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Antioxidants and condition-dependence of arrival date in a migratory passerine

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Early arrival at the breeding area after spring migration determines several fitness benefits to migratory birds, including an early start of reproduction and a greater seasonal reproductive success. Environmental conditions determine the onset of migration at the population level, while physiological costs associated with migration and imposed on individuals determine the level of asynchrony in the pattern of arrival. These costs include several physiological changes, suppression of immune response, and an increased risk of oxidation by production of free radicals due to energetically expensive activities. According to the condition-dependent hypothesis of arrival date, only healthy individuals in prime condition can afford to arrive and thus reproduce early in the season.

We investigated condition-dependence of arrival date in male barn swallow (*Hirundo rustica* L.), a migratory passerine, in two years that differed in environmental condition, and the role of carotenoids in mediating arrival date. We show that male tail length, the main secondary sexual character, body condition, and hematocrit were negatively correlated with arrival date in the first year, indicating better quality of early arriving males. In the second year, better environmental conditions advanced arrival of the entire population by weeks. In this season we could show no relationship between arrival and tail length or body condition. Moreover, a lower value of sedimentation rate and a brighter colour of the red throat feathers indicated better health status of the population. Arrival date is a condition-dependent character in barn swallows, since we found high repeatability of arrival date, lower values of carotenoids in blood and a lower depletion of carotenoids in blood shortly after arrival in early arriving males. This suggests that early arriving males either use fewer antioxidants for free radical scavenging, or they have differential access to antioxidants.

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Selection has shaped migration strategies to maximise fitness benefits to migratory species. Endogenous and genetic factors determine the onset of migratory behaviour, but environmental cues in terms of photoperiodicity and temperature are known to play a main role before and during migration, and at arrival (Brown and Brown 1999, Berthold 2001).

Early arrival at the breeding grounds after spring migration is associated with several fitness components in birds (Forstmeier 2002), including access to the best breeding sites (Hasselquist 1998) and mates (Møller 1994a, Lozano et al. 1996). Physical condition declines with arrival date as does breeding success (Slagsvold and Lifjeld 1988, Hasselquist 1998). An early start of

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reproduction allows individuals to raise more offspring by experiencing superior environmental condition in terms of quantity and quality of food (Møller 1994a, Potti 1998, Verboven and Visser 1998, Naef-Daenzer et al. 2001), and early reproduction confers additional time to replace failed clutches. A strong selection for early arrival is common in many species, and males usually reach the reproductive area earlier than females (Morbey and Ydenberg 2001).

Migration is also a highly costly activity. Several costs are imposed on migrating birds before and during migration, and at arrival, because specific metabolic processes and physiological and behavioural modifications take place (Battley et al. 2000, Berthold 2001). Increase in metabolic rate is known to occur (Berthold 2001, Kvist and Lindström 2001), which can be up to 30 times that of basal metabolic rate during flapping flight (Berthold 2001). Hyperphagia and the resulting replenishing of fat reserves, nutritional adaptations, and changes in body composition are common and take place constantly during migration (Berthold 2001, Schaub and Jenni 2001). Energetically expensive activities, such as migration and mating displays that take place as birds reach the reproductive area, are known to be associated with a reduced immune response (Råberg et al. 1998), and other negative effects on individual health status. In particular, strenuous physical exercise can increase the production of free radicals (Bejma et al. 2000). These compounds are the normal product of metabolism, as generated in mitochondria and during cell respiration, but also by activated immune defences. Due to their highly reactive nature, they can combine with enzymes and receptors, causing oxidation directly and thus damage several critical molecules including DNA, proteins, and lipids, leading in extreme situations to direct cell dysfunction and death of the individual (Punchard and Kelly 1996, von Schantz et al. 1999, Møller et al. 2000). Finally, hormones may be involved in controlling migration although their role is not clear. In particular, testosterone seems to play a role especially during spring migration (Berthold 2001), but it is known also to impair antioxidant defences exerted by enzymes and directly induce oxidative stress (von Schantz et al. 1999).

Carotenoids may be essential for coping with many of these costs. Carotenoids are pigments that birds ingest through their diet, since only plants, algae, fungi and bacteria can synthesise them (Goodwin 1984). They are known to act as free radical scavengers, reduce lipid peroxidation and enhance several aspects of immune function (von Schantz et al. 1999, Møller et al. 2000). They are one of the determinants of bright colours of plumage and fleshy tissues in several animal species, but can also be stored in the liver, blood, or fat depots from which they might be mobilised. Because of the wide array of different activities they can exert, and some evidence that they might not be available ad libitum to birds (Møller et al. 2000, McGraw et al. 2001, Hill et al. 2002), individual decisions to allocate carotenoids to a certain function and/or to store them in a certain tissue might affect fitness.

Independent of environmental conditions, which determine the general pattern of migration at the population level, the balance between costs imposed by migration and potential benefits ultimately shapes arrival. Individuals of high quality arrive early, pay relatively lower costs and obtain a net fitness gain during the reproductive season, assuring that arrival date is a condition-dependent character (Møller 1994b, Kokko 1999, but see Brown and Brown 2000).

In this study we investigate costs and benefits of early arrival to males of a migratory passerine during two seasons that differed in environmental condition. We tested the hypothesis that early arrival remains a condition-dependent character by studying the pattern of morphological and haematological variables shortly after arrival at the breeding area. In particular, we tested if carotenoids had a role in shaping arrival date by studying their blood concentration at arrival during the two seasons and how individuals that arrived at different times depleted their reserves shortly after arriving to the breeding area.

Methods

The study species

The barn swallow (*Hirundo rustica* L.) is a trans-Saharan migratory passerine, which winters in Africa, arriving at the breeding grounds in Europe from February to May depending on latitude of the breeding area (Møller 1994a). At southern latitudes, like Spain, early arriving birds can be seen already in December with the entire population having arrived by the end of April. Arrival date is strictly correlated with the date of start of breeding, and an early reproduction allows pairs to have a second and often a third clutch at southern latitudes (Møller 1994a).

The barn swallow is a socially monogamous species, where the female is the choosy sex. Strong selection for early arrival exists and males usually arrive earlier than females. The length of the outermost tail feathers seems to be the main character that females use to assess male quality and to choose their partner. Long-tailed males arrive earlier than shorter tailed ones, have fewer parasites and have higher reproductive success. Old males have longer tails and are present at the beginning of the season, while yearlings usually arrive later in the season. The benefits of early arrival include the possibility for males to acquire a better territory, and females of better body condition, and to experience superior environmental conditions for raising nestlings. Early reproduction allows a larger number of breeding attempts, resulting in more fledglings (Møller 1994a).

Barn swallows feed on the wing on several species of flying insects, including hover-flies and several other species that contain carotenoids (Goodwin 1984, Møller 1994a). Lutein is the main carotenoid in the plasma of adult barn swallows (Saino et al. 1999), and it partially determines the colour of the red feathers of the throat patch (Stradi 1998), a sexually dichromatic character.

Field procedures

The study was conducted in two farms in Badajoz (38°53′N, 6°49′W), southern Spain (de Lope and Møller 1993), during the breeding seasons 1999–2000. A total of 196 and 183 adults (among which 103 and 102 were males) were captured in the two years, respectively.

Birds were captured during early morning with mist-nets. Capture sessions at the beginning of the season took place once per week in each farm. At first capture birds were sexed according to Svensson (1984) and ringed with a numbered aluminium ring on one leg and a plastic colour ring on the other. Standard morphological measurements were taken (Møller 1994a), and a blood sample was taken from the brachial vein in heparinized capillaries. At the time of first capture a sample of the red throat feathers was taken from the central part of the badge, and stored in a plastic bag in a dark place. Before being released each bird received a unique colour marking on the belly feathers to allow individual recognition in the field. All measurements and ringing took a few minutes, and they were made by the same experienced person. In both years birds arrived over a period of two months. For this reason and because of frequent capture sessions, we considered the date of arrival to equal the date of first capture.

In 2000, during successive capture sessions birds already marked had a second and a third blood sample taken with an interval of approximately one week and three weeks from the first capture date. Capture sessions stopped when all birds in the colony were marked and females started laying. Birds without rings were considered yearlings because breeding philopatry is very high with no adult bird moving to another farm to breed during the long-term study from 1980–2002.

Blood samples were immediately stored in a cold bag in the field during capture sessions. The same day they were taken to the laboratory and placed in vertical position at 4°C for four hours to measure sedimentation rate. Immediately after, they were centrifuged, and after measurement of haematocrit, plasma and red cells were separated and frozen. Sedimentation rate was expressed as the ratio between the volume of the part of the capillary not occupied by blood cells and the total blood volume of the capillary. Haematocrit was calculated as the ratio between the volume of the red cells and the total volume of blood. Both these measurements have been used as indicators of health status in birds. In particular sedimentation rate, which is based on the fact that increased levels of fibrinogen and immunoglobolins reduce the rate of sedimentation of red blood cells in plasma (Merilä and Svensson 1995, Svensson and Merilä 1996, Coles 1997) have been shown to indicate health condition both in adults and nestlings (Szép and Møller 2000, Hoi-Leitner et al. 2001, Acquarone et al. 2002).

We estimated the total concentration of carotenoids in plasma by spectrophotometry. We followed the procedure used by Bortolotti et al. (1996, Tella et al. 1998). A total of 20 μl plasma was diluted in 180 μl ethanol. After 1 min of vortex, samples were centrifuged at $1500 \times g$ for 10 min 100 μl of supernatant was placed in a microtiter with two replicates of the standard curve obtained by progressively diluting xanthophyll in ethanol (Sigma, ref. X6250, 70%). The microtiter was immediately examined in a spectrophotometer and the optical density of the standard and the samples was determined at 450 nm. The concentration of total carotenoids in the plasma was estimated on the standard curve of each microtiter. A total of 15 samples were analysed both by spectrophotometry and by HPLC by J. Blount using the method described by Surai and Speake (1998), which allows determination of the absolute concentration of carotenoids in the blood. The correlation between the absolute carotenoid concentration and the optical estimation by spectrophotometry of the plasma was high ($r = 0.80$, $n = 15$, $p < 0.0001$), and demonstrated the validity of the method used.

The colour of the red throat feathers was analysed with a portable spectroradiometer (Ocean Optics Europe), following the methods described in Saino et al. (1999) and Perrier et al. (2002).

We followed the reproduction of all pairs breeding in the two farms. Nests were checked each week if empty and every second day when the pair started furnishing the nest to record the date of the first egg laid. After the entire clutch had been laid, the nest was checked every day as the date of hatching approached. Nestlings were ringed at the age of 12 days and the number of nestlings at this stage was considered to represent the number of fledglings since mortality is negligible during the late nestling period (Møller 1994a).

The number of reproductive events was estimated as the number of clutches that a given female laid during the season. The number of eggs and fledglings of each pair were used as estimates of reproductive success in that season. An independent experiment carried out in 1999 prevented us from using reproductive success for the entire breeding season. In 1999 the number of reproductive events and fledgling success were thus unavailable.

To calculate repeatability of arrival date we considered only males that were captured as adults in 1999 (i.e. captured as yearlings or older in 1998) and returned in 2000 ($n = 15$).

When body condition was considered in the analysis we used body mass as the dependent variable while tarsus length was entered in the model as a covariate to control for differences in body size (Garcia-Berthou 2001).

To compare plasma concentration of carotenoids of early and late arriving birds, we choose birds arriving on 10th March and those that arrived three weeks earlier and had a second capture the 10th March, because this procedure maximised sample size.

A total of 45 males that were already present the first year were recaptured in the second field season. When analysis included data of both years we randomly chose data for an individual from only one year to assure that each individual only appeared once in the analyses.

In some cases not all the parameters could be recorded causing differences in sample size among analyses.

Results

Arrival date differed between years, with individuals arriving earlier in 2000 than in 1999 (Wilcoxon two sample test, $z = -5.60$, $n = 152, 147$, $p < 0.0001$). Mean arrival date in 1999 was 17th March, while it was 6th March in 2000. Median arrival date in 1999 was 18th March, while in 2000 it was 3rd March (Fig. 1).

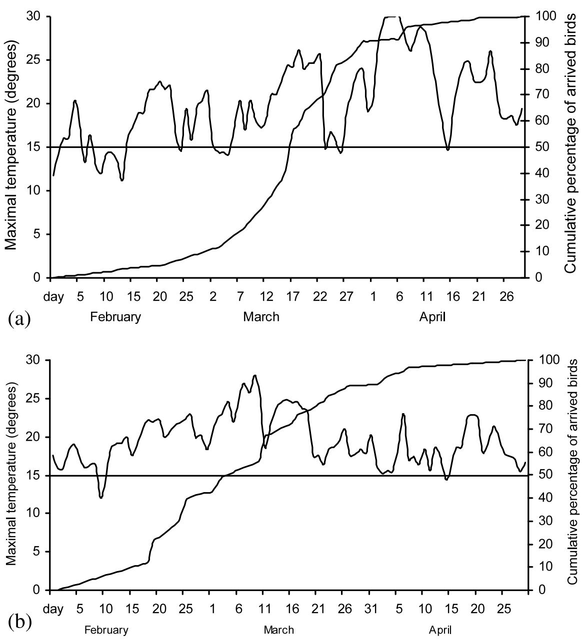

Fig. 1. Maximal daily temperature (degrees) and cumulative percentage of birds that arrived in the two seasons considered, (a): 1999; (b): 2000.

Repeatability of arrival date was 0.50 ($F_{14,\ 29} = 3.29$, $p = 0.014$) indicating that individuals that arrived early the first year were also early the second year.

First, we investigated the relationship between arrival date and laying date. Laying date was earlier in 2000 than in 1999 (Wilcoxon two sample test, $z = -6.37$, $n = 63,57$, $p < 0.0001$). Mean laying date was 12th April in 1999 and 25th March in 2000. Laying date was strongly positively correlated with arrival date (Spearman rank correlation 1999: $r_s = 0.577$, $n = 63$, $p < 0.0001$; 2000: $r_s = 0.676$, $n = 57$, $p < 0.0001$) showing that early arriving birds reproduced early.

Second, we tested if early arriving males had a net benefit over the season in terms of reproductive success. Since tail length has previously been shown to influence female choice and determine an advantage in terms of reproductive success to males with more elaborate character we corrected statistically for this character in the following analyses. Number of clutches laid by females was negatively correlated with arrival date (2000: Kendall partial correlation coefficient $\tau = -0.295$, $n = 56$, $p < 0.001$), as was the number of eggs laid: (1999: $\tau = -0.229$, $n = 53$, $p < 0.010$; 2000: $\tau = -0.232$, $n = 57$, $p < 0.010$) and the number of fledglings that the pair raised (2000: $\tau = -0.225$, $n = 57$, $p < 0.010$). Early arriving males had higher reproductive success in both years.

Third, we tested for condition-dependence of arrival date by determining how several morphological (tail length, body condition, colour of the red patch), haematological (haematocrit, sedimentation rate) and biochemical (carotenoid concentration in plasma) variables varied with arrival date in the two years of study. We tested for independence of the analysed variables by correlation analysis. None of the variables was significantly correlated with any of the others (all $r < 0.223$, $n > 77$) after correction for the number of test performed. As expected, sedimentation rate correlated with hematocrit in both years ($r > -0.576$, $n > 93$, $p < 0.01$).

Mean male tail length was slightly but not significantly different in the two years ($F_{1,156} = 2.32$, $p = 0.13$; mean (SE): 1999: 97.68 mm (0.68); 2000: 99.36 mm (0.66)). Early arriving males in 1999 had longer tails than late arriving individuals, while a much weaker tendency was found in 2000 (ANCOVA controlling for age, 1999: $F_{1,101} = 10.46$, $p = 0.0016$; 2000: $F_{1,97} = 2.97$, $p = 0.089$, with pooled data, interaction term: arrival date $\times$ year: $F_{2,156} = 4.19$, $p = 0.017$).

Mean body condition on average was better in 2000 ($F_{1,154} = 5.86$, $p = 0.017$, mean (SE): 1999: 17.73 (1.086), $n = 101$; 2000: 18.37 (1.274), $n = 99$). In 1999 early arriving individuals had better body condition than late arriving individuals, while no significant relationship was found in 2000, after controlling for tail length in an analysis of covariance (1999: $F_{1,99} = 5.16$, $p = 0.025$; slope $= -0.0191$ (SE $= 0.0084$); 2000: $F_{1,82} = 0.59$, $p = 0.44$ (slope $= -0.0069$ (0.0090)).

The colour of the red facial feathers showed great inter-annual variation ($F_{1,155} = 38.66$, $p < 0.0001$), with brighter colours in 2000 than in 1999 (mean (SE): 1999: 1.156 (0.007); 2000: 1.228 (0.006)), and an opposite pattern with arrival date in the two years, independent of age and tail length in an analysis of covariance (interaction term: arrival date x year: $F_{1,155} = 19.78$, $p < 0.0001$). In 1999 birds that arrived early had a more dull colour than birds that arrived later in the season ($F_{1,100} = 21.08$, $p < 0.0001$, slope $= 0.0024$ (SE $= 0.0005$)). In the second year the relationship showed an opposite trend ($F_{1,96} = 6.55$, $p = 0.012$, slope $= -0.001$ (SE $= 0.0004$)).

Mean hematocrit was larger in 2000 than in 1999 ($F_{1,154} = 7.51$, $P = 0.007$; mean (SE): 1999: 0.550 (0.004); 2000: 0.566 (0.004)). In both years hematocrit was negatively correlated with arrival date (1999: $F_{1,81} = 6.49$, $p = 0.013$, slope $= -0.0008$ (SE $= 0.0003$); 2000: $F_{1,72} = 3.80$, $p = 0.055$, slope $= -0.0005$ (SE $= 0.0003$)), although in the second season the relationship was weaker and did not reach significance. This showed that in the first year early arriving individuals had higher levels than late arriving individuals, independent of tail length.

Sedimentation rate showed large inter-annual variation, with lower values in 2000 than in 1999 ($F_{1,147} = 15.93$, $P < 0.0001$; mean (SE): 1999: 0.201 (0.008); 2000: 0.146 (0.007)), indicating better health status in the second season, but no correlation was found with arrival date ($F_{1,147} = 0.06$, $p = 0.810$), when controlling for tail length. Results did not change qualitatively while controlling for haematocrit values.

In both years birds that arrived in the middle of the season showed a higher concentration of carotenoids in the blood than those that arrived early in the season (Fig. 2), independent of age and tail length, although in 1999 the relationship was not as clear as in 2000 due to lack of blood sample data from birds that arrived at the very beginning of the season (1999: arrival date: $F_{1,78} = 4.76$, $p = 0.0324$; (arrival date)^2: $F_{1,78} = 3.49$, $p = 0.0656$; 2000: arrival date: $F_{1,93} = 6.05$, $p = 0.016$; (arrival date)^2: $F_{1,93} = 13.38$, $p = 0.0004$).

We tested if plasma concentration of carotenoids at arrival was independent of pigment availability in food resources at arrival by comparing male plasma carotenoid concentration sampled the same day from individuals that just arrived at the breeding grounds and individuals that arrived 20 days earlier. We measured concentration of carotenoids at arrival of 16 late individuals and that of 8 individuals that arrived approximately three weeks earlier on 10th March (see Methods). The variance of the two groups was slightly different (Levene's test $F = 4.45$, df $= 1,22$, $p = 0.046$), so

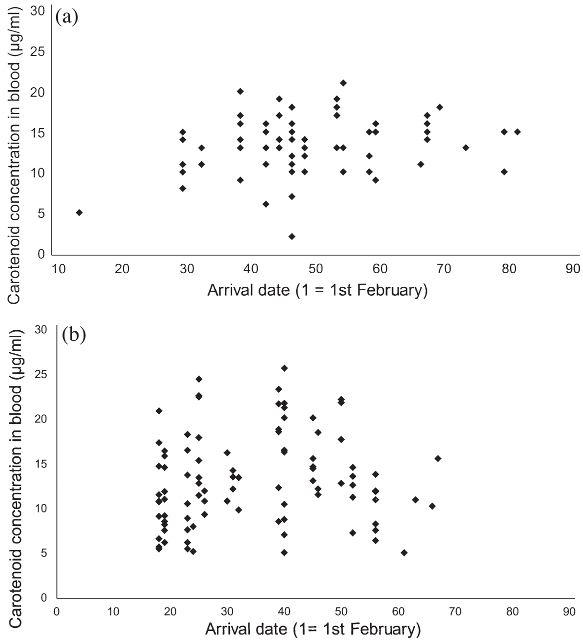

Fig. 2. Carotenoid concentration (µg/ml) at arrival of male barn swallows, (a): 1999; (b): 2000.

we tested for a difference between the means of the two groups by Welch's ANOVA (Zar 1996). Birds that arrived earlier had a lower concentration of carotenoids in the plasma compared to those that arrived later ($F = 9.59$, df $= 1, 21.52$, $p = 0.0053$, Fig. 3).

Finally, to test whether early and late arriving males had depleted antioxidant level to different extents, when they reached the reproductive area, we investigated change in carotenoid concentration from arrival to one week or three weeks later. We found a significant variation of carotenoid blood concentration in relation to arrival date (Table 1) after one week spent in the reproductive area. Males that arrived early on average did not change their concentration (Fig. 4), while those that arrived late (and had a higher concentration of carotenoids in blood) showed a greater decrease in their

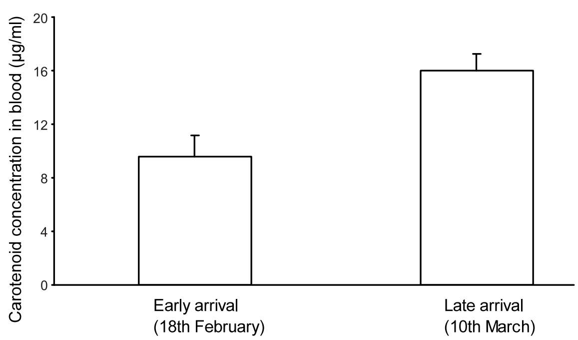

Fig. 3. Carotenoid concentration in blood (µg/ml) of early and late arriving male barn swallows, measured 10th March 2000. Values are means (+SE).

Table 1. Results of a repeated measures analysis of variance of the concentration of carotenoids in the plasma of male adult barn swallows within one week after arrival at the breeding site.

Source	df	MS	F	P
Carotenoid variation	1	2.029	0.11	0.740
Carotenoid variation × Date	1	55.027	3.01	0.089
Carotenoid variation × $(Date)^2$	1	157.53	8.63	0.005
Carotenoid variation × Age	2	2.72	0.15	0.862
Carotenoid variation × Tail length	1	1.49	0.08	0.776
Error	49	18.26		

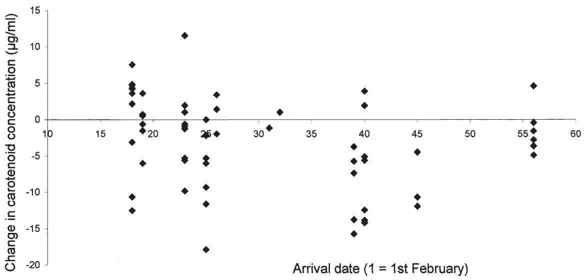

Fig. 4. Change in carotenoid concentration (µg/ml) in blood of male barn swallows from arrival to one week after arrival.

concentration (Fig. 4), independent of tail length and age. Males sampled between three and four weeks after their arrival showed a linear variation in their plasma carotenoid concentration dependent on their arrival date (Table 2).

Discussion

Environmental control of arrival

Early arrival is partly determined by temperature at the breeding grounds in the barn swallow with birds arriving early during warm springs (Huin and Sparks 1998). Spring was warmer in the second study season, and the population responded to environmental conditions by arriving and starting to reproduce on average two weeks earlier (Fig. 1).

Arrival time in migratory birds is determined by several factors (Berthold 2001). Departure schedule of long distance migrants relies on endogenous rhythms and environmental cues related to photoperiodicity at least at the beginning of migration (Gwinner 1996, Both and Visser 2001). For some species both migration and arrival are sensitive to temperature (Huin and Sparks 1998, Tryjanowski et al. 2002), which can exert direct and indirect effects on bird migration (due to effects of temperature on flowering and leafing time of trees and the emergence of invertebrate prey). Barn swallows respond to insect abundance and temperature locally during migration, and these are the environmental factors that determine the onset of breeding (Møller 1994a). Although individuals in prime condition can afford longer periods with poor weather and an arrival at the breeding area before optimal conditions are available, after pair formation, birds start building the nest only when weather is fine, and it is common that females on cold days stop laying for one or more days (Møller 1994a). Better condition in the area visited during the last part of migration and warmer temperature at arrival may have allowed more birds to arrive early in the second year.

The pattern of arrival that we observed in the two seasons could be due to phenotypic plasticity (Przybylo et al. 2000), with males performing differently according to external cues. Such responses following changes in environmental conditions have been demonstrated both in life-history traits and in a secondary sexual trait in a long-term study of a population of collared flycatchers (*Ficedula albicollis*, Qvarnström 1999, Przybylo et al. 2000). Alternatively, selection might eliminate very poor quality individuals and affect arrival date. We do not believe that different selection acted on the population in winter and during migration in the two years for several reasons. First, we could not detect a significant difference in mean values of morphological characters of the population in the two years. Moreover, in the two seasons approximately the same number of individuals appeared in the two colonies studied, with a survival probability of 0.36 from 1998 to 1999, and 0.47 from

Table 2. Results of a repeated measures analysis of variance of the concentration of carotenoids in the plasma of male adult barn swallows three weeks after arrival at the breeding site.

Source	df	MS	F	P
Carotenoid variation	1	3.11	0.20	0.654
Carotenoid variation × Date	1	141.50	9.32	0.005
Carotenoid variation × Age	2	30.73	2.02	0.149
Carotenoid variation × Tail length	1	0.292	0.02	0.89
Error	35	15.19		

1999 to 2000 (T. Szép and A.P. Møller unpubl.). The difference between years represents 25% and if it targeted mainly poor quality individuals, it could have contributed to the difference observed. If that was the case, firstly we would have recorded a lower rate of recruitment and fewer yearlings, which are known to suffer more during migration due to lack of experience (Møller 1994a). However we did not record any difference in recruitment in the two years (39 and 40, respectively), and moreover the total number of yearlings that returned to the reproductive area was more than 100 in both years, (1999: 156 yearlings; 2000: 107 yearlings). Finally, to verify that selection did not eliminate poor quality individuals during the early part of the reproductive period, thus acting only on the adult population, we tested for a difference in morphological parameters of individuals that were already adults in 1999, and in 2000, excluding from the analysis those that were captured in both years. None of the parameters (tail length, tarsus length or body condition) differed between years (all $F_{1,46} < 1.01$, $p > 0.32$).

Benefits and costs of early arrival

Advantages of early arrival at the breeding grounds are reported in several different avian and other vertebrate species (Kokko 1999, Forstmeier 2002). In the barn swallow selection for early start of reproduction is high (Møller 1994a), because benefits in terms of partner quality, nest site availability, and time to raise several clutches are known to occur. Here we confirmed that early arriving males had an advantage over late arriving individuals, in terms of number of breeding events, eggs laid and number of fledglings.

While environmental control of migration and arrival is imposed at the population level, physiological processes associated with migratory behaviour impose different costs on individuals depending on their quality (Kokko 1999). We showed that costs imposed on recently arrived individuals are higher for late arriving birds. In the first year early arriving birds had better body condition and a longer tail, which has been shown to be a condition-dependent secondary sexual character (Møller et al. 1998). Early arriving birds had a duller colour of the red throat feathers indicating a lower investment in this signal. In the second year, we found generally better body condition independent of arrival date, lower sedimentation rate which indicated better health status (Merilä and Svensson 1995, Coles 1997), a weaker relationship between haematocrit and arrival and tail length, and brighter colour of the red facial feathers, with early arriving birds showing more red colour than late arriving birds. Fewer costs were imposed on males the second year, but all birds could not advance their arrival date, since we found a significant repeatability of arrival date and a clear cost in terms of different concentration of carotenoids in blood and a higher depletion of carotenoids shortly after arrival. This indicates higher costs for late arriving, lower quality birds.

The negative effects of migration, reinforced by costs associated with the start of expensive mating displays (Andersson 1994), may still be acting and affecting the performance of recently arrived males. Moreover, high levels of testosterone, a sexual hormone that early in the breeding season is known to reach its maximum annual concentration (Ketterson and Nolan 1992), have been shown to increase metabolic rate in birds, which in turn causes an increase in oxidative stress in a range of different tissues, resulting in suppression of the immune system (Råberg et al. 1998, von Schantz et al. 1999). Carotenoids as well other metabolites like fat stores, that are accumulated before and during migration, may represent reserves once birds arrive at the breeding site, and individual decisions to allocate these pigments to certain activities and/or to store them in certain tissues may determine condition at arrival and, finally, directly affect fitness. Animals differ in absorption, transport, and deposition of carotenoids (Hill 2000), independent of the types and amount of pigment ingested. Several factors like parasitism, androgen level, sex and age have been related to intra- and inter-sexual differences in carotenoid content of different tissues (Hill 1992, 1993, Bortolotti et al. 1996, Thompson et al. 1997, Negro et al. 1998, 2001a, McGraw et al. 2002), and a trade-off between the expression of carotenoid-based characters and health status has been proposed (Lozano 1994, von Schantz et al. 1999, Møller et al. 2000, Blount et al. 2001, Hõrak et al. 2001). Although it still remains unknown whether carotenoid concentration in plasma is a reliable indicator of amount stored in other tissues, and how it is taken up for different functions (Negro et al. 2001b), variation in its concentration in the plasma might indicate the need for carotenoids on a short term.

The evidence that late arriving barn swallows had a higher level of carotenoids in the blood and depleted a larger amount of carotenoids shortly after settling in the breeding area, probably indicated a higher cost of oxidative stress for these birds, while early arriving individuals can afford a lower concentration of antioxidants in the blood. This pattern was consistent between the seasons, thus independent of environmental conditions before and at arrival at the breeding grounds. Although our findings are not conclusive, they describe natural variation in antioxidant concentration in blood that will be the base for further predictions concerning the role of carotenoids during migration and other highly expensive activities to be tested by experimental studies. Moreover, they are in accordance with the results of a supplemental feeding experiment on lesser black-backed gulls (*Larus fuscus,* Blount et al. 2002) where high plasma carotenoid concentration was associated with high antioxidant activity.

High carotenoid concentration of late arriving birds could be due to greater availability in the environment. If that was the case, we should expect early arriving birds to increase their concentration compared to late arriving conspecifics because they are foraging in the same environment as late arriving birds. This prediction was not supported, since early arriving individuals had lower concentration of carotenoids later in the season compared to late arriving birds, indicating less need and better health status. An alternative explanation might be that late arriving birds acquire a greater amount of pigments during the last part of migration. However the decrease in carotenoid concentration shortly after arrival of late arriving birds is consistent with greater need for carotenoids by such individuals for other activities related to their health status (Blount et al. 2003, Faivre et al. 2003). Our results suggest that antioxidant levels in the blood may reflect a trade-off between arrival date and condition, thus affecting the performance of migrating birds, leading to a delay in arrival of low quality individuals, even when environmental conditions seem not to be a limiting factor. Blount et al. (2001) proposed a mechanism, based on antioxidants availability, which linked male ornamental display and fertility of sperm during the reproductive period. They hypothesized that "males with high quality antioxidant-dependent ornamental display are those with high body supply of antioxidants, and hence also effective antioxidant protection of sperm" (Blount et al. 2001). In principle, any male ornamental character is subjected to this trade-off, and arrival at breeding grounds after long migration, like in the case of the barn swallow, could be a strong candidate. Antioxidant availability might affect both arrival date, as proposed by this study, and the protection of sperm from oxidative stress during a period (i.e. female choice and reproduction) when fertility of the male is a priority. Better quality males will be able to arrive early and assure the highest rate of fertilization of eggs.

Conclusions

Kokko (1999) developed a game-theoretic model to describe arrival of migrating, territorial species depending on condition. The model predicts advanced arrival date when competition for priority-dependent advantages of early arrival exists, in addition to seasonal effects that determine the optimal onset of breeding. Although the model originally dealt with territorial species, it also applies to other cases in which benefits of early arrival include the possibility to mate with a higher quality mate and to have more extra-pair copulations, as might be the case in the barn swallow. In all cases, arrival remains condition-dependent only if costs of advanced arrival are greater for low quality individuals, independent of environmental conditions. In accordance with the model, we showed that arrival time in the barn swallow is a condition-dependent character even when favourable environmental conditions allowed the population to arrive and thus reproduce early.

When environmental conditions are more favourable an advanced optimal date of arrival is predicted. In this scenario lower costs are imposed on the population for a given date (Kokko 1999). We would under such conditions expect more birds to be able to arrive early resulting in a higher degree of synchrony, which would reduce a repeatability of arrival date (Møller 1994b). A higher degree of synchrony would cause greater competition for benefits at the beginning of the season, since low quality individuals, which can advance arrival, have better access to territories and mates. For arrival date to remain condition-dependent, high quality individuals should always have greater benefits and pay fewer costs than low quality individuals (Kokko 1999). In the second year, half of the population arrived two weeks earlier than the first year, but, in accordance with the condition-dependent hypothesis (Møller 1994b), we found a high repeatability of arrival date, clear fitness benefits for early arriving birds, and higher costs for late arriving individuals in terms of rapid depletion of carotenoids at arrival.

Alternatively, if variation in costs varies stochastically, condition-dependence could be weaker (Forstmeier 2002), and other forces might shape arrival. In this view, condition-dependent selection should act only when early arriving birds face particularly unfavourable circumstances, that may appear rarely, and benefits could then control the arrival pattern (Forstmeier 2002). In the barn swallow, females prefer long-tailed males, but arrival date also provides advantages in terms of mating, independent of tail length, as was the case in this study. Although the population could arrive and reproduce early, higher costs of early arrival seemed to be imposed on low quality individuals, suggesting that arrival date remained a condition-dependent character.

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