Research article

Reproduction and migration in relation to senescence in the barn swallow *Hirundo rustica*: A study of avian ‘centenarians’

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Abstract

A. P. Møller, F. de Lope and N. Saino: Reproduction and migration in relation to senescence in the barn swallow *Hirundo rustica*: A study of avian ‘centenarians’. Senescence reflects the decrease in age-dependent residual reproductive value, and a previous study of a cohort of migratory barn swallows (*Hirundo rustica*) that had reached advanced ages for this species (at least five years old) provided evidence consistent with senescence such as reduced reproductive performance and increased abundance of parasites. We studied a population of migratory barn swallows over a number of years. Several questions were asked including 1) how do longer lived birds compare to shorter lived individuals of the same species, 2) is there a difference in individuals of the same species that live in different geographical regions, and 3) how do patterns of reproduction change during aging? We compared the phenotypes of 87 individuals from three populations in Denmark, Italy and Spain that had reached at least five years old with those of control individuals matched for sex, arrival date and breeding site. These controls only became one year old, i.e., they were not found thereafter and both groups of individuals were compared in their first year of life. Results showed that there were no significant differences in any of 11 morphological characters or in the abundance of three ectoparasites between the two groups of birds. Females that eventually became very old had relatively smaller first clutches as one year olds than did one year old controls, while males that eventually became very old had mates that laid clutches that were relatively larger than those of controls. Annual fecundity showed a similar pattern, with an increased sex difference in fecundity between birds from Spain than in Italy and in Denmark. Danish barn swallows that achieved older ages had relatively lower haematocrits than control individuals of the same sex, age and arrival date captured at the same site, while the difference in haematocrits between these two categories of individuals was small for Italian birds. Spring arrival date was relatively earlier in Danish males that eventually became very old compared to control males of the same age and tail length (a correlate of fitness) that arrived at the same site, while the two age categories of females did not differ in arrival date. In the Spanish population there was no difference in arrival date between the birds that aged most successfully and controls for either sex. We will discuss these findings in relation to current senescence theories.

Introduction

Senescence reflects a decrease in age-dependent residual reproductive value (Fisher 1930), usually

giving rise to a deteriorating phenotype and an increase in mortality with increasing age. Deterioration of the phenotype with increasing age is assumed to be a sign of senescence, and several different evo-

lutionary theories of senescence have been proposed (Rose 1991; Partridge and Barton 1993; Kirkwood and Austad 2000; Finch and Kirkwood 2000). First, deleterious mutations have been proposed to accumulate during the lifespan of an individual and cause an increase in age-dependent mortality (Medawar 1953), particularly among long-lived species. Second, antagonistically pleiotropic mutations may have beneficial effects early in life, but are costly later on, and would not be selected against because late-acting mortality would only to a small extent affect the fitness of most individuals (Fisher 1930; Rose 1991; Williams 1957; Hamilton 1966; Charlesworth 1994). Age-specific sensitivity of fitness to changes in reproduction or survival has been associated with age-specific selection (Hamilton 1966). Such pleiotropic genes with beneficial effects early in life and later detrimental effects should generally be associated with deteriorating performance with increasing age, and this process could be accelerated by the costs of early reproduction or other expensive activities. Third, deleterious mutations expressed only late in life are able to accumulate because of their negligible effects on overall fitness, reflecting the low probability of survival until the age at which they are expressed (Rose 1991; Partridge and Barton 1993). Fourth, antioxidants play important roles as quenchers of free radicals, thereby preventing damage to DNA and other molecules (Bendich 1989; Chew 1993; Edge et al. 1997; Møller et al. 2000). Since antioxidants also have additional functions, and since they can be of dietary origin, individuals may trade their availability or use for free-radical scavenging against other functions. The antioxidant theory of senescence suggests that individuals with large amounts of antioxidants, either due to greater availability or to infrequent use for functions other than free radical scavenging, may enjoy an extended lifespan (Finkel and Holbrook 2000; Melov et al. 2000). Fifth, the 'disposable soma' hypothesis suggests that individuals able to allocate limiting resources for competing activities according to evolutionary strategies that maximize lifetime fitness would be able to survive longer than others. Thus, there is a trade-off between adaptive life-history traits and metabolic efficiency of the individual (Roff 1992; Stearns 1992; Shanley and Kirkwood 2000).

Recently, several studies demonstrating senescence in free-living organisms through measurements of age-dependent mortality, reproduction and repro-

ductive value have been published [e.g., Gustafsson and Pärt (1990); Perdeck and Cavé (1992); Newton and Rothery (1997); Bérubé et al. (1999); Møller and de Lope (1999); Espie et al. (2000); Sanz and Moreno (2000); Pyle et al. (2001); Robertson and Rendell (2001); Cam et al. (2002); Laaksonen et al. (2002); Orell and Belda (2002); Reid et al. (2003)]. Several of these studies have investigated patterns of age-specific fecundity and mortality, showing how reproductive performance and survival late in life are related to early performance. For example, Gustafsson and Pärt (1990) in a now classical study of the collared flycatcher, *Ficedula albicollis*, showed how an experimental increase in clutch size during early life accelerated senescence later on. However, such studies do not provide information on the phenotypic traits associated with early aging or the potential mechanisms that cause senescence. Individuals may die of old age due to an inability to repair damaged strands of DNA, a deterioration of the immune system – and hence an increase in the frequency of parasitism and disease – and the negative effects of mutations at late life stages (Kirkwood and Austad 2000). Birds have very high survival rates for a given body size compared to mammals, but paradoxically also very high metabolic rates (Williams 1957; Comfort 1979; Nesse 1988; Promislow 1991). High metabolic rates should result in high rates of DNA damage. Even among the numerous studies of senescence in free-living birds, we are unaware of a single study that has contrasted the phenotypic characteristics of young individuals that later reached very old ages with those of matched young individuals that never reached old age.

While retrospective comparisons of the phenotypes of extremely old individuals with matched controls that fail to reach advanced ages is a standard technique in gerontology (Miller 1966), such comparisons have to the best of our knowledge never been done on animal populations in nature. Comparative studies of human centenarians and control individuals have revealed that various aspects of immune function including cytokines, T lymphocytes, MCH genes and susceptibility of erythrocytes to oxidation differ between groups (Argentati et al. 2002; Bruunsgaard et al. 2002; Lio et al. 2002; Rabini et al. 2002). Here we adopt this technique for the first time in studies of free-living animals, attempting to disentangle the phenotypic traits that

differ between individuals that succeed in becoming very old from those of controls matched with respect to sex, age, site of reproduction and tail length when both categories of individuals were in their first year of life. By studying individuals before and during their first reproductive event we were able to avoid the confounding effects of costs of reproduction that may otherwise have affected the conclusions if we had compared individuals after reproduction. This procedure was adopted in an attempt to pinpoint potential variables that may ultimately predict the ability to reach very old age and hence potentially indicate the mechanism(s) responsible for senescence.

The barn swallow, *Hirundo rustica*, is a ca. 20-g migratory, insectivorous bird that breeds in large parts of temperate and subtropical zones of the northern hemisphere. Males and females have similar morphology with the exception of the outermost tail feathers, which are longer in males due to a female preference for mates with longer tails review in Møller (1994a). Males with long tails live longer than males with short tails, they have higher annual reproductive success than short-tailed males, and therefore their lifetime reproductive success is higher than that of short-tailed males (Møller 1994a). The mating system is social monogamy with frequent extra-pair paternity, mainly by females mated to short-tailed males (Saino et al. 1997). Migration, reproduction, and ability to resist parasites are all associated with long tails in male barn swallows (Møller 1994a). Previous studies of senescence in the barn swallow were based on a cohort of individuals that eventually attained at least five years of age. The length of wings and tails, that reflect the ability to fly, but also sexual attractiveness in males (Møller 1994a), reached a maximum at intermediate age with the same individuals at very young and old ages having short wings and tails (Møller and de Lope 1999). Two measures of developmental instability of feather characters have been shown to reflect a reduced ability to control growth processes when young and very old (Møller and de Lope 1999). Spring arrival date from the African winter quarters was the earliest at intermediate ages, and reproductive performance also peaked at these ages (Møller and de Lope 1999). Parasitism by two different ectoparasites peaked at young and very old ages (Møller and de Lope 1999). A subsequent study of senescence showed that offspring body size, rate of feather development and T cell-mediated immunity

had already declined with paternal and/or maternal age after the first breeding season (Møller 1994a). Therefore, barn swallows suffered an age-dependent decline in offspring quality, and apparently traded quality of current offspring against that of offspring in subsequent reproductive events (Møller 1994a).

The aims of the present study were to make a comparison of the phenotype of individual barn swallows that eventually became at least five years old (very old for a passerine bird with an annual survival rate of 34.3% (Møller and Szép 2002), since only 1.4% of all individuals will reach that age) and control individuals that were matched with respect to sex, age, site of reproduction and tail length shown in previous studies to correlate with fitness. We chose five years because choosing a later cutoff age would have given us a sample of older birds that was too small and more likely to include individuals that had survived by sheer chance alone. This threshold also allows for comparison with a previous study of senescence in the barn swallow that showed clear evidence of senescence for reproduction, parasite abundance, migratory performance and developmental performance during annual molt among individuals that reached an age of five years (Møller and de Lope 1999). The first specific aim was to compare an array of 11 phenotypic variables (including morphology, parasite load, reproductive parameters) of old individuals when one year old to those of control individuals of the same age and sex arriving on the same day at the same site. This matched comparison automatically controlled for a large number of factors that are associated with certain other phenotypic differences. If very old age is caused by a particular mechanism, this should therefore be associated with a particular phenotype differing from that of matched controls, even when individuals are in their first year of life.

The second aim was to compare the arrival date at the breeding site at one year of age of individuals that eventually became very old with that of individuals of the same sex, age and tail length arriving at the same site. This analysis was performed to explore the possibility of phenotypic bias in the first analysis because individuals were matched with respect to arrival date. Migration is condition-dependent in the barn swallow, with individuals in prime condition arriving earlier than others, particular under adverse environmental conditions (Møller 1994b). Thus, we expected individu-

als in prime condition with a higher than average probability of survival to arrive earlier than controls. We included tail length as a control variable because long-tailed males have been shown to arrive earlier than short-tailed males due to their superior body condition (Møller 1994b). In addition, our earlier work has shown that arrival date of male barn swallows is correlated with circulating levels of antioxidants (Ninni et al. 2004). This suggests that antioxidant levels may be correlates of both migratory success and senescence: they may play a crucial role in neutralizing free radicals produced during long-distance migration (Ninni et al. 2004), and have also been shown to play a role in senescence (Finkel and Holbrook 2000; Melov et al. 2000). Hence, use of antioxidants for migration may be traded against use of antioxidants for preventing premature aging.

Materials and methods

Study sites

We studied barn swallows at Badajoz (38 °50' N, 6 ° 59' W), Southern Spain (since 1976), Milano (45 ° 28' N, 9 ° 10' E), Northern Italy (since 1993), and Kraghede (57 ° 12' N, 10 ° 00' E), Denmark (since 1970), as part of a long-term project. The study site at Badajoz consists of open farmland with pastures, cereals and fruit plantations, and most barn swallows breed in barns. The study site at Milano consists of open farmland with pastures, cereals and hedges. Barn swallows are mainly restricted to dairy farms, where they breed inside buildings. The study site at Kraghede consists of open farmland with pastures, cereals, potatoes and rape with mixed plantations, hedges and ponds. Barn swallows breed within barns and other buildings (see Møller 1994a).

General field procedures

Barn swallows were captured regularly from their arrival at breeding sites in early spring until the end of the breeding season using mist nets at all entrances to barns containing breeding birds. Mark-recapture analyses of the data have revealed that capture probability of adults exceeds 97% in Spain and Denmark (Møller and Szép 2002; F. de Lope, A. P. Møller and T. Szép, unpublished data). Capture probability in Milano was presumably equally high, based on the

absence of unbanded birds (N. Saino, unpublished data). Birds were assigned to nests by identifying color bands and color marks on the white breast feathers using binoculars.

This study is based on 87 individuals that reached at least five years old (32 from Spain, 33 from Italy and 22 from Denmark). With an annual survival rate of 34.3%, individuals that become very old only comprise 1.4% of all adult barn swallows. For this study we deliberately chose individuals that became very old and a sample of controls that were matched with respect to sex, age, site of reproduction, and arrival date because in this way we would be more likely to pinpoint specific differences in phenotype between the two categories of individuals. This procedure provided two advantages. First, we were able to analyze a sample of extremely old individuals that was highly unlikely to contain any birds that had managed to survive to such old ages by chance alone. Second, the paired design provided a very powerful statistical approach by deliberately controlling for a number of known confounding variables. In addition, it also *automatically* controlled for numerous additional potentially confounding variables that are likely to be similar in the two samples of individuals. For example, such individuals are likely to be of similar age and body condition and experience similar environmental conditions during migration and reproduction. Alternatively, we could have performed a multivariate analysis of all our data, while controlling statistically for known confounding variables. However, this approach would have been inferior to the one we adopted because (1) we would not be able to focus as precisely on the phenotypic characteristics of a sample of individuals that reached very old age, and because (2) we would be unable to control for a number of unidentified potentially confounding variables that are controlled automatically by adopting the paired design of analysis. The control sample of individuals only contained individuals that were never included in the analysis again after the first breeding season.

We assumed that the age of adults could be determined from the number of years present in the study population, given that unbanded birds are yearlings when first captured. This assumption is supported by the observation that less than 0.1% of the more than 10,000 adults ringed since 1970 ever moved to another farm in the same or subsequent years. Similarly, all but one of 400 local recruits

were captured in their first year of life. Thus, our assumption that unbanded birds can be reliably assigned an age of one year was justified. Likewise, old individuals that reached five years of age also had a high probability of capture, exceeding 97%, suggesting that the sample of individuals that became very old would be representative since only very few individuals would have remained alive without being captured.

Phenotypic characters

Upon capture all adults were measured for a number of phenotypic characters, recorded in the same, standardized way in all three populations. These were: length of the flattened left and right wings, the left and right outermost tail feathers, and the two central tail feathers, measured with a ruler to the nearest mm. Tail and wing asymmetry were subsequently calculated as the absolute differences in length between left and right characters. Tail and wing length were calculated as the mean of the left and the right character value. Length, width and depth of the bill, and length of tarsus and keel were measured with a digital caliper to the nearest 0.01 mm. Body mass was recorded with a Pesola spring balance to the nearest 0.1 g. The abundance of chewing lice of the species *Hirundoecus malleus*, as well as feather mites and louseflies, were recorded in a standardized way as described in Møller (1994a). Repeated measurements of the same individuals the same season revealed that all characters were highly repeatable, with all repeatabilities reaching values above 80% (Møller 1991, 1994a, A.P. Møller et al., unpublished data). Hence, the characters were measured in a highly consistent way.

Individuals with broken or damaged feathers were excluded from the analyses. The tips of the outermost tail feathers and the longest primaries of barn swallows are rounded, being composed of small barbs, and any broken barb leaves an irregular shape of the feather that is readily visible. The number of birds with damaged feathers was less than 3% in any given year, and is thus unlikely to have biased the analyses.

Upon first capture a blood sample was taken from the brachial vein by filling 1–2 heparinised capillary tubes. These samples were kept in a cooling box in the field and later centrifuged at 12,000 rpm for 10 min in a haematocrit centrifuge. The length of the blood column and the length of the fraction contain-

ing red blood cells were measured to the nearest 0.1 mm. Haematocrit was calculated as the length of the red blood cell column divided by the length of the entire blood column and multiplied by 100. A high value implies that a given individual has a blood profile indicative of a high level of activity, while a low value is indicative of anemia. The measure of haematocrit that we used is highly repeatable among capillary tubes taken from the same individual ($r > 0.98$ in tests made annually), and of the same individual at different stages of the breeding cycle (A. P. Møller et al., unpublished).

Arrival date was defined as the date of the first capture, although this provides an underestimate of the actual arrival date. The precision of this estimate was assessed from data collected from 1985 to 1990. Barn swallows spend the night within their small breeding territory, and males sit next to their partner if mated, or alone if unmated, during the first couple of hours in the morning and late in the evening (own observations). For six years adult barn swallows were watched daily for 1 h from sunrise until 1100 hours, and the identity of all color-banded individuals was recorded with binoculars. The first observation of the season of each individual provided the first estimate of arrival date, while mist net captures provided a second estimate. The Pearson correlation coefficient between these two estimates for each year separately varied from 0.982 to 0.997, $N = 10\text{--}46$, $P < 0.01$. A paired t -test revealed no significant differences between the two estimates for any year or sex ($t < 1.23$, $P > 0.22$). Thus estimates based on first capture provide reliable estimates of arrival date (see also Møller et al. 2004). The repeatability of arrival date of male barn swallows among years was statistically significant [$F = 6.12$, $df = 22, 97$, $P < 0.001$; $r = 0.51$; Møller (2001)], despite young males arriving later than males of intermediate age (Møller and de Lope 1999).

Reproductive variables were recorded by regular weekly nest checks, around the time of laying, hatching and fledging every day or every second day. This allowed us to estimate start of laying for first and second clutches under the assumption that one egg is laid per day [which is the case for more than 95% of all eggs (Møller 1994a)]. Size of first and second clutch was determined as the number of eggs present after laying had ceased, while brood size was estimated as the number of nestlings present on the last nest check when nests were inspected on day 13 days. Annual fecundity was the sum of brood

size in first and second clutches. Annual fecundity was unavailable for the sample from Italy because several broods were experimentally manipulated for other studies in one way or another.

Selection of individuals for statistical analyses

In the first series of analyses we selected all individuals that reached at least five years old and retrospectively matched each of these at one year old with an individual of the same age, sex, arrival date and breeding site that failed to reach five years of age or more. If more than one control individual was available, we chose an individual at random. These pairs of individuals were subsequently compared with respect to a set of phenotypic variables, including 11 morphological variables, ectoparasite loads and measures of reproductive performance.

In the second series of analyses we selected all individuals that reached at least five years old and matched these when one year old with an individual of the same age, sex and tail length from the same breeding site. We controlled for tail length in this analysis because long-tailed males are sexually more attractive and enjoy higher mating success and reproductive success than short-tailed males (Møller 1994a; see also Introduction). These pairs of individuals were subsequently compared with respect to arrival date to test if individuals that eventually became very old arrived earlier than the control individuals.

Statistical analyses

Differences in 11 morphological variables, ectoparasite loads and reproductive variables were calculated between individuals that eventually became very old and matched control individuals were used as units of analysis (Table 1). Heterogeneity in differences between individuals that became very old and controls was tested using two-way ANOVAs with sex and country (breeding location) as main effects.

Not all phenotypic traits were measured in all individuals (for example, a few individuals had broken feathers and haematocrit values were only available for Italian and Danish birds), and sample sizes thus differ among analyses.

If multiple statistical tests are performed on a large number of variables, the probability of achieving significance is inflated, since each test has a 5% probability significance by chance. We therefore

used the sequential Bonferroni correction of the tableside Type I error rate, adjusting the significance level downwards in relation to the number of tests made (Holm 1979; Wright 1992). Strict application of this method severely reduces statistical power, (Wright 1992), but this can be avoided by choosing an experiment-wise Type 1 error rate higher than the standard .05 level. Hence we used the .01 alpha level as suggested by Wright (1992) and Chandler (1995). Values reported are means (SE).

Results

Phenotypes of extremely old individuals and controls

We tested whether the difference in phenotype between individuals that subsequently reached very old ages and that of control individuals of the same age and sex arriving to the same site on the same date varied with respect to sex, country and sex by country interaction. None of the 11 morphological characters showed significant differences (Table 1). None of the three parasites differed in abundance between the two categories of barn swallows (Table 1). Among life history variables we found a significant effect of sex for size of the first clutch (Tables 1, 2). The effects of sex and sex-by-country interaction remained significant after sequential Bonferroni correction. Males consistently showed positive differences between individuals that became very old and control individuals, while females showed negative values (Figure 1a). Thus, males that eventually became very old had mates that laid more eggs in their first clutch than controls, while females that eventually became very old laid fewer eggs than controls.

Annual fecundity showed a pattern similar with respect to sex differences to that for size of the first clutch (Tables 1, 2). However, in this case there was also a significant sex-by-country interaction (Tables 1, 2), with the sex difference increasing from Spain to Italy to Denmark. The effects of sex and sex-by-country interaction remained significant after sequential Bonferroni correction. This implies that females that eventually became very old had increasingly smaller fecundities than controls from Spain to Italy to Denmark, while males that became very old had increasingly larger fecundities than controls over the same latitudinal gradient.

Table 1. Summary statistics for phenotypic variables of individuals that eventually reached at least five years of age and a matched sample of control individuals of the same age, sex and site of reproduction.

Variable	Spain		Italy		Denmark	
Individuals that subsequently became very old	Male	Female	Male	Female	Male	Female
Tail length	98.53 (1.50)	85.75 (0.78)	106.65 (1.96)	87.38 (0.91)	105.30 (3.26)	88.00 (1.07)
Wing length	120.41 (0.80)	120.50 (0.72)	123.35 (0.62)	122.38 (0.59)	125.90 (0.92)	123.88 (0.72)
Central tail length	42.31 (0.35)	43.69 (0.42)	44.59 (0.26)	45.25 (0.27)	43.30 (0.63)	44.17 (0.51)
Tail asymmetry	0.77 (0.14)	1.53 (0.39)	1.24 (0.65)	1.75 (0.50)	1.40 (0.37)	0.92 (0.23)
Wing asymmetry	0.50 (0.09)	0.56 (0.10)	0.41 (0.12)	0.50 (0.16)	0.20 (0.13)	0.25 (0.13)
Beak length	7.48 (0.13)	8.10 (0.29)	7.49 (0.11)	7.42 (0.13)	7.79 (0.18)	7.66 (0.15)
Beak width	11.60 (0.16)	11.42 (0.16)	11.65 (0.15)	11.30 (0.15)	11.89 (0.22)	11.91 (0.25)
Beak depth	2.82 (0.06)	2.83 (0.04)	2.80 (0.06)	2.83 (0.05)	2.85 (0.13)	2.77 (0.06)
Tarsus length	11.31 (0.15)	11.07 (0.26)	11.09 (0.10)	11.23 (0.08)	11.29 (0.24)	11.34 (0.17)
Keel length	21.58 (0.16)	20.15 (0.43)	21.21 (0.15)	20.85 (0.15)	21.58 (0.28)	21.07 (0.20)
Body mass	17.88 (0.23)	18.44 (0.38)	17.45 (0.24)	18.36 (0.41)	19.19 (0.32)	20.10 (0.61)
Chewing lice	21.44 (4.70)	21.47 (4.23)	19.47 (3.90)	22.75 (5.20)	12.40 (2.06)	6.58 (2.65)
Feather mites	80.17 (23.16)	82.44 (24.32)	84.41 (28.91)	81.50 (27.30)	51.50 (13.81)	52.33 (9.71)
Louseflies	0.00 (0.00)	0.00 (0.00)	0.59 (0.26)	0.44 (0.22)	0.00 (0.00)	0.00 (0.00)
Clutch size	4.88 (0.16)	4.50 (0.20)	4.94 (0.21)	4.19 (0.25)	4.90 (0.18)	4.92 (0.19)
Annual fecundity	8.81 (0.29)	8.25 (0.17)	—	—	7.30 (0.78)	6.08 (0.71)
Haematocrit	—	—	56.18 (1.02)	56.06 (1.10)	52.00 (0.52)	52.26 (1.16)
Arrival date	−0.31 (1.62)	3.25 (2.96)	—	—	70.40 (2.24)	81.75 (2.15)
<i>N</i>	16	16	17	16	10	12
Control individuals that only became one year old						
Tail length	99.31 (1.35)	86.84 (1.56)	106.53 (1.82)	87.06 (1.20)	105.75 (2.76)	89.33 (1.42)
Wing length	121.38 (0.49)	119.41 (0.49)	123.35 (0.53)	122.56 (0.60)	125.95 (0.50)	124.58 (0.77)
Central tail length	43.50 (0.32)	43.69 (0.32)	44.77 (0.28)	45.25 (0.30)	44.00 (0.63)	45.00 (0.56)
Tail asymmetry	1.03 (0.23)	1.28 (0.57)	2.53 (0.58)	1.38 (0.30)	1.70 (0.78)	0.83 (0.27)
Wing asymmetry	0.66 (0.18)	0.47 (0.10)	0.59 (0.17)	0.81 (0.28)	0.30 (0.21)	0.75 (0.18)
Beak length	7.45 (0.11)	7.68 (0.07)	7.40 (0.14)	7.23 (0.07)	7.76 (0.13)	7.84 (0.13)
Beak width	11.57 (0.17)	11.40 (0.16)	11.69 (0.12)	11.03 (0.17)	12.16 (0.20)	11.98 (0.15)
Beak depth	2.78 (0.05)	2.82 (0.05)	2.84 (0.04)	3.09 (0.28)	2.77 (0.04)	2.85 (0.09)
Tarsus length	11.17 (0.24)	11.28 (0.08)	11.06 (0.12)	10.90 (0.09)	11.28 (0.22)	11.70 (0.19)
Keel length	20.72 (0.38)	21.15 (0.32)	21.57 (0.19)	20.48 (0.19)	21.38 (0.20)	20.99 (0.18)
Body mass	17.94 (0.20)	18.29 (0.33)	17.62 (0.36)	18.63 (0.49)	19.21 (0.55)	19.99 (0.38)
Chewing lice	20.67 (4.48)	20.33 (4.09)	16.18 (3.70)	23.31 (3.18)	9.00 (3.02)	11.17 (2.97)
Feather mites	78.22 (19.64)	80.21 (25.19)	72.65 (44.24)	73.13 (19.31)	42.00 (9.40)	34.75 (6.73)
Louseflies	0.00 (0.00)	0.00 (0.00)	0.18 (0.13)	0.56 (0.20)	0.00 (0.00)	0.00 (0.00)
Clutch size	4.81 (0.10)	4.88 (0.09)	4.13 (0.19)	4.73 (0.21)	4.20 (0.20)	5.17 (0.11)
Annual fecundity	8.44 (0.34)	8.81 (0.14)	—	—	4.60 (0.52)	7.75 (0.69)
Haematocrit	—	—	56.00 (0.65)	54.94 (1.07)	56.00 (0.52)	56.12 (0.59)
Arrival date	−0.13 (1.96)	4.81 (3.40)	—	—	82.00 (2.24)	82.08 (2.52)
<i>N</i>	16	16	17	16	10	12

Values are means (SE). Arrival date is days since March 1. Annual fecundity was not estimated for Italy because some broods were experimentally manipulated for other studies. See “Materials and methods” for further information.

Haematocrits showed significant differences between categories of birds (Tables 1, 2), with both Danish males and females that eventually became very old having lower values than controls (Figure 1b). This implies that Danish individuals that ultimately became very old consistently had relatively low haematocrit values.

Arrival date of extremely old individuals and controls

We tested whether arrival date when one year old differed between individuals that subsequently attained at least five years old and control individuals of the same sex that arrived at the same site. An

Table 2. Two-way analyses of variance of the effect of sex and country on difference in fecundity, clutch size, haematocrit and arrival date between individuals that became at least five years old, when one year old, and a control individual of the same sex, age and arrival date (the latter for the first three comparisons only) from the same site.

Factor	Mean square	df	F	P
<i>Clutch size</i>				
Sex	18.444	1	26.460	0.0001
Country	1.118	2	1.604	0.208
Sex x Country	2.050	2	2.942	0.059
Residual	0.697	78		
<i>Fecundity</i>				
Sex	91.246	1	20.884	0.0001
Country	4.834	1	1.106	0.298
Sex x Country	38.138	1	8.729	0.0048
Residual	4.369	50		
<i>Haematocrit</i>				
Sex	3.901	1	0.157	0.693
Country	275.400	1	11.115	0.0016
Sex x Country	2.137	1	0.086	0.770
Residual	24.776	51		
<i>Arrival</i>				
Sex	189.839	1	4.259	0.042
Country	311.482	1	6.988	0.0016
Sex x Country	313.473	1	7.033	0.0015
Residual	44.571	81		

analysis of variance revealed significant effects of sex, country and sex-by-country interaction (Tables 1, 2). The country and the sex-by-country interaction were still significant after Bonferroni correction. Inspection of mean values revealed that Danish males that subsequently became very old arrived earlier than control males, while the two categories of females did not differ significantly from each other (Figure 1c). Furthermore, there was no difference between categories in the Spanish birds. Thus, the ability to survive until very old age was associated with early spring arrival when young in Danish males, but not in females.

Discussion

The main findings of the present study were as follows: (1) Males that successfully reached old age had mates with a larger clutch size than controls that never exceeded one year of age. Females that became very old had a smaller clutch size than controls, and this sex difference increased from Spain over Italy to Denmark. (2) Haematocrits were significantly lower among individuals that became very old compared to controls in Denmark, but not in Italy. (3) Arrival date

in males that became very old was earlier than that in controls, while there was no difference between these two categories of females. The difference between successfully aging males and controls varied significantly between countries. (4) There were no significant differences in morphology or abundance of three species of ectoparasites between the individuals that reached old age and those that did not. We discuss these findings in relation to current theories of senescence.

The first analysis suggested that patterns of natural selection are different for the sexes with respect to first clutch size and annual fecundity. While males that became very old had larger clutch size than controls when yearlings, very old females when yearlings had smaller clutch size than controls, and this sex difference increased from Spain over Italy to Denmark. These observations suggest that egg production (or incubation) is responsible for the sex difference, since males and females feed the young equally frequently (Møller 1994a). Once the effects of male tail length have been controlled statistically by matching individuals with respect to that variable, thereby controlling for differences among males in sexual attractiveness and hence male reproductive investment (Møller 1994a), there are no other clear predictors

Figure 1. Effects of sex and country on **a** difference in size of the first clutch between one-year-old individuals that reached at least five years old and a control individual of the same sex, age and arrival date from the same site, **b** difference in haematocrits between one-year-olds that became at least five years old and a control individual of the same sex, age and arrival date from the same site, and **c** difference in arrival date between individuals that became at least five years old when one-year old and a control individual of the same sex and age from the same site. Values are means (SE).

of amount of parental care by either sex (Møller 1994a). Lifespan and lifetime reproductive success measured as number of fledglings are strongly positively correlated in the barn swallow (Møller 1994a). It seems unlikely that female incubation is responsible for the sex difference since eggs by definition are laid before incubation starts. In addition, the differences cannot be attributed to previously incurred reproductive costs since yearlings have not yet reproduced. However, egg production and the associated maternal investment in incubating eggs may impose important costs. Reid et al. (2003) reached a similar conclusion for red-billed choughs, *Pyrrhocorax pyrrhocorax*. Maternal effects in terms on differential deposition of carotenoids, androgens and lysozyme have been reported for the barn swallow (Saino et al. 2002a, b; 2003; Gil et al. 2005). Moreover, an experimental study of the lesser black-backed gull, *Larus fuscus*, has shown that maternal provisioning with carotenoids increased the egg content of carotenoids (Blount et al. 2002). A subsequent experimental study demonstrated that carotenoids directly limited egg production ability in the lesser black-backed gull (Blount et al. 2003). In addition, challenge of the immune system of female barn swallows with a novel antigen reduced carotenoid deposition in eggs, while an experimental increase in sexual attractiveness of males increased female allocation of carotenoids to eggs (Saino et al. 2002a, b). Thus, maternal allocation of energy to eggs depends on the costs and benefits of such maternal effects to mothers.

The amount of carotenoids in a clutch of five barn swallow eggs equals more than 60 times the amount circulating in the plasma of an adult female (Saino et al. 2002a, b). We suggest that maternal investment in eggs trades against maternal self maintenance, possibly through the effects of carotenoids as antioxidants or immunostimulants (Bendich 1989, Chew 1993, Edge et al. 1997, Møller et al. 2000). This would not be the case for males. This explanation could account for the significant sex difference in clutch size between individuals that eventually became very old and controls (Figure 1a).

The larger sex difference with increasing latitude in fecundity between individuals that became very old and controls suggests that migration distance and the associated selection pressures also play an important role. Bird migration is costly in terms of time and energy (Alerstam 1990, Berthold 1996), and

the high metabolic activity needed for migration might be expected to result in the production of large amounts of free radicals that must be neutralized by antioxidants (Møller and Saino 2005). While the Spanish population migrates to the Ivory Coast and Nigeria 3100 km away, the Italian population migrates to Nigeria 3900 km away and the Danish population migrates 9,400 km to South Africa (F. de Lope, N. Saino and A. P. Møller, unpublished data). Early arriving barn swallows have very low plasma concentration of carotenoids, which increases considerably during the following weeks compared to late-arriving birds (Ninni et al. 2004). These observations are consistent with a trade-off between early arrival and high concentration of antioxidants. Therefore, the much larger distance traveled by more northern populations should result in arrival date more strongly correlated with phenotypic quality than that in southern populations. If such quality differences reflect abilities to perform maximal amounts of exercise without detrimental effects to the phenotype, perhaps through more efficient acquisition or use of antioxidants, this could explain a larger difference in fecundity between individuals that became very old and controls in Denmark compared to Italy and particularly to Spain. We suggest that the negative differences for clutch size for females (Figure 1a) are due to the costs of maternal reproductive investment. The positive differences for males (Figure 1a) may reflect that such males are more attractive and hence mated to very fecund females, which have been shown to invest differentially in reproduction when mated to more attractive males (Møller 1994a).

Haematocrits differed significantly between Danish barn swallows that became very old and controls matched with respect to sex, age, arrival date and site, while that was not the case for Italian barn swallows (Figure 1b). Haematocrit estimates the relative number of erythrocytes in the blood (Carpenter 1975), which increases during heavy exercise as in migratory birds (Sealander 1962; Palomeque and Planas 1978; Clemens 1990) such as those with trans-equatorial migration (Jones 1983; Morton 1994; Saino et al. 1997a, b). Studies of the barn swallow have found that adult males with long tail feathers (a secondary sexual character) have higher haematocrits than short-tailed males (Saino et al. 1997a). While males with their natural tails had reduced haematocrits following spring arrival at the

breeding sites, males with experimentally elongated outermost tail feathers maintained their high haematocrits (Saino et al. 1997b). This difference among experimental treatments suggests that flight is more costly with an elongated rather than a normal or a shortened tail. The benefits of a low haematocrit may be the lower transport costs of erythrocytes and lower costs of maintenance of a smaller number of erythrocytes. We suggest that in the Danish population, with a longer distance of migration than the Italian population, individuals may have become more efficient in locomotory performance due to more intense selection for migratory ability.

The second analysis suggested that Danish males that eventually became very old arrived very early compared to control males of the same age and tail length, while that was not the case for females from any of the populations, or for males from Spain. The objective of this analysis was to test if migratory ability was associated with the ability to eventually become very old. Males that arrived very early were indeed more likely to become very old, while that was not the case for females (Figure 1c). Arrival date in male barn swallows is strongly condition-dependent with long-tailed males arriving earlier than other males and earlier than females (Møller 1994a). After controlling for tail length, there was still a significant difference in arrival date between males that became very old and controls, suggesting that quality features other than those associated with tail length accounts for this difference (Møller 1994a). If only males of high phenotypic quality are able to arrive very early, we should expect such individuals to have superior abilities promoting successful aging. This would be the case for males to a greater extent than for females because competition for early arrival in female barn swallows is much less intense than in males (Møller 1994a).

Ninni et al. (2004) have recently shown that early arrival in the barn swallow indeed is associated with higher than average amounts of carotenoids in the blood. We suggest that the findings concerning arrival date and ability to reach very old age can be interpreted in terms of a role of antioxidants in the senescence process, consistent with the antioxidant hypothesis.

We also investigated a range of morphological characters and the abundance of three ectoparasites in our comparisons of individuals that eventually reached very old age and controls, but found no evidence of a significant difference in these traits.

This implies that these two categories of individuals do not differ in overall phenotype, but that only certain traits are associated with the ability to survive many years. Although wing and tail length have been shown to change during the annual molt in response to changes in environmental conditions in Africa (Saino et al. 2004), such effects did not appear to be of importance for the ability to reach very old age.

In conclusion, comparison of the phenotypes of yearling barn swallows that later became very old with controls matched with respect to several potentially confounding variables showed that fecundity, haematocrit and arrival date differed between these two categories of birds. These findings suggest that even for individuals matched with respect to sex, age, site of reproduction and arrival date there are clear phenotypic differences between individuals that eventually reach very old age and individuals that do not manage to do so.

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