the surface of the flight feathers, termed as plumicoles, but the taxa diversity within this ecological group is high (Proctor 2003), thus pointing out the importance of treating different groups and species separately. Due to

the scarcity of studies, we have no data about divergent roles of different mite groups and species.

Considering the nature of the host-symbiont relationship (see above), the effect of feather mites on the fitness of the host is crucial in the appearance and evolution of defense mechanisms of the host against symbionts. Ultimately, this can affect the abundance of mites on the host. For example, if a symbiotic organism diverts important resources from host's resources, typical for many parasites, a quick response from the host is expected, leading to the decrease in number of the parasitic population (e.g. Møller 2000). The huge diversity of immunological, ecological and behavioral forms of defense in birds support this theory (Clayton and Moore 1997). Contrary, if the symbionts have no or low effect on the fitness of the host, the evolution of defense mechanisms is not expected. Apparently the lack of response to the intensity of infestation by feather mites, measured by the grooming behavior of the Seychelles warblers, *Acrocephalus sechellensis*, and the lack of relationship between bill deformities and the number of feather mites of the red-billed choughs (*Pyrrhocorax pyrrhocorax*) come to support this assumption (Blanco et al. 1997, Dowling et al. 2001). In this case, factors other than host defense are responsible for the variation in the number of feather mites between hosts, like the resources available or the reproduction capacity of the mites (e.g. Blanco and Frías 2001).

Feather mites of the barn swallow (*Hirundo rustica*) are supposed to be commensals, with no harmful effect on the fitness of the birds (Blanco and Frías 2001, Møller et al. 2004). Mites feed on algae, fungi, pollen and secretion of the uropygial gland covering the feathers, removing the oil and the microorganisms (O'Connor 1982, Blanco et al., 2001). Feather mites live primarily on the ventral surface of the wing and tail feathers, between the barbs of feathers. They are generally oviparous symbionts with a slow reproduction rate, and from the large eggs produced by females only one is maturing at each reproducing event (Proctor 2003). Some species of mites lay thin-shelled eggs in spring and summer, and produce thick-shelled eggs in autumn that hatch the following spring. The low reproduction potential of feather mites should be reflected in the colonization speed of the new hosts.

Taking into consideration the lack of experimental studies on the role of feather mites on the fitness of the host, we address the following questions in our study of the barn swallow - feather mites (*Trouessartia spp.*) relationship: (1) do feather mites affect the fitness of the host and (2) which factors are responsible for the variation in the number of feather mites between individual barn swallows. We manipulated the number of mites of adult breeding barn swallows by fumigating the wing and tail feathers with an insecticide (experimental group), and compared the change in their morphological, reproductive parameters and survival one year later with a control group (sprayed with water). Furthermore, we analyzed the relationship between

condition indices of the birds and the number of feather mites in the same breeding population during the breeding season, and on a different population during the post-breeding season, respectively. The difference between age classes of the birds in the number of mites was tested for both populations.

METHODS

Study areas and measurements

Barn swallows were studied in two different areas, namely a breeding population near Balmazújváros (47°37'N, 21°21'E), Hungary between 2002 and 2004, and a post-breeding population c.a. 200 km east from the first study area, near Sic (46°55'N, 23°52'E), Romania in 2004. Breeding barn swallows were captured by intensive mist netting and with nest trap during the first breeding period between May and July. After marking birds with a unique aluminum ring and color ring combination, standard measures were taken. We measured the length of the left and right wing, left and right outermost tail feathers, left tarsus and body mass. We averaged the left and right values of the wing and tail, and we calculated the fluctuating asymmetry (FA) of the tail, which is the unsigned value of the left minus the right tail. FA of bilateral characters is widely used as stress indicator (e.g. Brown and Brown 2002), and we supposed that any effect of mites on the fitness of birds should be reflected in an altered FA of the tail. We used the FA of only the outermost tail feathers due to the high accuracy of measurement and to the high variance in FA (CV male: 107%, CV female: 117.6%), which increases the perceivability of measurements. We frequently inspected the nests during the breeding period in order to determine the laying date, clutch size and brood size, and to assign the identity of nest owners by reading the color ring combination or by capturing adult birds at the nests. Post-breeding birds were captured during June and July 2004 with mist nets mounted in a large reed bed, taking advantage of the barn swallows communal roosting in reed beds during this period of the year. The age (adult or juvenile) of the birds in the post-breeding period was determined based on external characters (Svensson 1992).

After measurements, the number of feather mites was assessed by a semi-quantitative method in both study sites. Namely, we counted (PLP on breeding population and PLP and JT on post-breeding population) feather mites separately on individual primaries on both wings and on tail feathers until 10, and above this number the intensity of infestation was assessed by scoring the mite clusters on a 5 grade scale (e.g. 15, 20, 25, etc.). Feather mites live in the ventral surface of the feathers, on the vanes, and are individually visible without magnification. The use of semi-quantitative assessment was necessary, since barn swallows are heavily infested, with some of them holding hundreds of mites. The use of this visual inspecting semi-quantitative method holds

measurement errors, due to the difficulties in distinguishing live mites and skin casts (Proctor and Owens 2000), and due to the general problem with the repeatability of estimates. We verified the efficacy of this method in two ways. First, in a sample of barn swallows from the breeding colony in Hungary in 2003, we compared counts obtained using this technique with those obtained from the same birds using dust-ruffling method (Clayton and Drown 2001). To remove feather mites we used a common repellent against arthropod parasites developed for veterinary use (see below). The intensity of infestation by feather mites based on visual inspection of the three outermost primaries correlated significantly with the number of feather mites counted on the collecting plate (Spearman rank correlation: $r_{48} = 0.65$, $n = 48$, $P < 0.0001$), supporting the accuracy of the counting method used. Second, on a subset of breeding birds from Hungary in 2004, we recounted the number of feather mites on the flight feathers of the same birds several days after the initial measurement, in order to calculate the repeatability of our assessment. The high repeatability indicates again the accuracy of the method used ($R = 0.97$, $F_{10,11} = 75.3$, $P < 0.0001$). Two feather mite species were identified (*Trouessartia crucifera* and *T. appendiculata*, Acari: Analgoidea) by Ronald Schmäscke (University Leipzig, Germany), collected from the wing feathers of the Hungarian breeding barn swallows. We have no information about the identity of feather mites of the Romanian barn swallow population.

In 2002 and 2003, we counted feather mites on breeding barn swallows only on the three outermost primaries, while in 2004 both wings (primaries, secondaries and tertials) and the tail feathers were completely inspected at both study sites. Thus, except for the experimental part and the analyses of post-breeding birds, we used only the data from the three outermost primaries. Counting feather mites only on the three outermost primaries serves as an accurate predictor of the total number of mites on flight feathers, because 57% of the mites are on these three outermost primaries, and the relationship between the quantity of mites measured on the three outermost primaries and in total is strongly significant (Spearman rank correlation, $r = 0.92$, $n = 151$, $P < 0.0001$). The values obtained from the left and right wings were averaged in order to increase the robustness of the data.

In order to test the effect of feather mites on the fitness of the birds, we conducted an experiment in 2003, in which half of the adult birds captured during the first breeding period in the Hungarian colonies were fumigated with an insecticide (0.17% permethrin and 0.07% bioresmetrin (Insecticide 2000, Waldner GmbH Wien, Austria) (experimental group), while birds in the control group were sprayed with water. After the fumigation procedure, we put the birds in a bag for 10 minutes in order to dry. A sample of birds was re-captured several weeks after the first capture, in order to test the effectiveness of the insecticide used. A sharp difference in infestation between the fumigated and

water splashed birds was found, with significantly fewer mites on birds in the fumigated group (Mann-Whitney U test, $Z = 3.89$, $n_1 = 10$, $n_2 = 17$, $P = 0.0001$). In the following year, we recaptured the breeding barn swallows that had survived and measured the same variables as in the previous year. We measured the effect of the experiment on morphological variables, breeding performance and on the survival of the birds. Survival could be easily assessed, since the philopatry to breeding colonies of this species is very high, and the breeding dispersal is very low (Møller and Szép 2002, Pap et al. in press).

Data analyses

In order to analyze the effect of the experiment on the fitness of the host, we compared the morphological variables, breeding parameters and survival of the birds from the two groups in the year after the experiment, and the change in the wing and tail length between the year of experiment and the next year, in this way controlling for the initial size of the character. In the analyses of the relationship between the number of feather mites, condition indices and breeding parameters of the birds, we used the data collected from the three years between 2002 and 2004. In these analyses, we used the data from an individual only once in order to avoid pseudoreplication, by randomly including data from only one year. Several potential categorical and continuous predictor variables were included in a General Linear Model (GLM) in order to test factors responsible for the variation in number of feather mites between individuals. We used a backward stepwise procedure eliminating all non-explanatory variables included in the model, where the critical value was set to 0.05. Year, age and in the case of post-breeding birds the time of capture (the birds were captured at sunrise and at sunset) were included as categorical predictors, and the date of capture, time of capture (in case of breeding birds), wing-, tail-, tarsus length, and in case of breeding birds laying date and clutch size were included as continuous predictors of the number of feather mites (dependent variable). We included in the model year as a factor, since birds differed significantly in their number of feather mites between years (Kruskal-Wallis test, $H = 11.05$, $df = 2,451$, $P < 0.01$).

In the case of post-breeding birds, data of males and females was pooled due to the uncertainty in determining the sexes. The age of post-breeding birds was defined as adult (more than one year old) and juvenile (born in the study year), while breeding birds were categorized as one-, two-, three- and four-year-old birds. Ageing of breeding adult barn swallows was possible, since we have conducted intensive capturing and ringing of the breeding population since 1999, where more than 95% of the birds are marked, and as mentioned before, the breeding philopatry of the birds is high. So, newly ringed birds were defined as one-year-old, while the age of the recaptured birds was determined based on

ring number. We had only a few five-year-old birds, so their data could not be included in the analyses due to the small sample size. Whenever necessary, data transformation was used in order to normalize data, and in case the transformation did not normalize the data, we used nonparametric tests.

RESULTS

In both breeding and post-breeding populations studied in 2004, when the total number of feather mites were counted on flight feathers, birds were heavily infested, since 97% and 98% of adult birds had mites on the flight feathers. The mean intensity of feather mites was 125.4 and 106.2 for the breeding and post-breeding populations, respectively. We tested the consistency of infestation within individual birds by analyzing the changes in number of mites on the same individuals recaptured in different years between 2002 and 2004 in the Hungarian population. The repeatability of the number of mites within individuals is low, but significant ($R = 0.24$, $F_{54,61} = 1.97$, $P = 0.005$), indicating that once birds became infested, they will probably be infected with about the same intensity in the next years also. The low repeatability could be caused, at least partially, by the fact that the number of mites increases after the first year of life of the birds until the second year (see below), decreasing the consistency of the number of mites within individuals. Analyzing separately the data from those birds being at least two-years-old, the repeatability increases considerably ($R = 0.44$, $F_{16,21} = 3.47$, $P = 0.004$). We found a significant difference between individual barn swallows in the total number of mites counted in 2004, with skewness of 0.64 ($n = 157$) and 0.82, ($n = 101$), and kurtosis of -0.14 and -0.33, respectively in both breeding and post-breeding populations. This indicates that most of the birds were either not infected or only moderately infected, while just a few birds harbored many mites.

Next we analyzed the relationship between the number of mites and factors, which could affect their number. First, considering the low reproductive potential of the feather mites (Proctor 2003), we hypothesized that juveniles have less mites than adults during the post-breeding season. As we expected, juvenile barn swallows had fewer mites on the flight feathers than adults ($F_{1,99} = 107.5$, $P < 0.0001$; Figure 1a), but unexpectedly, the difference between age classes remained significant also between the one-year-old birds and more than one-year-old birds of the breeding Hungarian barn swallows ($F_{1,188} = 16.49$, $P < 0.0001$, Figure 1b). However, after the second year of life, the number of feather mites did not change (Tukey post-hoc test for unequal sample size, in all cases $P > 0.05$). We repeated these analyses on the same individuals captured as one-year-old bird and recaptured one year later. Again, birds in their first year harbored fewer mites than one year later ($F_{1,39} = 23.63$, $P < 0.0001$), corroborating the previous analyses. Second, we analyzed the relationship

between the number of feather mites and condition indices of the birds both on the breeding and post-breeding populations. Our hypothesis was that the symbiotic nature of the mites should be reflected in the relationship between their number and the condition indices of the host. The GLM analyses showed that among the condition indices and breeding parameters, only the laying date of females predicted significantly the number of mites, birds laying earlier being more heavily infested than late breeders (Table 1, Figure 2).

Fumigation with the insecticide had a significant effect on the total number of feather mites on the wing and tail feathers one year after the experiment, since birds in the experimental group had significantly fewer mites than those in the control group ($F_{1,59} = 34.84$, $P < 0.0001$). Comparing the breeding performance and condition indices of the birds the year after the experiment, we found no significant difference between the two groups (Table 2). Similar results were found when comparing the change in wing length and tail length between the two years, following the experimental manipulation (Table 1). The survival of the birds was similar in the experimental and control groups (male: $\chi^2 = 0.10$, $n_1 = 21$, $n_2 = 21$, $P = 0.76$, female: $\chi^2 = 0.17$, $n_1 = 23$, $n_2 = 20$, $P = 0.28$), indicating again the harmless effect of feather mites. Thus our results from the experiment confirm the previous findings on the relationship between number of mites and condition indices of the host.

DISCUSSION

Correlative studies on the relationship between the intensity of infestation by feather mites and condition of the host generated antagonistic results. Some authors emphasize the parasitic nature of feather mites (e.g. Thompson et al. 1997, Harper 1999), which is supported also by an experimental study (Figueroa et al. 2003). Others, based on the positive association between condition and number of feather mites, bring support for the commensal and/or mutualistic nature of feather mites (Blanco et al. 1997, 1999, Dowling et al. 2001, Møller et al. 2004). On the other hand, feather mites are supposed to be harmless or have little effect on the fitness of the host (e.g. Proctor 2003), which results in no or weak associations between the abundance of mites and the condition of the host. These contradictory data results are the drawback of the correlative studies, for e.g. in a host-parasitic association positive, negative, and even no associations are expected between the population size of the parasites and the condition of the host. Our experimental results support the commensal life of feather mites, as we found no difference between the fumigated and control groups in the condition indices of the birds, and in change in wing and tail length following the experimental year. Furthermore, it is worth mentioning that the absence of difference in the number of mites between experimental and control groups persisted even after one-year following the manipulation. We

consider this finding to be important, since some authors explained the absence of relationship between the number of feather mites and condition of the host with methodological drawbacks. Namely, most studies followed the host-symbiont association during the non-molting period, but the effect of symbionts on condition indices can be expressed only during the developing period of the character in case (e.g. during molting for periodically replaced characters) (Thompson et al. 1997, Pérez-Tris et al. 2002, Figuerola et al. 2003). Blanco et al.'s (2001) preliminary data about the food composition of an other mite species (*Pterodectes rutilus*) of the same host species, that in the gut of the mites they found mostly algae, fungi and pollen, contained by the feather oil from the feathers, is in accordance with our results, considering the absence of effects of *Trouessartia* spp. mites on barn swallow. It has been hypothesized that host may even benefit from the interaction with feather mites, due to mites removing the old preening oil and the pathogenic microorganisms such as fungi and bacteria accumulated on feathers (Blanco et al. 1997). In this respect we would expect that by eliminating the population of the mutualistic symbionts, the fitness of the host should decrease. Our experimental results do not support the beneficial effect of feather mites on the fitness of the barn swallows, as we did not find any effect of the fumigation on the condition indices of the birds related to the control group. As our data support the commensal nature of feather mites, other aspects than host's defense should explain the difference in the number of mites between individual barn swallows.

Our results on the difference between the number of feather mites on juvenile and adult barn swallows during the post-breeding season is in accordance with previous findings on several different host species, namely birds in their first year of life have fewer mites than adults (Blanco et al. 1997, 1999, Blanco and Frías 2001, Jovani and Blanco 2000). This difference between age classes can be explained by the fact that after infection of juvenile birds, probably by their parents in the nest (Proctor 2003), or during the social life of the birds (Blanco and Frías 2001), the time available for the population of mites to build up is short. However, the difference between age classes persisted even one year after hatching, since one-year-old breeding barn swallows had fewer mites than birds being at least two-years-old. This difference could be explained by the low reproductive rate of feather mites, since only one egg matures at a reproductive event (Proctor 2003). The lack of difference in the number of mites between two-years-old and more than two-years-old age classes of barn swallows suggest, that after the second year of life of the host, the number of mites reaches an upper limit. Above this limit their population size is probably limited by other factors than their reproductive potential. The number of mites one year after the experimental treatment was significantly lower in the case of birds fumigated with an insecticide. This difference could be explained also by the low population growth rate of feather mites. The insecticide used has a long-

term effect, however barn swallows undergo a complete annual molt on the wintering grounds, are freed from the effect of the insecticide and are exposed to infection with mites from other group members during the migratory or wintering periods. An alternative explanation for the pattern of differences between the age classes is that mites obtain fewer resources from the young swallows than from older birds, resulting in smaller mite populations on young birds. An indirect evidence for this explanation is that condition indices and breeding performance of the barn swallows increase during the first two years of life, but afterwards their performance remain unchanged at least until they are four-years-old (Møller and de Lope 1999). However, a direct test in this respect is required, by for e.g. analyzing the difference between age classes in the quantity and quality of food supply of feather mites. The positive relationship between laying date of female barn swallows and the number of feather mites apparently supports the condition dependent abundance of mites on the host, since laying date in birds generally is regarded a good index of condition (e.g. Møller et al. 2004). But it is important to mention, that none of the other condition indices of males and females showed significant association with the number of mites. This can be explained by the assumption that condition indices of the barn swallows are weakly related to the preening oil and microorganism's abundance on the feathers. Unfortunately, we have no data on birds concerning this association intimating speculations about the pattern of abundance of feather mites between hosts.

In conclusion, we demonstrate that two *Trouessartia* species of feather mites have no deleterious or beneficial effect on the fitness of the barn swallow, supporting the previous suggestions on the commensal nature of feather mites. Juvenile barn swallows had fewer mites than adults, and this difference between age classes persisted until the second year of life of the birds. The difference in population size of mites between age-classes could be attributed to the slow reproductive potential of mites. Alternatively, the difference in food supply for feather mites could be responsible for the smaller population size of mites on one-year-old hosts. The positive relationship between the abundance of feather mites and the laying date of female barn swallows suggests the condition dependent abundance of mites between hosts.

ACKNOWLEDGEMENTS

The Association for Bird and Nature Protection "Milvus Group" supported our fieldwork in Romania. We specially thank to István Csongor Vágási and István Kovács for their help in ringing birds. The administration of the Hortobágy National Park gave permission to work in the breeding colonies in Hungary. We thank Ronald Schmäscke for identifying feather mites, Eszter Ruprecht for her help and to Kyle Kenyon for correcting English of an earlier version of the

manuscript. P.L.P. was supported by the Domus Hungarica Foundation and by a research grant offered by the Hungarian Ministry of Education. T.Sz. was supported by the OTKA T042879.

REFERENCES

- Blanco, G. and Frías, O. 2001. Symbiotic feather mites synchronize dispersal and population growth with host sociality and migratory disposition. – *Ecography* 24: 113-120.
- Blanco, G., Tella, J. L. and Potti, J. 2001. Feather mites on birds: costs of parasitism or conditional outcomes? – *J. Avian. Biol.* 32: 271-274.
- Blanco, G., Seoane, J. and de la Puente, J. 1999. Showiness, non-parasitic symbionts, and nutritional condition in a passerine bird. – *Ann. Zool. Fennici* 36: 83-91.
- Blanco, G., Tella, J. L. and Potti, J. 1997. Feather mites on group-living Red-billed Choughs: a non-parasitic interaction? – *J. Avian Biol.* 28: 197-206.
- Brown, C. R. and Brown, M. B. 2002. Ectoparasites cause increased bilateral asymmetry of naturally selected traits in a colonial bird. – *J. Evol. Biol.* 15: 1067-1075.
- Clayton, D. H. and Moore, J. eds. 1997. Host-parasite evolution: general principles and avian models. – Oxford University Press, Oxford.
- Dowling, D. K., Richardson, D. S. and Komdeur, J. 2001. No effects of a feather mite on body condition, survivorship, or grooming behavior in the Seychelles warbler, *Acrocephalus sechellensis*. – *Behav. Ecol. Sociobiol.* 50: 257-262.
- Figuerola, J. 2000. Ecological correlates of feather mite prevalence in passerines. – *J. Avian Biol.* 31: 489-494.
- Figuerola, J., Domènech, J. and Senar, J. C. 2003. Plumage colour is related to ectosymbiont load during moult in the serin, *Serinus serinus*: an experimental study. – *Anim. Behav.* 65: 551-557.
- Harper, D. G. C. 1999. Feather mites, pectoral muscle condition, wing length and plumage coloration of passerines. – *Anim. Behav.* 58: 553-562.
- Jovani, R. and Blanco, G. 2000. Resemblance within flocks and individual differences in feather mite abundance on long-tailed tits, *Aegithalos caudatus* (L.). – *Ecoscience* 7: 428-432.
- Lindström, K. M., Foulfopoulos, J., Pärn, H. and Wikelski, M. 2004. Immunological investments reflect parasite abundance in island populations of Darwin's finches. – *Proc. Royal. Soc. London B* 271: 1513-1519.
- Møller, A. P. 2000. Survival and reproductive rate of mites in relation to resistance of their barn swallow hosts. – *Oecologia* 124: 351-357.

- Møller, A. P., deLope, F. and Saino, N. 2004. Parasitism, immunity and arrival date in a migratory bird. – *Ecology* 85: 206-219.
- Møller, A. P. and deLope, F. 1999. Senescence in a short-lived migratory bird: age-dependent morphology, migration, reproduction and parasitism. – *J. Anim. Ecol.* 68: 163-171.
- Møller, A. P. and Szép, T. 2002. Survival rate of adult barn swallows *Hirundo rustica* in relation to sexual selection and reproduction. – *Ecology* 83: 2220-2228.
- Pap, P. L., Tökölyi, J. and Szép, T. in press. Frequency and consequences of feather holes in Barn Swallows *Hirundo rustica*. – *Ibis*.
- Pérez-Tris, J., Carbonell, R. and Tellería, J. L. 2002. Parasites and the blackcap's tail: implications for the evolution of feather ornaments. – *Biol. J. Linn. Soc.* 76: 481-492.
- Poulin, R. 1998. Evolutionary ecology of parasites. Chapman & Hall.
- Proctor, H. C. 2003. Feather mites (Acari: Astigmata): ecology, behavior, and evolution. – *Annu. Rev. Entomol.* 48: 185-209.
- Proctor, H. and Owens, I. 2000. Mites and birds: diversity, parasitism and coevolution. – *Trends Ecol. Evol.* 15: 358-364.
- Rózsa, L. 1997. Wing feather mite (Acari: Proctophyllodidae) abundance correlates with body mass of passerine hosts: a comparative study. – *Can J Zool* 75: 1535-1539.
- Shutler, D., Mullie, A. and Clark, R. G. 2004. Tree swallow reproductive investment, stress, and parasites. – *Can. J. Zool.* 82: 442-448.
- Svensson, L. 1992. Identification guide to European passerines. Stockholm.
- Thompson, C. W., Hillgarth, N., Leu, M. and McClure, H. E. 1997. High parasite load in house finches (*Carpodacus mexicanus*) is correlated with reduced expression of a sexually selected trait. – *Am. Nat.* 149: 270-294.

Table 1. Results of the stepwise GLMs of the relationship between number of feather mites (dependent variable), age (categorical predictor) and condition indices and breeding performance (continuous predictor) in male and female Barn Swallows. For the confounding variables included in the model see text.

Breeding barn swallows in Hungary				
Variable	MS	Df	F	P
Male				
Capture time	14077.7	1	5.47	0.02
Age	18865.5	1	7.33	0.008
Model	$F_{1,99} = 6.68, r^2 = 0.12, P = 0.002$			
Female				
Laying date	10.93	1	10.33	0.002
Model	$F_{1,106} = 10.33, r^2 = 0.09, P = 0.002$			

Post-breeding barn swallows in Romania				
Variable	MS	Df	F	P
Day of capture	35786.2	1	9.73	0.002
Time of capture	17305.0	1	4.70	0.03
Age	445537.1	1	121.10	< 0.0001
Model	$F_{3,92} = 46.29, r^2 = 0.60, P < 0.0001$			

Table 2. ANOVA results for comparing measures of morphological variables and breeding performance of the barn swallows in the fumigated and control group following the experimental year, and the difference in change in wing length and tail length of birds between the two years in the two groups. None of the interactions between the variables in effect and sex was significant.

	MS	F _{df}	P
Wing length			
Fumigation treatment	9.00	1.11 ₅₈	0.30
Sex	38.58	4.77 ₅₈	0.033
Tail length			
Fumigation treatment	30.45	0.64 ₅₈	0.43
Sex	4614.69	96.55 ₅₈	< 0.0001
FA tail length			
Fumigation treatment	0.87	0.28 ₅₀	0.60
Sex	14.77	4.77 ₅₀	0.34
Δ wing length (2003–2004)			
Fumigation treatment	0.36	0.44 ₅₇	0.51
Sex	3.92	4.86 ₅₇	0.031
Δ tail length (2003–2004)			
Fumigation treatment	10.22	1.26 ₅₇	0.27
Sex	66.46	8.16 ₅₇	< 0.01
Laying date in 2004			
Fumigation treatment	165.97	1.86 ₄₉	0.18
Sex	80.98	0.91 ₄₉	0.35
Clutch size in 2004			
Fumigation treatment	0.47	0.52 ₅₀	0.47
Sex	0.94	1.03	0.31

Figure 1. Difference in the number of feather mites between juvenile and adult post breeding barn swallows in Romania (a), and between one-, two-, three- and four-years-old birds of breeding barn swallows in Hungary (b). The number of feather mites of the post breeding birds is for the total number of mites counted on wing and tail feathers, while for the breeding birds the infection of only the three outermost primaries were used. Numbers in the bars show the sample size within each group. Mean \pm SE.

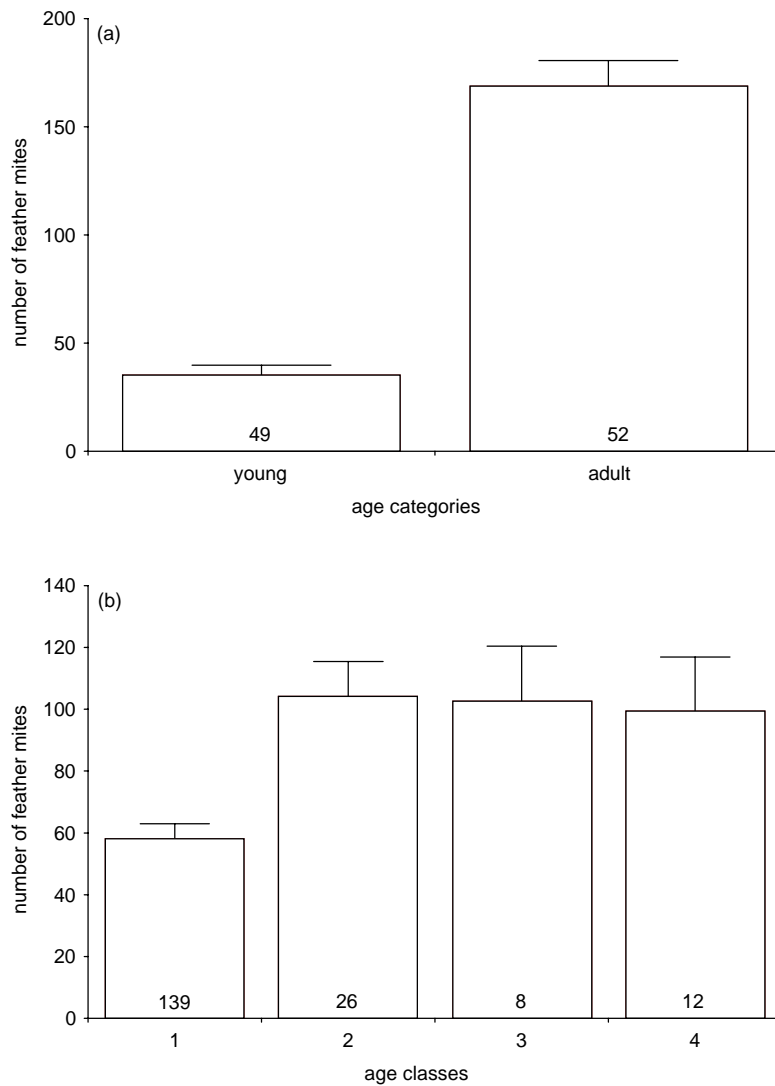
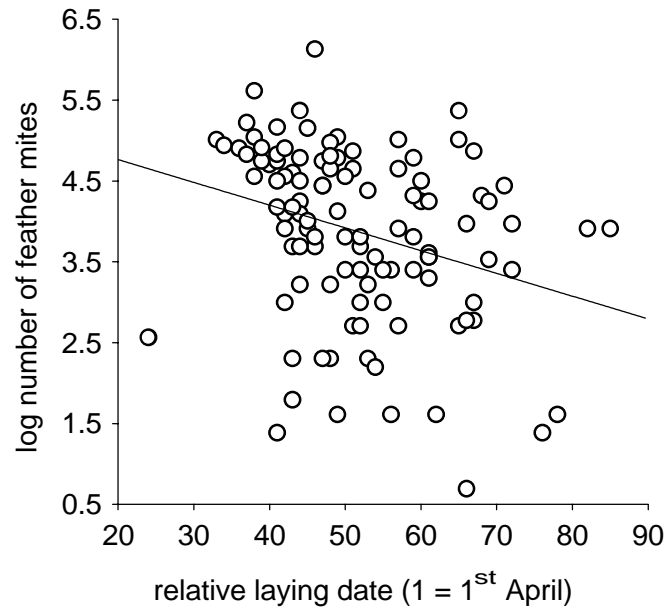


Figure 2. Arrival date of Hungarian female barn swallows in relation to the intensity of infestation by feather mites between 2002 and 2004. The line is a linear regression line with the equation $Y = 0.53 - 0.03 X$.



Paper 6

Manuscript (Submitted in *Behavioral Ecology*)

**Microhabitat preference, escape behavior and cues used by feather mites to
avoid molting wing feathers: an experimental test**

by
Péter László Pap, Tibor Szép, Jácint Tökölyi and Steven E. Piper

Microhabitat preference, escape behavior and cues used by feather mites to avoid molting wing feathers: an experimental test

Péter László Pap^{1,2*}, Tibor Szép³, Jácint Tökölyi² and Steven E. Piper⁴

¹Behavioural Ecology Research Group, Department of Evolutionary Zoology, University of Debrecen, H-4010 Debrecen, Hungary

²Department of Taxonomy and Ecology, Babeş-Bolyai University, RO-3400 Cluj Napoca, Clinicilor Street 5-7, Romania

³Department of Environmental Sciences, College of Nyíregyháza, P.O. Box 166, H-4401 Nyíregyháza, Hungary

⁴School of Botany and Zoology, University of KwaZulu-Natal, Private Bag X01, Scottsville, Pietermaritzburg, 3209 KwaZulu-Natal, South Africa.

ABSTRACT

We analyzed the pattern of distribution and the effect of molting on the escape behavior of feather mites on the wing feathers during the non-molting and molting season of the barn swallow *Hirundo rustica*. Feather mites showed consistent preference for the second outermost primary, with a steady decrease in proximal distance and avoidance of the outermost primary. Several explanations are suggested to explain this unusual distribution. Further, analyzing the escape behavior of feather mites on molting primaries, we show that mites avoid the feathers destined to be dropped next on molting barn swallows, and in the case of the outermost primary, mites use the “last moment” strategy, namely leaving feathers shortly before it is dropped. Next, we performed an experiment in which we simulated shedding feathers or feathers about to be shed on non-molting barn swallows, in order to test cues used by feather mites in avoiding molting primaries. Both the vibration of the incised feather, and the gap of the pulled feather, induced mites to leave primaries situated distally, at two-feathers distance from the manipulated primary, related to the control group. Our results show that feather mites have the ability to perceive the signal produced by the feather that will drop next and by the gap of the missing feather. It remains to be demonstrated, whether feather mites have the ability to perceive the vibration of the feather *per se*, or they perceive the altered airflow caused by the vibrating feathers.

INTRODUCTION

Host-symbiont relationships have received much attention during the last decade (e.g. Clayton and Moore, 1997) due to the important driving force of parasites on the evolution of host's life history and behavior (Hart, 1997; Møller, 1997). Because symbionts can decrease the fitness of the host by diverting important resources from vital physiological processes, they are important selective forces for the evolution of efficient host defense (Clayton and Moore, 1997). Alternatively, physiological and behavioral counter adaptations of the parasites are expected in order to avoid the fitness decreasing effect of the defending host. 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 an evolutionary response in the parasite. Numerous organisms, defined as obligate symbionts, are closely connected to the host, hence for their survival and reproduction a permanent close association with the host is essential. Considering the defense mechanisms of the host, counter adaptations of the obligate symbionts to maintain the close association with the host is vital. These adaptations to obligatory symbiont life include morphological, physiological, ecological and behavioral modifications of the symbiotic organisms (Clayton et al., 1999; Marshall, 1981; O'Connor, 1982; Proctor, 2003).

Birds host a rich diversity of arthropod symbionts, of which the greatest diversity is made up of feather mites, Acari: Astigmata, a small-bodied symbiotic acarine group dwelling in/on the skin and feathers (Proctor and Owens, 2000). Little is known about the relationships of birds and their feather mites (O'Connor, 1982; Proctor and Owens, 2000), these being considered as parasites, commensals or mutualistic symbionts (Figuerola et al., 2003; Proctor and Owens, 2000). Data available for the barn swallow *Hirundo rustica* indicate that the feather mites of this host are commensals with no harmful effects on the fitness of the birds (Blanco and Frias, 2001; Møller et al., 2004; Pap PL et al., in prep). They feed on algae, fungi and pollen contained in feather oil found on the surface of the feathers (Blanco et al., 2001). Feather mites live primarily on the ventral surface of the wing and tail feathers, between the barbs of feathers. Their distribution seems to be affected by the morphology and structure of the feathers, the life history and social behavior of the host, and several external environmental factors such as humidity, air temperature and possibly the airflow on the surface of the feathers (Blanco et al., 1997; Bridge, 2003; Choe and Kim, 1989; Figuerola, 2000; Jovani and Blanco, 2000; Rózsa, 1997). Despite the limiting locomotor capacity of feather mites due to the adaptation to symbiotic life, seasonal and daily movement of mites has been observed (e.g. Wiles et al., 2000). This probably reflects the response of mites to the changing

environment, or in case of molting birds, an adaptive behavior to avoid molting feathers (see below).

Among the various behavioral defense mechanisms of hosts, the periodic shedding of feathers, fur (known as molting), and the external epidermal layer were considered as an effective way to escape from the ectosymbionts (see Moyer et al., 2002). However, studies on lice, quill mites and feather mites indicate that these symbionts have the ability to escape from the shedding parts of the host by modifying their behavior during the molting period. For example, quill mites abandon old feathers and enter the quills of new ones (Casto, 1974; Kethley, 1971), and lice actively seek refuge inside the sheath of the developing feather during the molt, where they are safe (Moyer et al., 2002). Feather mites of several passerines abandon wing feathers next and also the second one to be molted (Jovani and Serrano, 2001). But, despite the importance of molt in disrupting the life cycle of mites, little is known about the behavior of feather mites and the way they deviate from their normal pattern of distribution on the molting feathers. The response of feather mites to the molting feather has been investigated in only two studies (Dubinin, 1951; Jovani and Serrano 2001). The aforementioned study has the inherent defect of low sample size. In the second study, different species with generally small sample sizes were pooled together without controlling, for example, for the different patterns of distribution of feather mites on the wing feathers of different bird species. Apparently, nothing is known about the mechanisms by which these organisms detect the shedding external parts of the host (e.g. Proctor, 2003). Dubinin (1951; cited in Jovani and Serrano, 2001) and others (e.g. Blanco and Frías, 2001) proposed several hypotheses explaining the capacity of feather mites to perceive molting wing feathers. However, except for Dubinin's pioneering study, to the best of our knowledge, the mechanisms responsible for the escape behavior have never been tested. Dubinin (1951) proposed that the vibration of loosening feathers prior to molt provides a cue to feather mites to leave the shedding part of the wing ("vibration hypothesis"). Alternatively, feather mites detect the altered airflow produced by the gap of the missing adjacent feather ("window hypothesis") and leave the feather.

In this study, we first describe the distribution patterns of feather mites on the primary wing feathers of the barn swallow during their non-molting breeding season in Hungary, which presumably reflects their adaptive microhabitat preferences. Second, we analyze the distribution of feather mites on different wing feathers of molting birds in South Africa in relation to the molting stage of different primary feathers, in order to analyze the escape behavior of feather mites. Third, in an experimental study on a breeding barn swallow population in Hungary, we tested the efficacy of two not exclusive hypotheses, the window and vibration hypotheses as cues used by two feather mite species (*Trouessartia spp.*, Analgoidea) in avoiding the molting feathers.

That is, we mimicked a loosening feather by incising the base of the rachis of the 6th primary (e.g. the 5th primary from the distal part of the wing) (first experiment) in order to imitate the vibrating feather shortly before it was molted. By pulling out the same primary (second experiment) we mimicked the gap caused by the missing feather during molt. The change in the number of feather mites on each primary feather was computed as the difference between the number counted on first capture and those counted on the second capture approximately three weeks later. Our prediction was that in case of use as cues the vibrating feather or/and the altered airflow on gap of the fallen feather during escape, feather mites leave at least the next distal (normally the next to be molted) feather from the manipulated primary in the first or/and the second experimental group.

METHODS

Study areas and species

The barn swallow is a long distance migratory species, with the central-eastern European breeding populations probably wintering south of the equator, in the central and southern part of Africa (Cramp, 1988). Birds have a complete molt once a year during their Austral migration, when both adults and juveniles replace old wing and tail feathers. This study was carried on a breeding barn swallow population in eastern Hungary, near Balmazújváros (47°37'N, 21°21'E) between 24th of May and 30th of June 2004, and in three different wintering populations in South Africa, namely 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) between 25th and 30th of January 2004. The breeding area is characterized by scattered farms, where the birds breed alone or in loose colonies of up to 15 pairs. During the wintering period in Africa, hundreds or thousands of birds roost in reed beds, where the birds were captured in mist nets at dusk. After capturing and ringing the birds, one of us (P.L.P.) measured a number of morphological characters on the barn swallows, and on the birds in South Africa the molt of wing and tail feathers was recorded according to the scheme of Newton (1966). A dropped feather was scored as 1, a quarter-, half- or three quarter-regrown feather was scored 2, 3 and 4 respectively, and a full-regrown feather was scored 5. The molting pattern of barn swallow is similar to that of most passerines in that it sheds primary (P) flight feathers from the wing in sequential order (proximal to distal) (Jenni and Winkler, 1994). Barn swallows were about half way through their molt during our fieldtrip season in South Africa, presenting a high degree of synchronization in molt (e.g. 98% of captured birds molted the 5th, 6th, 7th or 8th primaries).

Examination of birds for mites

Feather mites are present on the vanes of wing and tail feathers and are individually visible without magnification. On completion of morphometric measurements, the number of feather mites on the whole vane of primaries, secondaries and tertials of the left and right wing and on the tail feathers was examined by the same person (P.L.P.) after the following procedure. The wing and the tail feathers were extended and held up to ambient or artificial light, and the feather mites attached to the feathers were counted until 10 on each feather, and above this number their intensity of infestation was assessed by scoring the mite clusters on a 5 grade scale (e.g. 15, 20, 25, etc.). The use of semi-quantitative assessment was necessary, since barn swallows are heavily infested, some of them holding hundreds of feather mites. The use of this visually inspecting semi-quantitative method holds measurement errors, due to the difficulties in distinguishing live mites and skin casts (Proctor and Owens, 2000), and due to the general problem with the repeatability of estimates. We verified the efficacy of this method in two ways. First, in some barn swallows from the breeding colony in Hungary in 2003, we compared counts obtained using this technique with those obtained from the same birds using dust-ruffling method (Clayton and Drown, 2001). To remove the feather mites we used a common insecticide developed for veterinary use, containing 0.17% permethrin and 0.07% bioresmethrin (Insecticide 2000, Waldner GmbH Wien, Austria). The intensity of infestation by feather mites based on visual inspection of the three outermost primaries (the most heavily infested feathers; see Results) correlated significantly with the number of feather mites counted on the collecting plate (Sperman rank correlation: $r = 0.65$, $n = 48$, $p < .0001$), supporting the accuracy of the counting method used. Second, on a subset of breeding birds in Hungary in 2004, we recounted the number of feather mites on the flight feathers of the same birds several days later after initial measurements, in order to calculate the repeatability of our assessment. The high repeatability indicates again the accuracy of the method used ($R = 0.97$, $F_{10,11} = 75.3$, $p < .0001$).

Two species of feather mites, *Trouessartia crucifera* and *T. appendiculata* collected from the Hungarian birds were identified by Ronald Schmäscke (University Leipzig, Germany), while we have no information about the identity of feather mites from the South African barn swallows.

Experimental manipulation

Adult male and female breeding barn swallows from the Hungarian population were captured by mist nets during breeding, and after taking measurements and counting feather mites, they were selected randomly to be in one of the three experimental groups. In the first experimental group, we mimicked the vibration

of the feathers shortly before molt by laterally incising half of the rachis at the base of the left and right 6th primary. Our experiment most probably had an effect on the vibration of the feathers, since from the 26 experimental birds, 15 had broken their incised feathers on at least one side of the wing three weeks after the first capture. In the second experimental group, we pulled out the 6th primary on both sides of the wing, imitating in this way the gap produced by the missing feather during the molt. The third group was held as a control, where except for feather manipulation, we followed the same procedure in capturing and measuring birds as in the two other groups. The number of feather mites was counted on both capture sessions, and the change in number of mites between the first and second measure was used in the analyses (see below). The time elapsed during capture-recapture did not differ between the control, first and second experimental groups (20.1, 21.5 and 20.3 days, respectively; $F_{2,62} = 0.44$, $p = .65$).

Ethical considerations

Wing gap can reduce the maneuvering and flying capacity of birds (Rayner and Swaddle, 2000), and the effect of the gap is greater in wings of higher aspect ratio (long and narrow wing), as in the barn swallow. We made a compromise between manipulating the feathers as close as possible to the primary with the highest intensity of infestation (primary 8), and between reducing as little as possible the aerodynamic capacity of the birds, by handicapping them with the least important proximate primary. Thus primary 6 seemed reasonable to satisfy both expectations, since at least during the experimental period the manipulation seemed to have no effect on the condition of the birds, at least measured by the change in body mass between the first and last recapture (female: $F_{2,33} = 0.68$, $p = .52$; male: $F_{2,20} = 0.12$, $p = .89$). By incising the rachis of the feather the chance of breaking increased considerably (see above). Because these feathers are not replaced until the next molt, at the end of the experiment we pulled out these feathers in order to induce regrowing.

Data analysis

The mean intensity of infestation of barn swallows by feather mites differed between the three South African sites ($F_{2,110} = 42.98$, $p < .0001$), while birds in their first year of life did not differ from adults in this respect ($F_{1,110} = 1.41$, $p = .24$). The determination of sexes with accuracy was difficult, since most birds had broken or molting outermost tail feathers, a highly sexually dimorphic character. Hence, we pooled the data of males and females. Because we did not expect differences between populations and age classes in the pattern of distribution of feather mites on wing feathers and their response to molting, we

pooled the data of molting birds captured in South Africa. The pooling procedure was also followed in the Hungarian breeding population, but in this case we used only the data of adult birds, and the sexes did not differ significantly in their numbers of feather mites ($F_{1,152} = 0.49$, $p = .49$).

We included in the analysis only the data of the infected birds. Since the prevalence was high in each populations studied (e.g. 97% of the Hungarian population), we had to exclude the data of only a few birds. Feather mites show a strong preference for the primaries. Of the total number of feather mites counted on the wing and tail feathers of 151 non-molting adult infected birds in Hungary, 90% were on primaries, 3% on secondaries, 2% on tertials and 5% on tail feathers. Therefore, the distribution and movement of mites on secondaries, tertials and tail feathers were not analyzed, except when we followed the redistribution of the mites on flight feathers following the experimental manipulation. For the experiment, we included birds with at least 10 feather mites on the most preferred primary 8 (~ 5% of the mean intensity of infection). We used this arbitrary selection of birds for the experiment, because below this infection intensity the movement of mites is hard to follow due to the problem of statistical perceivability on low infestation level (Jovani and Serrano, 2004). In the data analyses of Hungarian birds we used the average values of the left and right wing in order to increase the robustness of the data, while for the South African birds we used the data obtained only for the left wing (the average value for this dataset could not be calculated due to the asymmetry observed in molting for some birds). The correlation in the number of feather mites between the left and right wing is highly significant (Behnke et al., 1999; Jovani and Serrano, 2004; see Results), therefore we consider appropriate to use the data of only one wing for the molting birds.

The data for 8 out of 25 birds from the first experimental group (incised primaries) with broken primary 6 on one side of the wing was excluded initially from the analyses. Similarly, data for 7 birds from the same group recaptured after several days following the manipulation with both primaries broken was definitely excluded from the analyses. First, we analyzed the effect of manipulation on the distribution of feather mites on the wing feathers on the subset sample excluding these birds with broken primaries. In the following, we repeated all analyses on the large data set including birds with one primary 6 broken in the first experimental group (using the data of feather mites for the same side of the wing), and including birds with primaries broken on both wings in the second experimental group. This transfer was done by pulling out the calamus of the broken feathers of the birds recaptured within a few days after the initial measurements. Because none of the results differed between the two analyses on small and large datasets, we kept the data of these birds, increasing the samples size in the control, first and second experimental group to 19, 28 and 18, respectively.

Due to the nature of dispersion of symbionts, feather mites showed a negative binomial distribution. Hence, we used non-parametric statistical analyses whenever it was necessary. Parametric tests were used for analyzing the results of the experiment, where because of excluding birds with no or very low level of infestation the data were normally distributed. Medians + lower quartile ranges or means + SE (for parametric tests) are given for the data presented.

RESULTS

Distribution of feather mites on non-molting barn swallows in Hungary

The distribution of the number of feather mites on the primary feathers was far from random (Figure 1), with primaries differing significantly among each other in the intensity of their infestations (Friedman ANOVA test: $\chi^2_{8,151} = 702.5$, $p < .0001$). The Wilcoxon matched-pairs signed-rank test indicates that mites showed a high consistency in their preference for the primary 8, and interestingly they consistently avoided the outermost primary (Table 1). The consistency in preference of feather mites for the primaries 7 to 1 was also high, with a gradual decrease in their number from distal to proximal.

Distribution of feather mites on molting barn swallows in South Africa

The distribution of feather mites on molting wing feathers apparently differed from the pattern on the non-molting birds (Figure 2), feathers unoccupied by mites or with low infestation in the case of non-molting birds can be highly infected in molting birds (e.g. primary 9). In order to test the effect of molt on the distribution of feather mites on primaries, we performed separate analyses for the primaries 7, 8 and 9, where we included the data of those birds which had a dropped or growing feather neighbor, second proximal and in the case where we had sufficient data the third proximal from the target (7, 8 and 9) primaries. For the primary 7 we had enough data to test the effect of only the neighbor and second proximal primary on the number of feather mites of the target feather (see Figure 3a). The number of mites on primary 7 decreased significantly when the neighboring primary was dropped related to when the second proximal feather was in molt (Mann-Whitney U-test: $Z = -2.68$, $n_1 = 43$, $n_2 = 21$, $p < .01$, Figure 3a). We found a similar effect of the molt of the neighbor and second or third proximal feather on the intensity of mites on primary 8, namely only the neighbor feather influencing negatively the number of mites on the target primary ($F_{2,88} = 9.06$, $p < .001$, Figure 3b), and the abundance of feather mites on primary 8 was not affected by molting of the second and third primaries next to the neighbor primary (Tukey post-hoc test: $p = 1.0$). The number of feather mites on the outermost primary (P. 9) did not

change significantly whether the dropped primary was neighbor, second or third proximal from the primary in case ($F_{2,88} = 0.14$, $p = .87$, Figure 3c). However, when we separated the data of birds to feathers in pin (molt category 1) and expanded feather (molt category 2 and 3) of the neighbor primary, we found a significant difference between the two groups in the number of mites on the outermost feather ($F_{1,20} = 7.65$, $p = .01$, Figure 4).

We performed separate analyses for the primaries 6 and 7 for which we had sufficient data in order to test the re-colonization capacity of mites by analyzing the change in the number of feather mites before molting (i.e. on an old primary) and after molting (i.e. on a new primary) in the same position. The results clearly show the rapid re-colonization of these two primaries, since in both cases the number of mites increased in number on new primaries relative to the old feathers on the same position (ANOVA planned comparison of the number of mites on old and new feathers; primary 6: $F_{1,60} = 13.07$, $p < .001$; primary 7: $F_{1,69} = 8.12$, $p < .01$).

Effect of experiment on the movement of feather mites

We performed separate repeated-measure ANOVA analyses for different primaries, in order to test the effect of the experiment on the change in number of feather mites on primary feathers 7, 8 and 9 when primary 6 was manipulated. We found no difference between the three experimental groups for any of the three primaries in the number of feather mites (Table 2), but in case of primary 8 the significant interaction between the treatment and repeated measure show the significant difference in the change in number of mites between the groups (Figure 5a). The Tukey post-hoc test for unequal sample size indicates that the decrease between the first and second count of the feather mites was significant for both experimental groups, but not for the control one ($p < .05$, $p < .001$ and $p = ns.$ for the first and second experimental group and for the control group, respectively). Surprisingly, the number of feather mites did not change significantly between the groups on the distal neighbor feather (P. 7) of the manipulated primary, as we would expected based on the response of mites to the nearest primary feather that had been dropped. However, this could be a problem of statistical perceivability of mites in low number (Jovani and Serrano, 2004), since the change in the number of feather mites on distal primaries during the three weeks of the experiment was proportional to the initial abundance of mites (Spearman rank correlation, P7: $r = 0.50$, $p < .0001$; P8: $r = 0.60$, $p < .0001$, $n = 65$; P9: $r = 0.30$, $p < .05$), and primary 7 held significantly less mites than the most preferred primary 8 (see Table 1, Figure 1).

Feather mites leaving the most preferred distal primary 8 due to the experiment raise the question of where these mites disperse to. Accordingly, we

performed the same repeated measure ANOVA test on the total number of feather mites counted on all wing and tail feathers (Table 2, Figure 5b). Again, the significant interaction between the treatment and repeated measure indicates that the two experimental groups differed in the degree of decrease in number of mites from the control group ($p < .01$, $p < .001$ and $p = \text{ns.}$ for the first and second experimental group and for the control group, respectively), revealing the fact previously observed (see Proctor, 2003), namely the capacity of feather mites to distribute on body feathers. Furthermore, the significant relationship between the changes in the numbers of mites during the experiment of the outermost primaries of the right and left wing within the same individual indicates that feather mites have a fine-tuned movement to changing environment (Spearman rank correlation, P2: $r = 0.72$, $p < .001$; P3: $r = 0.84$, $p < .001$; P4: $r = 0.56$, $p < .001$, $n = 65$).

DISCUSSION

Studies of the patterns of feather mites distribution have pointed out the importance of external factors (temperature, probably humidity, interspecific competition, social life of the host, etc.) in determining the host species-specific intensity of infestation and distribution of mites on wing feathers (Blanco et al., 1997; Choe and Kim, 1989; Figuerola, 2000; Jovani and Blanco, 2000; Wiles et al., 2000). The between-host differences in the number of feather mites seems to be condition dependent in at least some host-symbiont associations. The positive or negative correlation between the abundance of mites and the health and physical state of the host support both parasitic and non-parasitic life histories of feather mites (see review in Proctor and Owens, 2000). Interestingly, in some host-mite associations apparently no relationship was found between the condition of the host and number of feather mites (Blanco et al., 1999; Dowling et al., 2001), which raises the possibility that features other than condition determine the distribution of mites between hosts. The population growth capacity of the mites after colonization (adults have generally more mites than juveniles) and the nutrition supply differences between hosts are among other possible explanations for the differences observed in the number of mites between hosts (e.g. Blanco and Frías, 2001; Proctor, 2003).

Another characteristic of feather mites is their pattern of distribution on the wing feathers of the host. Regarding the within-host differences in the distribution of mites on wing feathers, external climatic factors such as humidity and temperature seem to be important. Changes in ambient temperature induce movement of mites living on remiges, with mites moving closer to the body during winter or cold periods (Wiles et al., 2000). Our preliminary observations on several different bird species, and the few data

from the literature we have (e.g. Bridge, 2003; Choe and Kim, 1989; Jovani and Serrano, 2004) suggest that there is a great variability between different host species in their feather mites' distribution on the wing. In addition, feather mites show a high consistency in their fine-tuned distribution on the wing within the same host species, as revealed by the present study and in a recent work by Jovani and Serrano (2004) on the blackcaps *Sylvia atricapilla*. The consistent distribution pattern of feather mites on the non-molting barn swallows, with a clear avoidance of the outermost wing feather, their preference for the second outermost primary, and the consistent decrease in their number to proximal primaries, raises the question of why these mites show this pattern of preference for specific feathers. There are probably a number of important factors that influence the microhabitat selection of feather mites, and at least three explanations may account for this distribution pattern. First, the morphological features of the feathers on which mites live differ (e.g. in barb height), as suggested by Bridge (2003) and Choe and Kim (1989). This means that differences in structure between feathers determines the way that mites can attach to the feather, which for example is important to resist air turbulences at the surface of the feather. This is less likely in our case for at least the two distal primaries, however, since the difference between neighbor primaries in barbs height is probably more uniform (Bridge, 2003), and this should be reflected in the distribution pattern of mites also. The sharp difference between the outermost and its proximal neighbor primary in their numbers of mites apparently contradicts this explanation. Second, the air turbulence at the level of feather surface could influence the site preference of the mites, as aerodynamically more stressful feathers are probably less appropriate for living. The air turbulence decreases from distal to proximal direction of the wing, with probably the greatest turbulence on the outermost primary (Rayner, 1988). This is in accordance with the reduced number of feather mites from the outermost primary, but inconsistent with their decreasing number to proximal direction, and their almost complete absence from secondaries and tertials, where the air turbulence is lowest. Furthermore, feather mites seem to prefer the outer half of the feathers (Bridge, 2003; Pap PL, pers obs), which does not support the prediction of spatial distribution based on aerodynamic considerations. However, the distribution of mites on the wing feathers probably reflects a compromise between the aerodynamically less stressful position and other factors, such as the barb height of the feathers. Third, the observed distribution pattern could be explained by the feathers differing in their nutritional content (e.g. preen gland oil), which ultimately determines the feather-specific distribution of feather mites. This difference can be caused by the preference of the host of preening for e.g. the distal part of the wing, leading a greater chance to smear preen oil on feathers of the respective part of the wing. Again, the sharp difference in the number of feather mites between the two outermost

primaries is hard to explain by this assumption, thus we consider this explanation to be implausible. The present study was not designed to distinguish between these alternatives. In order to understand the intriguing life of feather mites, we recommend further study.

Our observational data of feather mites on molting barn swallows confirm the previous findings (Dubinin, 1951; Jovani and Serrano, 2001), that mites avoid molting wing feathers. But contrary to Jovani and Serrano (2001), our results show that mites abandon feathers only when the proximal neighbor feather is molted, not when the second primary from the target feather is dropped, as previously reported (ibid.). However, our experimental results show that at least on the non-molting birds, *Trouessartia* spp. mites have the capacity to sense the molting feather when the second proximal feather is dropped or is close to being dropped (see below). The difference between Jovani and Serrano's (2001) findings, and our results on molting birds can be explained by the difference in hosts and their specific symbionts analyzed. Our detailed analyses of the escape behavior of mites on the three outermost primaries revealed that feather mites abandon the next to be molted feather only in the case of the second outermost and proximal neighbor feathers. This difference in escape behavior of feather mites between primaries suggests that their escape is limited in the case of the outermost primary. The logical assumption for this limited escape behavior of mites on the outermost feather is that in case of mites on inner primaries they have the possibility to redistribute themselves on the next distal primary. However, if this assumption is true, we would expect in case of primary 8 (for which we have sufficient data) a significant difference in number of feather mites between primary 8 with the second proximal feather dropped and primary 8 with the third proximal feather dropped (Figure 3b), which based at least on this subset of data is not true. Our results on the differences between the three experimental groups in the total number of feather mites on non-molting flight feathers indicate that mites during molt disperse away from the remiges, finding refuge probably within body feathers. This movement of mites is also supported by the observation on the transfer of mites from parents to fledglings, when initially feather mites are distributed over the body of the fledglings, from where they later redistribute to their typical positions, similar to that of adults (Dubinin, 1951; cited in Proctor, 2003; Kethley 1971). The difference between the outermost primary with the feather in pin and the expanded proximal neighbor feather in the number of feather mites indicates that feather mites use a "last moment" strategy, leaving the molting feather shortly before it is dropped. This is based on our observation on molting barn swallows and generally on birds, namely that after expanding the growing primary, the next to be molted feather is dropped within a short time. Since the neighbor growing feather in pin and expanded feather differ in just a few millimeters, this probably does not significantly modify the air turbulence

at this level, excluding the window hypothesis. Thus, it is reasonable to conclude, that at least at this level of outermost primary, the vibration hypothesis works. Connected with this, it remains to be demonstrated whether feather mites sense the vibration of the feather *per se*, or the airflow caused by the vibrating feather (see below). Our results on the re-colonization of feather mites suggest that mites rapidly respond to molting, quickly occupying preferred locations. The re-colonization of feathers and the adaptive escape behavior of feather mites suggest no, or at least little effect of molt, on the population size of mites.

Our experiment supports the observations of the escape behavior of feather mites on molting birds, since the number of mites on primary 8 declined after the manipulation of primary 6. Contrary to our expectation, our manipulations had no effect on the number of mites on the distal neighbor feather of the manipulated primary. As mentioned before, this could be caused by a statistical drawback to detect the change of the number mites in case of low intensity of infestation, since on this primary the number of mites is low, and the magnitude of change of mites is proportional with their initial number (see also Jovani and Serrano, 2004). The experimentally vibrating feather and the gap of the pulled primary both induced the movement of mites on primary 8, which resulted after a three week experiment in a significant decrease in their number related to the control group. Our results support both hypotheses suggested by Dubinin (1951), namely the vibration and window hypothesis. This would allow us to suppose, that feather mites have a developed “sense system” of detecting the altered vibration of the feather and the altered airflow produced by the gap of the molted feather. But, considering the fact that feather mites changed their behavior two feathers distal from the manipulated one, it is tempting to accept, that in both situations mites sense the altered airflow, caused by the vibrating feather or by the gap of the dropped feather. However, further research is required to understand the mechanisms used by feather mites.

ACKNOWLEDGMENTS

We are grateful for the help and assistance of Malcolm Gemmel, Rick Nuttal and Les Underhill during our fieldtrip in South Africa. We thank Ronald Schmäscke for identifying the two feather mites, Eszter Ruprecht and Kyle Kenyon for their help and Zoltán Barta for the statistical advices. The travel and fieldwork of T.Sz., S.P. and P.L.P. in South Africa was made possible by the financial support of the South African Government and the Government of Hungary, grant TÉT DAK-013. P.L.P. was supported by the Domus Hungarica Foundation and by a research grant offered by the Hungarian Ministry of Education. T.Sz. was supported by the OTKA T042879. The administration of the Hortobágy National Park gave permission to work in the breeding colonies.

REFERENCES

- Behnke J, McGregor P, Cameron J, Hartley I, Shepherd M, Gilbert F, Barnard C, Hurst J, Gray S, Wiles R, 1999. Semi-quantitative assessment of wing feather mite (Acarina) infestations on passerine birds from Portugal - Evaluation of the criteria for accurate quantification of mite burdens. *J Zoology* 248: 337-347.
- Blanco G, Frías O, 2001. Symbiotic feather mites synchronize dispersal and population growth with host sociality and migratory disposition. *Ecography* 24: 113-120.
- Blanco G, Tella JL, Potti J, 1997. Feather mites on group-living Red-billed Choughs: a non-parasitic interaction? *J Avian Biol* 28: 197-206.
- Blanco G, Seoane J, de la Puente J, 1999. Showiness, non-parasitic symbionts, and nutritional condition in a passerine bird. *Ann Zool Fennici* 36: 83-91.
- Blanco G, Tella JL, Potti J, 2001. Feather mites on birds: costs of parasitism or conditional outcomes? *J Avian Biol* 32: 271-274.
- Bridge ES, 2003. Densities and distributions of commensal feather mites (*Zachvatkinia caspica*) among the primaries of caspian terns. *Internat J Acarol* 29: 389-398.
- Casto SD, 1974. Entry and exit of syringophilid mites (Acarina: Syringophilidae) from the lumen of the quill. *Wilson Bull* 86: 272-278.
- Choe JC, Kim KC, 1989. Microhabitat selection and coexistence in feather mites (Acari: Analgoidea) on Alaskan seabirds. *Oecologia* 79: 10-14.
- Clayton DH, Moore J, 1997. eds. Host-parasite evolution: general principles and avian models. Oxford: Oxford University Press.
- Clayton DH, Drown DM, 2001. Critical evaluation of five methods for quantifying chewing lice (Insecta: Phthiraptera). *J Parasitol* 87: 1291-1300.
- Clayton DH, Lee PLM, Tompkins DM, Brodie ED, 1999. Reciprocal natural selection on host-parasite phenotypes. *Am Nat* 154: 261-270.
- Cramp S, 1988. The birds of western Palearctic, vol. 5. Oxford: Oxford University Press.
- Dawkins R, Krebs JR 1979. Arms races between and within species. *Proc R Soc London B* 205: 489-511.
- Dowling DK, Richardson DS, Komdeur J 2001. No effects of a feather mite on body condition, survivorship, or grooming behavior in the Seychelles warbler, *Acrocephalus sechellensis*. *Behav Ecol Sociobiol* 50: 257-262.
- Dubinin VB, 1951. Feather mites (Analgesoidea) Part I. Introduction to their study. *Fauna SSSR Paukoobraznye* 6: 1-363.
- Figuerola J, 2000. Ecological correlates of feather mite prevalence in passerines. *J Avian Biol* 31: 489-494.

- Figuerola J, Domènech J, Senar JC, 2003. Plumage colour is related to ectosymbiont load during moult in the serin, *Serinus serinus*: an experimental study. *Anim Behav* 65: 551-557.
- Hart BL, 1997. Behavioural defence. In: Host-parasite evolution: general principles and avian models (Clayton DH, Moore J, eds). Oxford: Oxford University Press; 59-77.
- Jenni L, Winkler R, 1994. Moult and ageing of European passerines. London: Academic Press.
- Jovani R, Blanco G, 2000. Resemblance within flocks and individual differences in feather mite abundance on long-tailed tits, *Aegithalos caudatus* (L.). *Ecoscience* 7: 428-432.
- Jovani R, Serrano D, 2001. Feather mites (Acarina) avoid moulting wing feathers of passerine birds. *Anim Behav* 62: 723-727.
- Jovani R, Serrano D, 2004. Fine-tuned distribution of feather mites (Astigmata) on the wing of birds: the case of blackcaps *Sylvia atricapilla*. *J Avian Biol* 35: 16-20.
- Kethley J, 1971. Population regulation in quill mites (Acarina: Syringophilidae). *Ecology* 52: 1113-1118.
- Marshall AG, 1981. The ecology of ectoparasitic insects. London: Academic Press.
- Moyer BR, Gardiner DW, Clayton DH, 2002. Impact of feather molt on ectoparasites: looks can be deceiving. *Oecologia* 131: 203-210.
- Møller AP, 1997. Parasitism and the evolution of host life history. Host-parasite evolution. In: Host-parasite evolution: general principles and avian models (Clayton DH, Moore J, eds). Oxford: Oxford University Press; 105-127.
- Møller AP, deLope F, Saino N, 2004. Parasitism, immunity and arrival date in a migratory bird. *Ecology* 85: 206-219.
- Newton, 1966. The moult of bullfinch *Pyrrhula pyrrhula*. *Ibis* 108: 41-67.
- O'Connor BM, 1982. Evolutionary ecology of astigmatic mites. *Ann Rev Entomol* 27:385-409.
- Proctor HC, 2003. Feather mites (Acari: Astigmata): ecology, behavior, and evolution. *Annu Rev Entomol* 48: 185-209.
- Proctor H, Owens I, 2000. Mites and birds: diversity, parasitism and coevolution. *Trends Ecol Evol* 15: 358-364.
- Rayner JMV, 1988. Form and function in avian flight. *Curr Orn* 5: 1-66.
- Rayner JMV, Swaddle JP, 2000. Aerodynamics and behaviour of moult and take-off in birds. In: Biomechanics and Animal Behaviour (Domenici P, ed). London: Bios Publishers. Seminar series of the Society for Experimental Biology; 125-157.

- Rózsa L, 1997. Wing feather mite (Acari: Proctophyllodidae) abundance correlates with body mass of passerine hosts: a comparative study. *Can J Zool* 75: 1535-1539.
- Wiles PR, Cameron J, Behnke JM, Hartley IR, Gilbert FS, McGregor PK, 2000. Season and ambient air temperature influence the distribution of mites (*Proctophyllodes stylifer*) across the wings of blue tits (*Parus caeruleus*). *Can J Zool* 78: 1397-1407.

Table 1. Wilcoxon matched-pairs signed-rank test of the consistency among non-molting birds in the difference in the number of feather mites between neighboring primaries (n = 151). Primary 9 = outermost primary.

Primary	Neighbor proximal feather of the target primary	
	Z	P
9	-10.41	< .0001
8	8.96	< .0001
7	6.31	< .0001
6	6.14	< .0001
5	5.60	< .0001
4	6.89	< .0001
3	7.48	< .0001
2	7.06	< .0001
1	-	-

Table 2. Results of repeated-measure ANOVA on the change in experimental groups of the number of feather mites on the 7th, 8th and 9th primaries and on flight feathers (wing and tail feathers).

Source	Df	SS	F	P
Primary 7				
Treatment	2	134.4	1.28	0.29
Subject within groups	62	6531.4
Repeated measure	1	1024.4	51.13	<0.0001
Treatment x Repeated measure	2	20.2	1.00	0.37
Repeated measure x Subjects within groups	62	1242.3
Primary 8				
Treatment	2	901.0	0.66	0.52
Subject within groups	62	42601.4
Repeated measure	1	1552.1	23.23	<0.0001
Treatment x Repeated measure	2	653.0	4.89	0.01
Repeated measure x Subjects within groups	62	4142.4
Primary 9				
Treatment	2	152.8	1.03	0.36
Subject within groups	62	4604.1
Repeated measure	1	63.2	2.23	0.14
Treatment x Repeated measure	2	29.2	0.51	0.60
Repeated measure x Subjects within groups	62	1758.3
Total number of feather mites				
Treatment	2	31042.1	1.48	0.24
Subject within groups	62	652398.8
Repeated measure	1	41278.2	45.09	<0.0001
Treatment x Repeated measure	2	6709.4	3.67	0.03
Repeated measure x Subjects within groups	62	56756.1

Figure 1. The distribution of feather mites on primary feathers of the non-molting barn swallows in Hungary (median + lower quartile range). Cross indicates the primary which was experimentally incised or pulled out (n = 151).

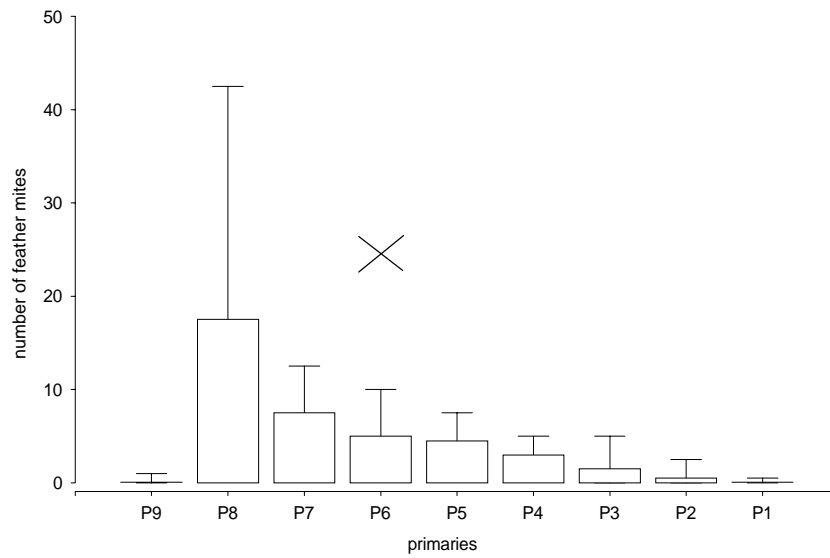
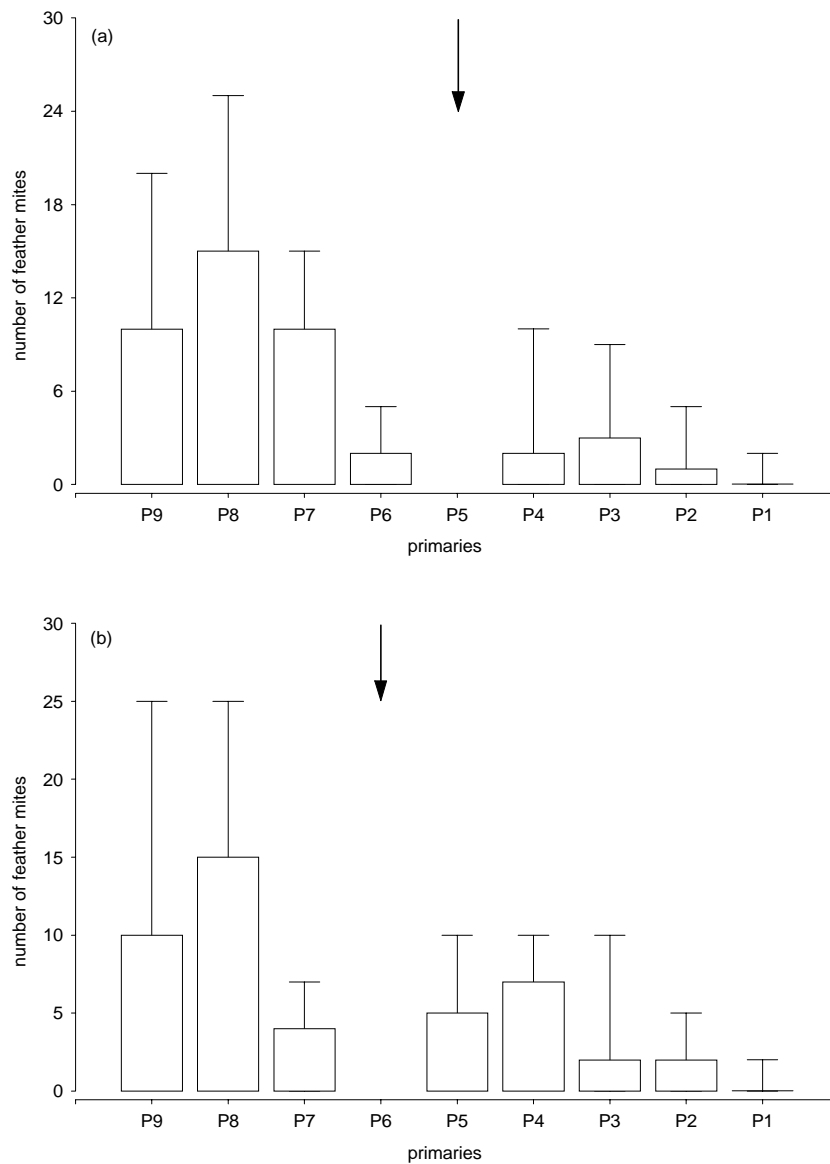


Figure 2. The distribution of feather mites on primary feathers in relation to molting of the 5th, 6th, 7th and 8th primaries (a, b, c and d in increasing order) of the barn swallows in South Africa (median + lower quartile range). Arrows indicate the gap of the dropped feather (sample size are 21, 43, 27 and 20, respectively).



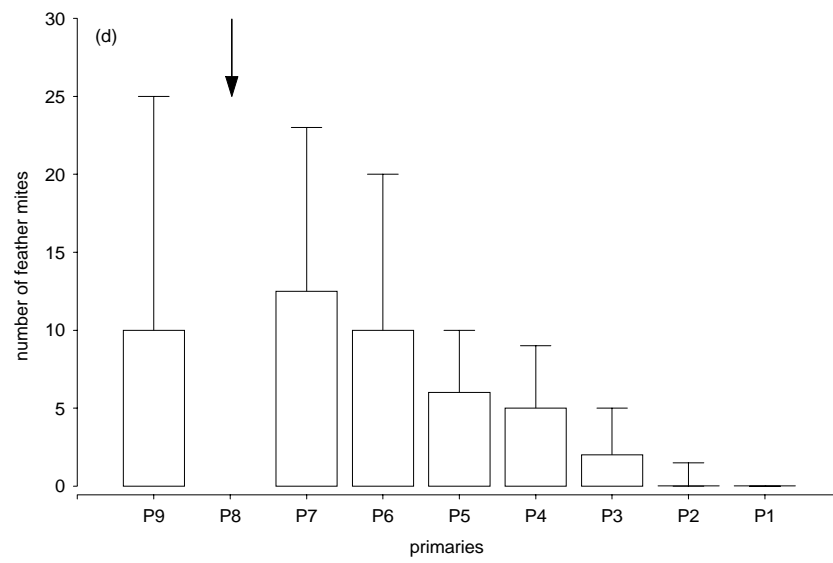
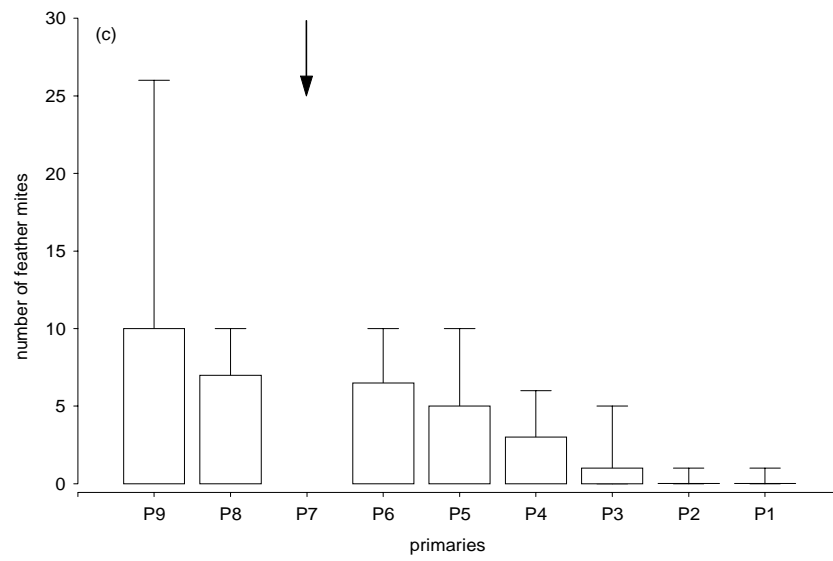
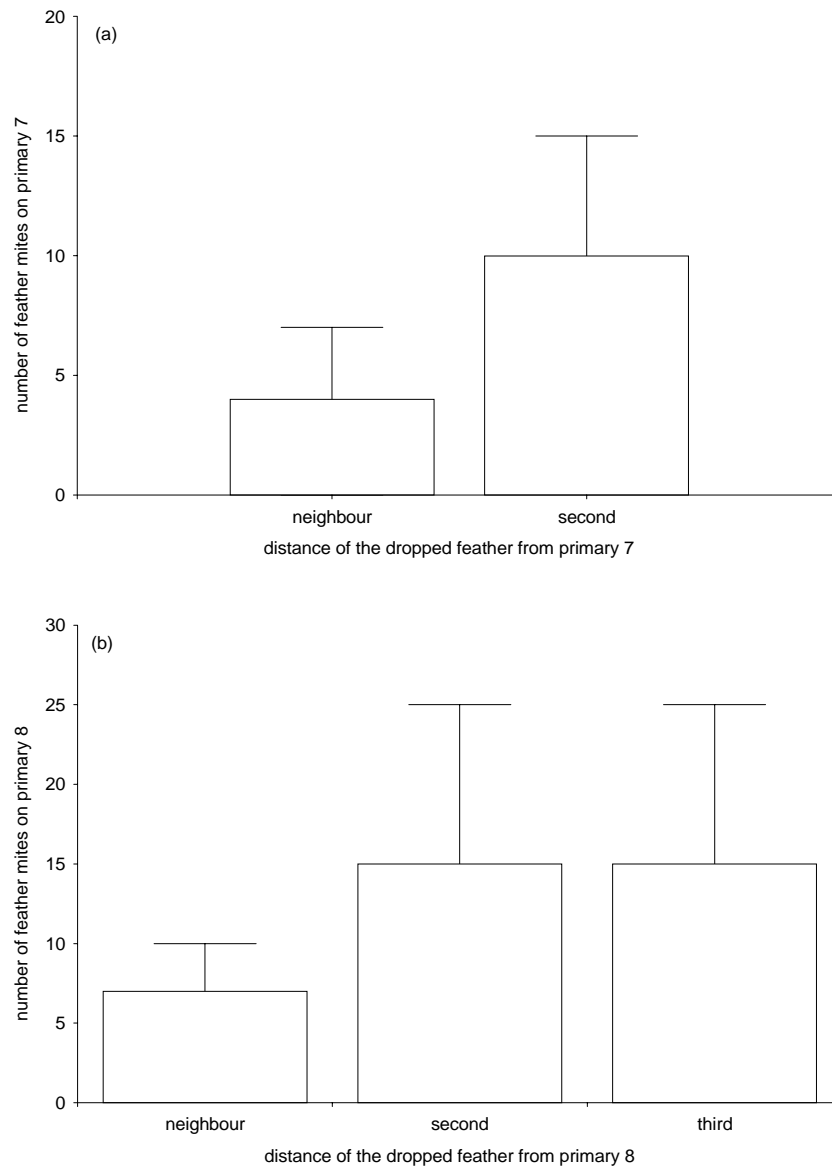


Figure 3. Number of feather mites on the 7th, 8th and 9th primaries (a, b and c) in the case where the dropped feather is a neighbor, the second one and the third one from the target primary (median + lower quartile range).



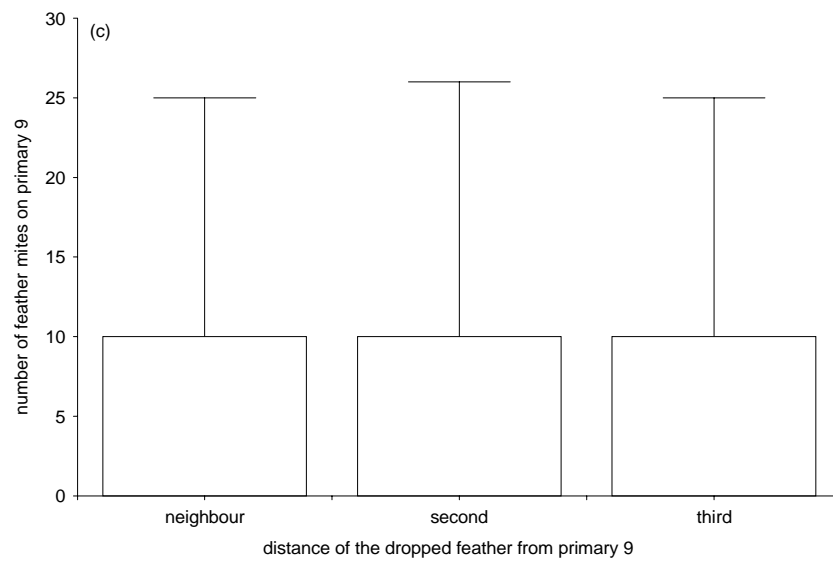


Figure 4. Number of feather mites on the outermost primary in relation to the developing stage of the neighbor proximal feather (mean + SE).

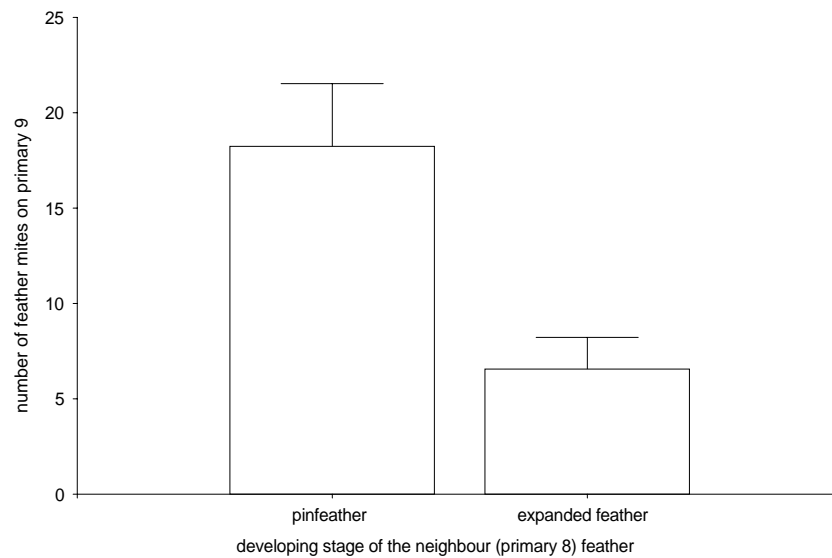
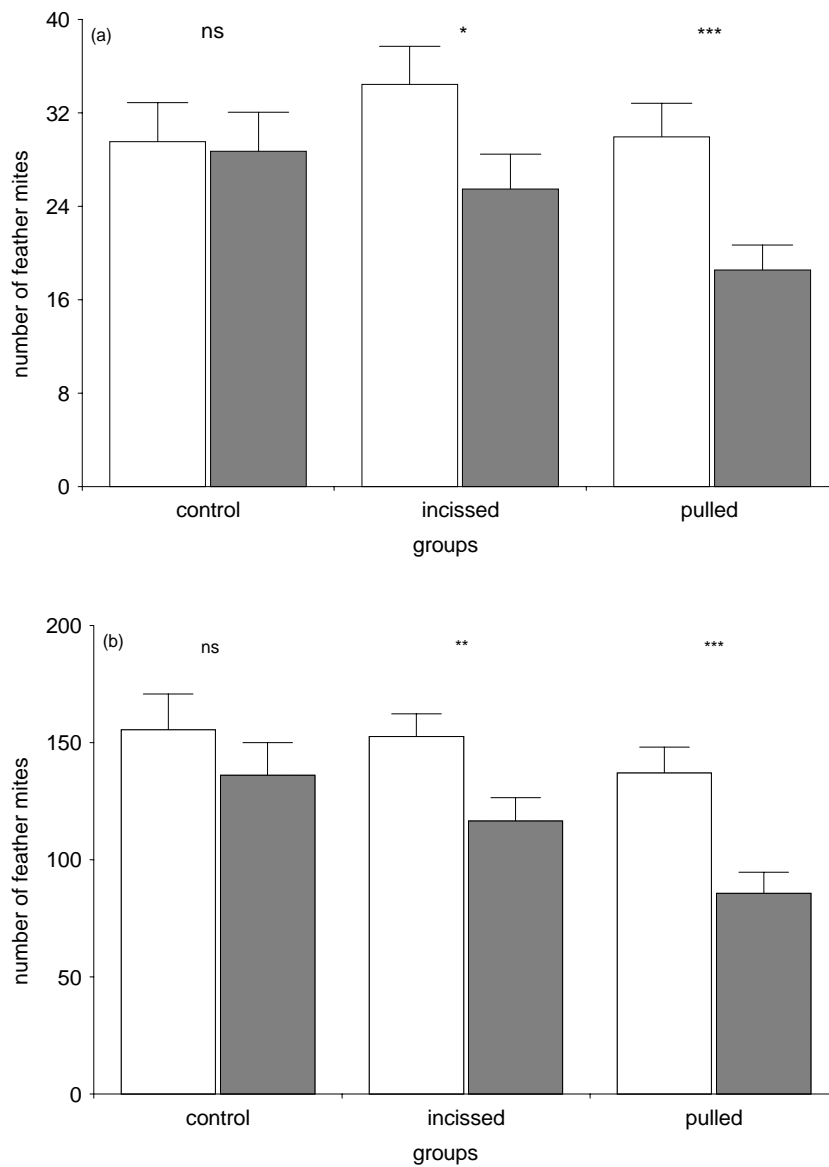


Figure 5. Change in the number of feather mites on the primary 8 (a) and on wing and tail feathers (b) between the first (white) and second (grey) measurements in the three experimental groups. The decrease of feather mites was significant for the incised and pulled groups. (For the statistical tests see text. * $p < .05$; ** $p < .01$; *** $p < .001$) (mean + SE)



ÖSSZEFOGLALÓ

Az életmenet vizsgálatokból tudjuk, hogy az egyed rendelkezésére álló források korlátozottak, így egy életmenet jellegbe fektetett többlet energia az ezzel csereviszonyban álló másik tulajdonság készletét csökkenti. A szaporodás és az élősködőkkel szembeni védekezés csereviszonyban, mint mediáló tényezőnek a különböző életkomponensek között a madár fiziológiai állapotának egy jelentős szerepe lehet. Az eredmények azt mutatják, hogy az erőteljes reprodukciós erőfeszítés a madarak magas fertőzöttségével jár, amely az ennek következtében legyengült immunrendszerrel áll kapcsolatban. Mivel a gazda egészségi állapota és immunkompetenciája közvetlenül meghatározza az egyed parazitákkal szembeni védekezését, és mivel a hatékony immunrendszer fenntartása költséges, ezért a fiziológiai védekezés vizsgálata és szerepe a cserekapcsolatokban, valamint az életmenet jellegek evolúciójában és kialakulásában kiemelkedő fontossággal bír. Ezen védekezési mechanizmusok evolúciójának egyik igen lényeges eleme, hogy e tulajdonságok örökölhetők legyenek. A gazda-parazita kapcsolat az utóbbi évtizedben különös figyelemben részesült a viselkedésszkológusok és az evolúcióbiológusok körében, hiszen a kérdéskörnek úgy gyakorlati, mint elméleti jelentősége igen nagy. A gazda-parazita vizsgálatokban egyrészt a gazdát, másrészt az élősködő életmódot folytató szervezetet figyelve tanulmányozhatjuk az együttélésből adódó alkalmazkodási jellegeket, valamint az együttélés során megjelent tulajdonságok evolúcióját. Az élősködő életmód feltételezi, hogy a parazita szervezet a gazdától fontos energiaforrásokat von el, ezáltal csökkentve az utóbbi rátermettségét. Ezen kényszerű együttélés hátrányából adódóan, evolúciós léptékben mérve várható, hogy a gazdaszervezet különböző védekezési mechanizmusokat fejleszt ki. Ez jelentheti például a madarak időszakos vedlését, melynek során a külső élősködőket "elhullatja". Elméleti szempontból igen jelentős azon parazita szervezetek életmódjának a vizsgálata, amelyek egyrészt teljes mértékben függenek a gazdától, másrészt fajspecifikusak, vagyis egyetlen gazdafajon vagy egy szűk csoporton élősködnek. A parazitizmus evolúciós következményeinek a vizsgálatában különös szerephez jutnak a madárfajokon végzett kísérletek. A tolltetvek és a tollatkák az egyik legfajgazdagabb szimbionta csoport, képviselőik nagyon elterjedtek a madarak körében. Gyakoriságuk ellenére ezen élősködő csoportokról igen keveset tudunk, vagyis a gazda életében betöltött szerepükről és arról, hogy miként befolyásolják a gazda állapotát, és ezáltal milyen szerepet játszanak például a párválasztásban és a szaporodási sikerben. Vizsgálatomban a fenti problémák vizsgálatára a füstli fecske (*Hirundo rustica*) igen gyakori tollatkáit választottam, valamint egy közvetett becslés alapján a tolltetvek szerepét vizsgáltam a madarak életmenetében.

Vizsgálatom első részében a madarak egészségi állapotának és immunkompetenciájának a költési idő meghatározásában játszott szerepét követtem nyomon. Ebben a dolgozatban kimutattam, hogy mind a hím, mind pedig a tojó füsti fecske esetében a belső, fiziológiai változók szoros összefüggést mutatnak a költési idővel. A költőterületre korán érkező, és ennek következtében korán költő madarak fiziológiai állapota jobb volt később érkező társaikhoz viszonyítva, amely leginkább a perifériás vérben keringő fehérvérsejtek alacsonyabb számában mutatkozott meg. Továbbá, egy fészekaljmanipulációs kísérletben a szaporodási erőfeszítés és a fiziológiai kondíció közötti cserekapcsolatot követtem nyomon, melyben az immunrendszer egyes elemeinek (pl. alakos elemek, T-sejtes immunitás) mennyiségét és egy antigénre adott válaszát vizsgáltam a tojó füsti fecskék különböző szaporodási erőfeszítése esetén. Kísérletemben kimutattam, hogy a megnövelt fészekaljméret nemcsak a tojó számára, hanem a fiókákra nézve is költséges, ugyanis mindkét esetben a T-sejtes immunitás a csökkentett fészekalj esetében nagyobb volt a növelt fészekaljhoz viszonyítva. Ezzel szemben nem találtam változást a manipuláció során a vele született immunitást jelző alakos elemek számában, valamint a madarak stressz állapotát jelző heterofil/limfocita arányban. Ez alátámasztani látszik azt a feltevést, miszerint a füsti fecskék csökkent immunkompetenciáját nem az emelkedett stressz okozza, hanem valószínűleg a megnövekedett aktivitás okozta energiahány (a felnőtt madarak esetében), illetve a fiókák esetében az elégtelen táplálékellátottság. Eredményeim továbbá alátámasztják azt a feltevést, miszerint enyhe stressz esetén a madarak először az energiaigényes szerzett immunitásukat csökkentik, majd ezt követően, hosszabb ideig tartó és nagyobb stressz esetén csökken a vele született immunitás is.

Tekintve a madarak morfológiai és fiziológiai jellegeinek szerepét a fitnessz meghatározásában, egy részleges fiókakicseréléses és egyben fészekaljmanipulációs kísérletet terveztem, melyben az egyes jellegek örökölhetőségét vizsgáltam. Vizsgálatomban kimutattam, hogy a füsti fecskék morfológiai és fiziológiai változóinak örökölhetősége szignifikáns de alacsony, és a fiziológiai tulajdonságok általában kisebb heritabilitással rendelkeznek, mint a morfológiai tulajdonságok. Az alacsony heritabilitás részben a magas környezeti varianciának, részben pedig az alacsony genetikai varianciának tulajdonítható. Eredményeim alátámasztani látszanak azt a feltevést, miszerint az erős szelekció alatt levő jellegek alacsonyabb heritabilitással rendelkeznek a szelekciós nyomásnak kevésbé kitett tulajdonságokhoz viszonyítva.

A füsti fecske evező- és faroktollain levő tolllyukak eredete még nem tisztázott. Éppen ezért, munkámban további adatokat kerestem a tolllyukak eredetére vonatkozóan. A tolllyukak gyakorisága és eloszlása megegyezik a füsti fecske tolltetveinek gyakoriságával és eloszlásával. Továbbá, a költő madarakon végzett öt éves korrelatív vizsgálatomban a tolllyukak száma és a

madár rátermettségét jelző változók, mint pl. a költéskezdés ideje, a fészekaljméret, a túlélés és a különböző morfológiai jellegek, között kerestem összefüggést. Vizsgálatomban kimutattam, hogy a tolllyukaknak szignifikáns hatása van a madarak költéskezdésére, vagyis szoros, negatív összefüggést találtam mindkét nem esetében a tolllyukszám és a költési idő között. A tolllyukak jelentős fitnessz-csökkentő szerepe a tojó füsti fecskék túlélésében is megmutatkozott, ugyanis az elpusztult madarak szignifikánsabb több tolllyukkal rendelkeztek túlélő társaikhoz viszonyítva. Ellenkező esetben, vagyis ha a tolllyuk kondíciójelző, akkor a magas tolllyuk számmal rendelkező egyedek feltehetően rossz állapotúak. Ezen gyenge minőségű madarak érzékenyebbek a környezeti tényezőkkel szemben, amely csökkent túlélésükben és kései költéskezdésükben nyilvánul meg. Elfogadva azt a feltevést, miszerint a tolllyukak okozói a tolltetvek, korrelatív vizsgálatom alapján feltételezhető, hogy az eddig csekély hatással rendelkező parazitáknak hitt tolltetveknek jelentős szerepük van a füsti fecskék szaporodási sikerében és túlélésében.

A gazda-szimbionta kapcsolatban egyrészt a tollatkák hatását vizsgáltam a madarak fitnesszére, másrészt a tollatkák vedlő tollakat elkerülő viselkedését követtem nyomon. Az eddigi szórványos ismeretek alapján mindhárom típusú szimbionta életmód feltételezhető volt, vagyis a kommenzalista, a mutualista és a gyenge virulenciával rendelkező parazita életforma. A költés után levő és a költő füsti fecskéken végzett korrelatív vizsgálatomban nem találtam összefüggést a madarak kondíciója és a tollatka fertőzöttség között. A tollatkák kommenzalista jellegét egy manipulációs kísérlet is alátámasztja. Eredményem fontos abban a tekintetben, hogy a kísérletes vizsgálatok hiányából adódóan fennálló ellentmondások között rámutat a tollatkák kommenzalista jellegére.

A tollatkák vedlő tollakat elkerülő viselkedéséről szintén szórványos megfigyelések állnak rendelkezésünkre. A kérdés jelentősége pedig igen nagy, ugyanis egészen a közelmúltig a madarak vedlésére úgy tekintettek, mint egy, a külső élősködőkkel szembeni hatékony védekezési adaptációra. E hiányt pótolandó, arra kerestem a választ, hogy képesek-e a tollatkák elkerülni a kihulló tollat, és ha igen, milyen inger, szignál képezi e felismerési képesség alapját. A vedlő füsti fecskéken végzett megfigyelésekből kitűnik, hogy a tollatkák képesek a soron következő vedlő toll elkerülésére, a felismerésben pedig – a kísérlet szerint – mind a rezgő toll, mind pedig a kihullott toll által létrehozott résen át keltett légáramlat egyaránt ingerként szolgál. Vizsgálatomban ezáltal bizonyítottam, hogy eltérően az eddigi elgondolástól, miszerint a madarak vedlése hatékony a külső élősködőkkel szembeni védekezésben, a külső szimbionták fejlett érzékelő képessége lehetővé teszi a gazdaszervezeten való állandó fennmaradást.

SUMMARY

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. 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. The results show that an increased investment in reproduction results in an elevated risk of infestation, which is related to a suppressed immune system. 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. 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. 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 and in theory. 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. As a consequence, a quick evolutionary response is expected from the host, leading to a decrease in the number of parasites.. 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. From the theoretical point of view, it is important to study the life history of those parasites that depend completely on the host and are species-specific, namely they parasitize only one or a few host species. In the study of host-parasite relationships, a special interest is devoted to birds. Lice and feather mites are one of the most diverse arthropod groups living in symbiotic relationship with birds. 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.

In the first part of my 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. In this study 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, 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. In this 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. 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. 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.

Considering the role of morphological and physiological traits in determining the fitness of the birds, in the following I studied the heritability of different traits of the nestling barn swallows in a partial brood-size manipulation 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 realize a lower heritability than traits exposed to less intense selection.

The origin of feather holes, which are on the wing and tail feathers of the barn swallow is still unclear. In order to elucidate this problem, I provide additional data related to the origin of feather holes. The abundance and distribution of feather holes among birds comply with the data for lice of this species. 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. 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, these parasites have an important role in determining the breeding success and survival of the birds. Alternatively, if feather holes are an indicator of quality, those birds with a high intensity of feather holes may have been of poor quality. These birds may have been less able to cope with the environmental conditions, resulting in the lower survival and later arrival of the birds to the breeding grounds.

In the 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, various symbiotic life forms could be assumed, namely the commensal, mutual and parasitic nature of mites. In this 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 an experiment, in which similarly I did not find any effect of these symbiontes on the fitness of the birds. 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.

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. 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. 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 I showed that mites have the capacity to sense the vibrating feather and 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.

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

A füsti fecske *Hirundo rustica* immunkompetenciájának, egészségi állapotának és parazitáltságának hatása rátermettségére

Értekezés a doktori (Ph.D.) fokozat megszerzése érdekében
a biológia tudományágban

Írta: Pap Péter László okleveles biológus

Készült a Debreceni Egyetem Biológia Doktori Iskolája
(Biodiverzitás programja) keretében

Témavezetők: Dr. Szép Tibor, Prof. Dr. Varga Zoltán

A doktori szigorlati bizottság:

elnök:	Dr.
tagok:	Dr.
	Dr.

A doktori szigorlat időpontja: 200... ..

Az értekezés bírálói:

Dr.
Dr.
Dr.

A bírálóbizottság:

elnök:	Dr.
tagok:	Dr.
	Dr.
	Dr.
	Dr.

Az értekezés védésének időpontja: 200.....