

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.

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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,

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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 Hørak 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 day-time 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 ± 5.08 versus 12.96 ± 8.45 , $t = -2.22$, $df = 53$, $p = 0.03$; lymphocytes: 17.72 ± 8.03 versus 24.74 ± 12.43 , $t = -2.46$, $df = 53$, $p = 0.02$; WBC: 28.30 ± 13.17 versus 39.23 ± 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 ± 15.65 versus 12.14 ± 7.74 , $t = 2.54$, $df = 40$, $p = 0.02$; lymphocytes: 22.57 ± 9.15 versus 35.32 ± 20.45 , $t = 2.98$, $df = 40$, $p < 0.01$; WBC: 56.96 ± 35.03 versus 34.78 ± 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

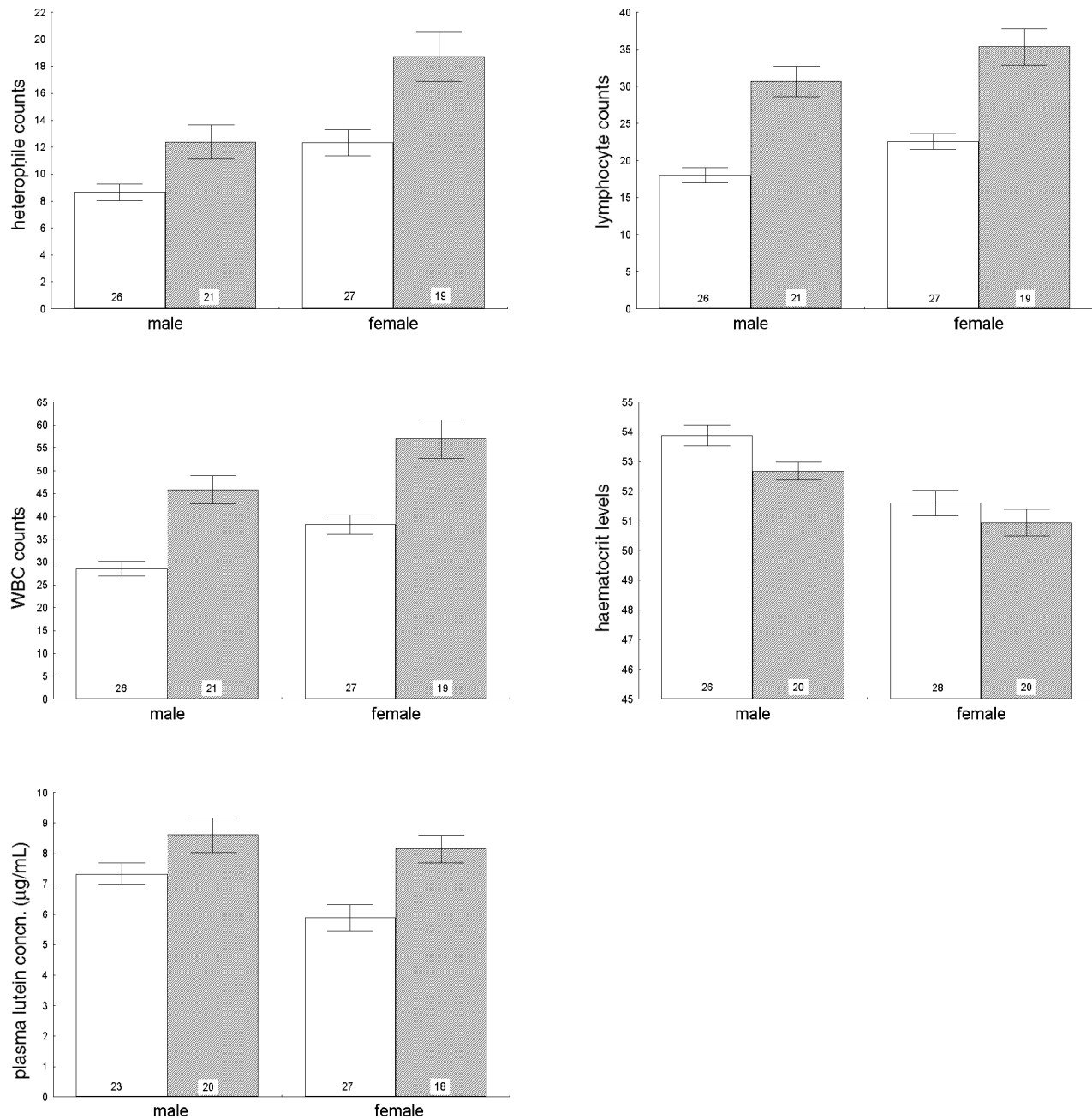
Haematocrit levels did not differ between early- and late-breeding males or females for the prelaying and nestling periods (Fig. 1, Table 2). Haematocrit was significantly higher for males than for females during both sampling periods. However, after introducing tail length as a covariate in an ANCOVA, 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 ± 2.81 versus 49.80 ± 2.61 , $t = 5.77$, $df = 52$, $p < 0.0001$) and late-breeding males (first versus second sampling: 52.85 ± 2.39 versus 49.57 ± 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 ± 4.65 versus 15.84 ± 2.83 , $t = -4.61$, $df = 31$, $p < 0.0001$) than for early breeders (7.33 ± 2.77 versus 10.82 ± 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 ± 3.72 versus 11.23 ± 3.51 , $t = -5.52$, $df = 52$, $p < 0.0001$; late breeders: 8.15 ± 3.84 versus 12.96 ± 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 immunomodulator 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.

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